Greater Mekong Subregion Medical Journal

ISSN 2730-3446 GMSMJ Vol. 2 No. 1 Jan - Apr 2022

Journal Name	Greater Mekong Subregion Medical Journal
Abbreviation	GMSMJ
ISSN (Online)	2730-3446
Owner	School of Medicine, Mae Fah Luang University
Aims and Scope	Greater Mekong Subregion Medical Journal is an online, peer reviewed international scientific journal published by Mae Fah Luang University. The journal aims to publish articles in the field of basic and advanced clinical research in medicine and related health sciences, medical education as well as community medicine in Thailand, international and especially in countries of Greater Mekong Subregion. Manuscripts submitted to Greater Mekong Subregion Medical Journal will be accepted on the conditions that the author must not have previously submitted that paper to another journal elsewhere. The journal will not charge for any submission. The reproduction or copy of the articles included the pictures should be under the permission of the publisher.
Language	Full text and Abstract in English
Abstracting and Indexing Information	Thai citation index (TCI) and Google scholar
Frequency	3 issues per year (Jan-Apr, May-Aug and Sep-Dec)
Editorial office	School of Medicine, Mae Fah Luang University 333 Moo 1 Thasud Sub District, Muang District, Chiang Rai 57100, THAILAND Phone: 053-916566 Fax: 053-916570 E-mail: med@mfu.ac.th, apichai.lee@mfu.ac.th Website: http://medicine.mfu.ac.th
Support Agency	Mae Fah Luang University

Published by:

Tana Press, Phone +6625304114, Fax +6621088950, Website www.tanapress.co.th

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Contents

Original Article

- **Melatonin Decreased Postoperative Pain after Abdominal Hysterectomy: A Randomized, Double-blind, Placebo-controlled Trial**
  Prok Laosuwan, Kamonwan Dechaworawut, Oraluxna Rodanant, Dhammika Leshan Wannigama, Somrat Charuluxananan 1

Special Article

- **Cyclosporine and Prednisolone as First-line Treatment of Subcutaneous Panniculitis-like T Cell Lymphoma: Clinical Features and Outcomes in 4 Patients**
  Natnicha Girdwichai, Narumol Nolwachai, Anan Promrattanakun 15

Medical Education

- **Implementation of Early Clinical Exposure (ECE) in Preclinical Teaching**
  Apichai Leelasiri, Ubolwan Charoonruengrit, Roger Timothy Callaghan 25

Integrated Medicine

- **Acupuncture in Medicine**
  Taksin Jiemthong 31

Pharmacology

- ***In Silico* Prediction of the Action of Ivermectin-like Compounds on Binding Sites of the SARS-CoV-2 Spike Protein and Receptor-binding Domain of ACE2**
  Shisanupong Anukanon, Narudol Teerapatarakarn, Chaiyong Rujjanawate 35

Oral Medicine

- **Okra Jelly Affecting Self-Perceived Xerostomia and Oral Health Related Quality of Life in The Elderly: A Preliminary Study**
  Chaninard Wiriyaprasitchai, Visarut Thangvaravut, Witsuta Pongphaladisai, Wilairat Worapamorn 43

Case Report

- **CMV Enteritis and Guillain-Barré Syndrome after Stem Cell Transplantation for Lymphoma**
  Wichean Mongkonsritragoon, Wimwipa Mongkonsritragoon 55

Original Article

GMSMJ Greater Mekong Subregion Medical Journal

Melatonin Decreased Postoperative Pain after Abdominal Hysterectomy: A Randomized, Double-blind, Placebo-controlled Trial

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Received 9 August 2021 • Revised 30 September 2021 • Accepted 30 October 2021 • Published online 1 January 2022

Abstract:

Background: Incidence of anxiety and pain in patients undergoing hysterectomy is significant and primarily due to postoperative pain. Most patients usually receive opioids for pain control. Melatonin is a natural hormone produced by the body. Synthetic melatonin is available over the counter for the management of insomnia and jetlag. Clinically, melatonin can also be used to reduce pain and analgesic requirement in patients undergoing surgery. The analgesic benefit of melatonin as primary or adjuvant agents has been reported in various studies.

Objective: We aimed to study whether melatonin could improve pain and other postoperative conditions after hysterectomy.

Methods: A randomized, double-blinded, placebo-controlled trial study was carried out on 54 women undergoing hysterectomy, with or without oophorectomy under spinal anesthesia. Patients were allocated randomly to receive either 4 mg prolonged-release melatonin at night and in the morning before surgery or 2 doses of placebo. Morphine consumption within 24 hours, visual analog scale (VAS) pain score, quality of sleep, anxiety level score, fatigue, general well-being and satisfaction score were measured.

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GMSMJ 2022; 2 (1): 1-13

Results: Morphine consumption in melatonin group was significantly low compared to placebo (33.04 ± 10.42 and 42.63 ± 8.21 mg, ($p < 0.001$). Also, postoperative VAS pain scale was lower in the melatonin group at recovery room arrival (23.41 vs 8.07, $p = 0.01$). Postoperative fatigue, general well-being and satisfaction scores in the melatonin group were better than the placebo group.

Conclusion: Prolonged-release formulation of melatonin decreased pain intensity in post anesthetic care room and reduced morphine consumption within 24 hours after surgery. Melatonin may be an additional choice of multimodal analgesia for hysterectomy.

Keywords: Melatonin, Hysterectomy, Postoperative pain

Background

The hysterectomy is one of the most frequently performed surgical procedures in gynecology. Especially hysterectomy provides a definitive cure for women with symptomatic fibroids resulting in complete resolution of symptoms, relieved significant pain and distress.[1] However, abdominal hysterectomy is associated with moderate to severe postoperative pain, particularly in the early postoperative period.[2] Traditional methods for postoperative pain management include opioids administered systemically using intravenous patient-controlled analgesia (PCA), or neuraxial via epidural or spinal injections.

However, pain relief, specifically on movement, is not always adequately controlled when using PCA, despite moderate–large doses of morphine. This is associated with side-effects such as postoperative nausea and vomiting, tiredness, pruritus, headache, and constipation.[3] Currently epidural or intrathecal analgesia considered by some to be the current analgesic preferences for pain management after abdominal surgery.[4,5] Although concerns remain regarding complications after central blocks, specifically in elderly women.[6] Common postoperative problems after hysterectomy are not only acute postoperative pain but also high anxiety levels among patient. Preoperative anxiety in hysterectomy is strongly related to postoperative pain score and quality of life.[7-9] Benzodiazepine is a common medication as preoperative anxiolytic in hysterectomy but may impair psychomotor performance.

There has been recent interest in alternative methods for analgesia with minimal side-effects. Melatonin (*N*-acetyl-5-methoxytryptamine) is a pineal hormone regulating sleep-wake cycle in mammals. Besides circadian rhythm stabilizing, exogenous melatonin has been investigated for other effects such as modulation of blood pressure, body temperature and cortisol control, immune function and anti-oxidative defense.[10] The strong chronobiotic properties and the ability to regulate circadian rhythm make melatonin a good choice for sleep disorders in the elderly.[11]

Analgesic mechanisms of melatonin are not known but may be involved with β-endorphins, GABA receptor, opioid receptors and nitric oxide-arginine pathway in brain were proposed.[12] Recent studies showed some benefits of perioperative short-acting melatonin in many aspects among different groups of patients such as quality of recovery after surgery, diminished

depressive symptoms and pain score reduction.[13-15] However, melatonin's analgesic effect remains controversial in the perioperative period and requires further investigation.[16] Therefore, the aim of the study was whether preoperative oral slow-release melatonin can potentiate the analgesic effects of intravenous morphine and improve sleep quality and anxiety levels compared to placebo, on morphine consumption in patients undergoing abdominal hysterectomy with or without oophorectomy. In addition, other therapeutic perspectives in clinical anesthesia such as anxiolytic effect, sleep quality, and also quality of life after hysterectomy were compared.

Methods

This study was registered in an international registry, clinicaltrials.gov (identification number TCTR20140516001) before patient recruitment. The study was performed at the Department of Anesthesiology, King Chulalongkorn Memorial Hospital, Thai Red Cross Society. After approval from the Institutional Review Board of Faculty of Medicine, Chulalongkorn University (IRB No.428/56), oral and written informed consent was obtained from 54 patients. ASA physical status I-II and age 18-65 years scheduled for elective abdominal hysterectomy (with or without ovarian surgery) were enrolled into the randomized, double blinded, placebo-controlled study. Patient exclusion criteria included history of heart disease, hepatic or renal failure, psychiatric disorders, sleep disorders, chronic pain syndromes, mental impairment, drug or alcohol abuse, patients receiving drugs with known analgesic and sedative properties, BMI over 30 kg/m^2 and patients who refused spinal anesthesia.

Randomization and blinding

The patients were randomly divided into 2 groups (27 patients each) as shown in figure 1 by using computer-generated randomized numbers inserted into sealed opaque envelopes and marked 1–54. All personnel involved in patient management were fully blinded to the method of analgesia until the study was completed. These patients received either 4 mg of prolonged-release formulation of oral melatonin (Circadin®) (M group) or placebo (P group) at the night (8 PM) before the procedure and another dose 2 hours before surgery from a pharmacist who generated the random sequence and was not involved to the study. No other preoperative medication was given. Blinding and randomization were performed by an investigator who was not involved in patient evaluation. Other personnel involved in the patient's care were unaware of patient group assignment.

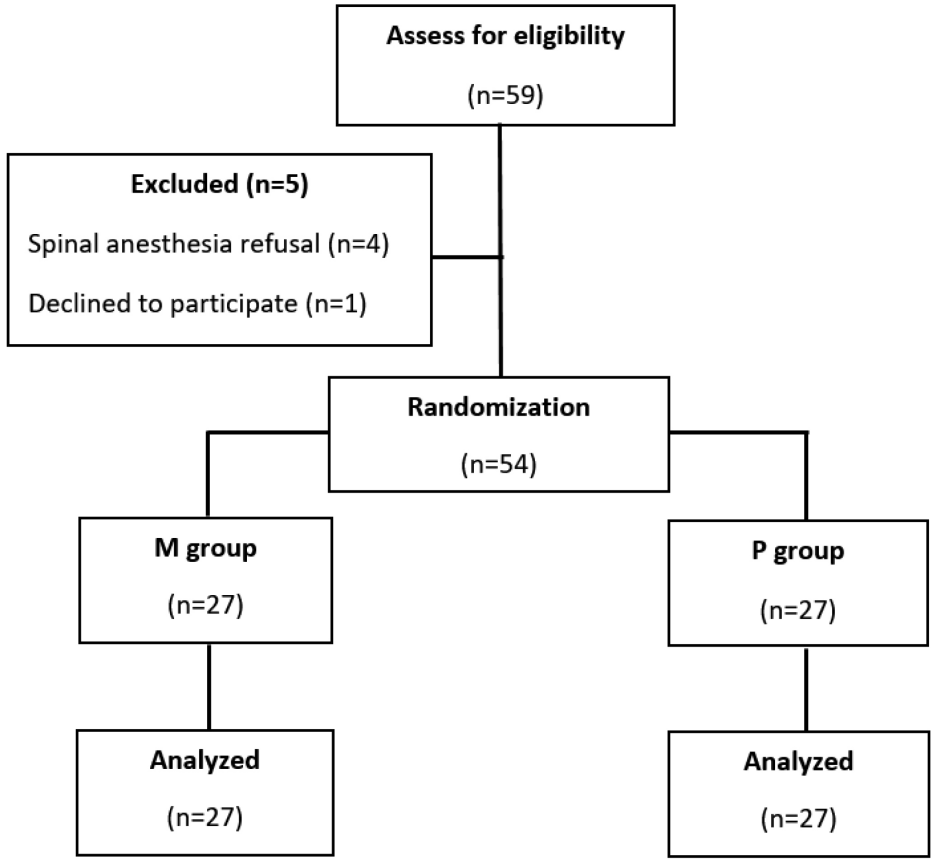

Figure 1 CONSORT diagram

Anesthesia and surgery

Preoperative visit was conducted the day before surgery. All patients were evaluated by the same anesthesia resident, who provided information on the preoperative course and instructed them on how to use the patient-controlled anesthesia (PCA) machine. Each patient was multidimensionally assessed; level of anxiety by the Amsterdam Preoperative Anxiety and Information Scale (APAIS) Thai version.[17] Anxiety score ranged from 4-20 (anxiety score >13 is possible to high level of anxiety). Sleep quality was measured by a questionnaire about subjective sleep quality using 100 mm VAS (visual analog scale; 0 = best conceivable sleep and 100 = worst conceivable sleep). Level of physical fatigue and general well-being were evaluated by using 10-point ordinal scale (1= least fatigue feeling and 10 = most fatigue feeling and 100 mm VAS (0 = extremely well and 100 = extreme malaise), respectively, at the night before and 24 hours after the surgery.

Upon arrival in the operating room, all patients underwent standard monitoring. Before spinal anesthesia, 10 ml/kg of physiologic crystalloid solution was administered intravenously. Spinal anesthesia was performed by spinal needle at lumbar segment L2/3 or L3/4 with 0.5% hyperbaric bupivacaine 16-20 mg according to attending anesthesiologists. If any patient had anxiety or discomfort, continuous propofol 0.08-0.1 mg/kg/min was given to maintain conscious sedation during the surgery. At the end of the surgery, sedation was stopped.

Postoperative pain management

At recovery room, all patients received morphine via patient-controlled analgesia (PCA) machine; the PCA dose was 1 mg, a 6-min lockout and maximum dose of 30 mg within 4 hours and no basal rate was applied. In the first 2-hour postoperative period, if the patients had VAS pain score more than 40 mm after being connected to morphine PCA, morphine 0.1 mg/kg was further injected. PCA pump was continued for 24 hours after surgery. Four mg of ondansetron every 6 hours was administered for nausea/vomiting as required. No other analgesic was allowed.

The primary outcome with respect to the efficacy of the study drug was postoperative morphine consumption in 24 hours. Secondary outcomes were postoperative pain score, anxiety, sleep quality, general well-being and satisfaction with pain treatment. Postoperative pain was assessed using 100 mm VAS (0 = no pain and 100 = worst imaginable pain) when arriving at recovery room (T0), 1 (T1), 6 (T6) and 24 (T24) hours. Satisfaction with pain treatment and nausea/vomiting were assessed using 100 mm VAS at 24 h postoperatively. In addition to the routine postoperative protocols, other adverse effects, surgical and anesthetic complications were recorded.

Statistical analysis

According to the previous study, the patients in the placebo group required 0.39 mg/kg/min of morphine during the first 24 hours after surgery.[18] The sample size of 25 patients in each group was required to detect difference between groups to reduce postoperative morphine consumption by 0.1 mg/kg/min with a confidence level of 90% and a significance level of 5% in order to achieve statistical significance for the primary endpoint. The sample size was calculated using the unpaired two-sided t-test. Assuming $\beta = 0.2$ (power 80%) and $\alpha = 0.05$, we determined that we would require 50 patients (25 per group) in order to achieve statistical significance for the primary endpoint.

We recruited 54 patients (27 in each group) in order to achieve adequate power in the case of missing data or allowing a 10% drop-out rate. Statistical analysis was calculated by using SPSS software version 22.0. Data are presented as mean ± standard deviation unless stated otherwise. Comparison of morphine consumption was analyzed using unpaired *t*-test. Pain score, anxiety and sleep quality were analyzed using repeated measure ANOVA. Satisfaction and nausea/vomiting were analyzed using Chi-squared test (Fisher's exact test if appropriate). A $p < 0.05$ was considered statistically significant.

Results

Fifty-four patients were enrolled into the study. No patient was excluded from the study after enrollment. The patient characteristics in each group, 27 patients, including diagnosis and types of operations were comparable between both groups (as shown in Table 1). Doses of bupivacaine, propofol and ephedrine were comparable. There was no significant difference in anesthetic level of bupivacaine and number of patients who required sedation. There was no statistical difference in surgical variables including operation time and amount of blood loss. The number of intraoperative events such as hypotension needed treatment, bradycardia (Heart rate < 60/min). were comparable in both groups and shown in Table 2.

Table 1 Demographic data and patient characteristics

Patient characteristics	P group (n=27)	M group (n=27)	*p*-value
Age (year)	42.85 ± 5.16	44.93 ± 4.13	0.11
Weight (kg)	59.07 ± 8.88	60.0 ± 7.86	0.69
BMI (kg/m^2)	23.77 ± 3.59	24.48 ± 3.22	0.45
ASA PS I/II	23/4	22/5	0.72
Diagnosis			0.49
Myoma uteri	20	21	
Adenomyosis	6	4	
ovarian cyst	1	2	
Operation			0.79
TAH	15	14	
TAH and SO	12	13	
Preoperative			
Level of anxiety	8.63 ± 3.48	8.11 ± 4.09	0.62
VAS sleep quality	26.89 ± 26.34	23.81 ± 26.41	0.67
VAS fatigue level	2.19 ± 2.15	1.85 ± 2.45	0.59
VAS general well being	20.70 ± 21.65	22.63 ± 23.11	0.75

Results showed in mean ± S.D., ASA PS, The American Society of Anesthesiologists physical status; BMI, Body Mass Index; TAH, Trans Abdominal Hysterectomy; SO, Salpingo-oophorectomy

Table 2 Intraoperative outcomes

Variable	P group (n=27)	M group (n=27)	*p*-value
Dose of bupivacaine (mg)	18.25 ± 1.05	18.75 ± 0.8	0.18
Anesthetic level (T4/T6)	18/9	22/5	0.19
Sedation requirement (Yes/No)	11/16	16/11	0.27
Propofol dose	90.37 ± 25.47	138.15 ± 27.61	0.21
Skin incision (Low midline/Pfannenstiel)	4/23	8/19	0.32
Duration of surgery (min)	105.56 ±19.23	103.52 ± 31	0.77
Blood loss (mL)	220.37 ± 23.90	247.04 ± 29.89	0.49
Intraoperative events (Yes/no)	18/9	20/7	0.83
Hypotension need treatment	16 (59.3%)	18 (66.7%)	0.64
Ephedrine dose (mg)	5 ± 6.17	6.15 ± 6.7	0.51

Variable	P group (n=27)	M group (n=27)	*p*-value
Bradycardia	0	0	
Nausea/Vomiting	2 (7.4%)	2 (7.4%)	0.56

Results showed in mean ± S.D. or n (%)

Cumulative 24–hour morphine dose of the patients in the M and P group were 33.04 ± 10.42 and 42.63 ± 8.21 mg, respectively ($p < 0.001$). Postoperative VAS of pain was significantly lower in the M group at recovery room arrival (T0) (23.41 vs 8.07, $p = 0.01$). However, there was no significant difference of VAS pain score between groups at 1 (T1), 6 (T6) and 24 hours (T24) postoperatively.

Satisfaction with pain treatment in the M group was significantly higher than in the P group. (8.56 ± 1.25 vs 7.78 ± 1.50, $p = 0.02$). (Table 3) There was no significant difference between groups in preoperative and postoperative anxiety level. On the first day after surgery, the patients in the M group reported fatigue VAS score lower than the P group (3.30 ± 2.22 vs. 5.15 ± 1.85, $p = 0.002$) Moreover, general well-being VAS scores was significantly lower in the M group compared with the P group. (31.59 ± 24.14 vs. 49.78 ± 14.87, p = 0.002) However, subjective sleep quality was not significantly different between both groups. (57.93 ± 21.08 vs. 49.33 ± 21.02, p = 0.14)

Table 3 Analgesic outcomes, other subjective scores and adverse effects

Outcomes	P group (n = 27)	M group (n = 27)	*p*-value
Morphine consumption in 24 hr. (mg)	42.63 ± 8.21	33.04 ± 10.42	< 0.01*
VAS pain score (mm)			
T0	23.41 ± 4.62	8.07 ± 3.39	0.001*
T1	41.67 ± 9.13	35.26 ± 5.88	0.19
T6	56.74 ± 8.87	48.89 ± 7.94	0.21
T24	34.37 ± 6.56	29.26 ± 4.91	0.12
VAS Satisfaction score	7.78 ± 1.05	8.56 ± 1.25	0.02*
Postoperative at 24 hr.			
Level of anxiety	5.44 ± 1.37	5.00 ± 2.00	0.35
VAS sleep quality	57.93 ± 21.08	49.33 ± 21.02	0.14
VAS fatigue	5.15 ± 1.85	3.30 ± 2.22	0.002*
VAS general well being	49.78 ± 14.87	31.59 ± 24.14	0.002*
Surgical complications	0	0	
VAS Nausea/Vomiting in 24 hr. after surgery	3.7 ± 2.52	4.1 ± 2.67	0.56

Results showed in mean ± S.D. or n (%)

Discussion

The present study demonstrates that long-acting oral melatonin improved VAS pain score and reduced cumulative dose of PCA morphine consumption in 24 hours. These results were similar to the previous studies in other procedures, such as prostatectomy,[19] dental surgery,[20] hand surgery,[21] cataract surgery under topical anesthesia[22] and abdominal hysterectomy.[18,23] In contrast, some studies failed to show the effectiveness of perioperative melatonin in terms of analgesic outcomes.[24,25] The variation of dose, route and timing of melatonin administration might affect these individual results, which remain inconclusive even after systematic review were conducted.[11,13,26,27] Caumo et al. revealed the analgesic effect of preoperative oral melatonin. Melatonin reduced pain scores on VAS scale within postoperative period of 48 hours and lowered morphine consumption for 24 hours after abdominal hysterectomy, compared to placebo.[18] Such a study proposed that postoperative anxiolytic effect of melatonin treatment led to anti-nociceptive effect.[18,23] In contrast, this study could not show a significant difference of anxiolysis, as well as VAS pain score after immediate postoperative phase at post-anesthesia care unit arrival.

The present study investigated a 4 mg of prolonged-release formulation of melatonin (Circadin®, Neurim Pharmaceuticals, Tel-Aviv, Israel). We chose this dose and form of melatonin because this was the only commercially available form in Thailand. This was a lower dose than other previous studies as premedication for analgesic effect. Forms of melatonin in all previous studies might be a short acting formulation or higher doses. However, from general clinical practice, 2-mg dose once daily of prolonged-release melatonin showed clinical benefits in terms of sleep quality and quality of life in patients aged 55 years and older without any side effects.[28] The therapeutic indication of this novel formulation melatonin is primary insomnia in elderly due to long duration of action and safety profiles.[27] Because exogenous melatonin modulates via activation of the MT1 and/or MT2 melatonin receptors in the central nervous system.[28,29] In addition, there were several in vitro studies which demonstrated that the anti-nociceptive effects of melatonin could be reversed by various mechanism such as flumazenil, naloxone, potassium or calcium ion-channel-blockers.[10] Moreover, a recent review of literature proposed the synergistic effects of melatonin combined with morphine in terms of hyperalgesia and morphine tolerance reduction.[30] In contrast, another recent meta-analysis could not show the significant association between melatonin use and acute postoperative pain outcome.[31] The present study is the first clinical study of prolonged-release formulation in perioperative period. A recent study in patients who underwent orthognathic surgery showed that prophylactic oral melatonin significantly decreased pain, numbness perception and were also correlated to lower serum hydrogen peroxide but higher antioxidant enzyme levels.[32]

Patients with postoperative sleep disturbance can suffer from delirium, delayed recovery and pain.[33] However, in our study failed to demonstrate the improved postoperative sleep quality. Similar to a recent meta-analysis in cholecystectomy, melatonin interventions showed no substantial impact on sleep quality and pain score after 1 and 3 hours.[34] However, Kirksey A. et al concluded melatonin did not have effect on subjective sleep assessment but improved sleep efficiency and sleep time by actigraphy wrist bracelet measurement.[35] But, correlation between pharmacologic sleep promotion and perioperative pain control are still controversial.[13]

Acute postoperative pain after hysterectomy may be complicated by anxiety state and psychological factors. A qualitative systematic review demonstrated that anxiety was a significant predictor for postoperative pain.[36] Such result was similar to another study in patients who underwent hysterectomy, in which preoperative anxiety was a positive predictor of immediate postoperative pain, pain on wards and also pain at home.[37] Moreover, Pinto et al. showed that anxiety predicted pain intensity at 48 hours after hysterectomy and also mediated pain catastrophizing.[38] In several clinical studies and systematic reviews, the outcome of preoperative melatonin administered to reduce preoperative anxiety was still controversial among varied population and doses.[15,18,20,39] Whereas another systematic review from Cochrane database concluded melatonin can reduce preoperative anxiety at the same rate as standard medication with midazolam if it was given within appropriate timing.[40] However, our study could not exhibit the benefit of melatonin as an anxiolytic.

In addition, the concept of immune-pineal axis influencing postoperative pain in patients who underwent hysterectomy was proposed. There was an inverse correlation between tumor necrosis factor (TNF) and nocturnal melatonin level. Moreover, the lower melatonin level was accompanied by lower cortisol levels and patients required higher doses of analgesics.[41] Therefore, exogenous melatonin might play a role for perioperative period especially in hysterectomy.

Fatigue has been defined as the lack of energy or exhaustion which is a complex, multifactorial symptom distinct from sleepiness or sadness.[42] The incidence of postoperative fatigue following hysterectomy was frequent regardless of general or spinal anesthesia.[43] Intensity of postoperative fatigue was the result of many biological factors, such as surgical stress response, anemia, declined nutritional status, psychological and social factors.[43] Fatigue was associated to poor quality of life in cancer patients who underwent surgery.[42] From the present study, melatonin enhanced subjective fatigue, general well-being VAS pain score and satisfaction score compared to placebo. These results were different from previous studies. Ivry M. et al. revealed melatonin improved quality of recovery following bariatric surgery in terms of sleep and pain levels.[14] Although differing in definition and measurement, the present study demonstrated advantages of melatonin administration in early postoperative fatigue and recovery, but no improvement of sleep quality. This may be due to lower morphine requirement.

Limitations of this study include the quality of recovery questionnaire in Thai version, which was not validated at the time the study was conducted. Likert and VAS pain score were measured to represent overall subjective recovery condition. The details of each standard domain may be inconclusive. Second, the results were focused only on perioperative and acute postoperative periods. Future studies should need to evaluate the effect of melatonin on chronic pain after hysterectomy. Third, the present study revealed only benefits of preoperative 2 doses of 4 mg of prolonged-release melatonin. Continuation of melatonin in postoperative period or earlier timing to load rather than one night before the surgery might be more appropriate with melatonin's pharmacokinetics and patient's metabolism. Moreover, to our knowledge, the appropriate dose and timing of oral prolonged-released melatonin was not established in perioperative period.

Conclusion

Preoperative orally prolonged-release melatonin had clinically relevant advantages in patients who underwent hysterectomy

with or without oophorectomy under spinal anesthesia in terms of decreased morphine consumption, pain score in PACU. Furthermore, this finding indicates that the postoperative fatigue, subjective general well-being, VAS pain score and patients' satisfaction score in treatment group were better than placebo without adverse effects.

List of abbreviations

GABA, γ-aminobutyric acid
ASA, American Society of Anesthesiologists
VAS, Visual analog scale
APAIS, Amsterdam Preoperative Anxiety and Information Scale
PCA, patient-controlled analgesia
PACU, Post-anesthetic care unit
TNF, tumor necrosis factor

Ethics approval and consent to participate

The approval of the Institutional Review Board of Faculty of Medicine, Chulalongkorn University (IRB No.428/56). Before study enrollment, all subjects reviewed and signed an informed consent document explaining the study procedures and potential risks.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

Funding

This study was supported by the Ratchadapisek Sompoch Endowment Fund, Faculty of Medicine, Chulalongkorn University, Thailand [RA57/097].

Authors' contributions

PL helped with development of research idea, writing the proposal, data collection, data analysis, a major contribution in writing the manuscript. KD collected and analyzed the patient data. OR collected the patient data and reviewed the literature and perform final review of the manuscript. DLW supervision, critical review and editing of the manuscript. SC performed critical reviewing of research idea and the manuscript.

Acknowledgements

We appreciate the contributions to this work from the following: Administrators of Research Unit and all anesthesia personnel at post anesthesia care unit of the Department of Anesthesiology, King Chulalongkorn Memorial Hospital.

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Special Article GMSMJ Greater Mekong Subregion Medical Journal

Cyclosporine and Prednisolone as First-line Treatment of Subcutaneous Panniculitis-like T Cell Lymphoma: Clinical Features and Outcomes in 4 Patients

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Received 25 May 2021 • Revised 27 June 2021 • Accepted 30 July 2021 • Published online 1 January 2022

Abstract:

Background: Subcutaneous panniculitis-like T-cell lymphoma (SPTCL) is a rare primary cutaneous T-cell lymphoma that can present with subcutaneous nodules mimicking panniculitis. Treatment protocols for SPTCL are varied, due to lack of agreement on standard treatment. Recent publications showed good response to treatment with cyclosporine and/or prednisolone as first-line treatment for SPTCL.

Objective: We aimed to study the outcome of SPTCL patients treated using cyclosporine and prednisolone as a first-line treatment, and also describe clinical presentations, histopathology, immunophenotype, molecular, treatment protocol, and treatment outcomes.

Results: Our study reported 4 SPTCL patients that presented with multiple subcutaneous nodules or indurated plaques, associated with fever and weight loss. All patients received cyclosporine and prednisolone as first-line treatment and achieved complete remission within 4-8 weeks. Three patients are still in complete remission. Relapse of SPTCL was suspected in one patient.

Conclusion: Our results suggest that cyclosporine and prednisolone are beneficial and could be use as first-line treatment in SPTCL.

Keywords: Subcutaneous panniculitis-like T cell lymphoma, Cyclosporine, Prednisolone

Introduction

Subcutaneous panniculitis-like T- cell lymphoma (SPTCL) is a rare primary cutaneous T-cell lymphoma. It typically presents with subcutaneous nodules or deeply seated plaques on the lower extremities and trunk.[1-3] It was first described in 1991 by Gonzalez, et al. as a new type of T cell lymphoma with clinicopathologic features simulating panniculitis, that was often associated with HPS *(Hemophagocytic syndrome)* and following an aggressive clinical course.[4] In the past, it was categorized into either

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GMSMJ 2022; 2 (1): 15-23

α/β T-cell phenotype or γ/δ T-cell phenotype. However, the World Health Organization-European for Research and Treatment (WHO-EORCT) classification in 2005 and WHO classification (2008/2016) defined SPTCL as a cytotoxic T-cell lymphoma, characterized by the presence of primarily subcutaneous infiltrates of small, medium-sized, or large pleomorphic T cells with an α/β T-cell phenotype, while γ/δ T-cell phenotype was defined as a primary cutaneous γ/δ T-cell lymphoma. In spite of their different clinical course, histology, immunophenotype, and prognosis.[1,5,6] SPTCL has an excellent prognosis. Unfortunately, it has no standard treatment protocol. Recently, many case reports and small case series suggested cyclosporine and/or systemic steroids as first-line or alternative treatments in SPTCL.[3,7-12] but their use as first-line treatment is still not well-established. This retrospective study aims to review clinical presentations, histopathology, immunophenotype, molecular, treatment protocol, and treatment outcomes in SPTCL patients, using cyclosporine and prednisolone as first-line treatment.

Methods

This is a retrospective study of 4 patients diagnosed with SPTCL, treated with cyclosporine and prednisolone as first-line treatment at Saraburi hospital, between January 2015-November 2020. We collected data from patients' medical records and analysed demographic data, clinical manifestations, and treatment outcomes by reviewing the patient's history, clinical manifestations, findings, physical examinations, histopathology (skin, lymph node, and bone marrow), immunohistochemistry, staging evaluations, treatment protocol, and treatment outcomes. The study was approved by the research committee at Saraburi hospital.

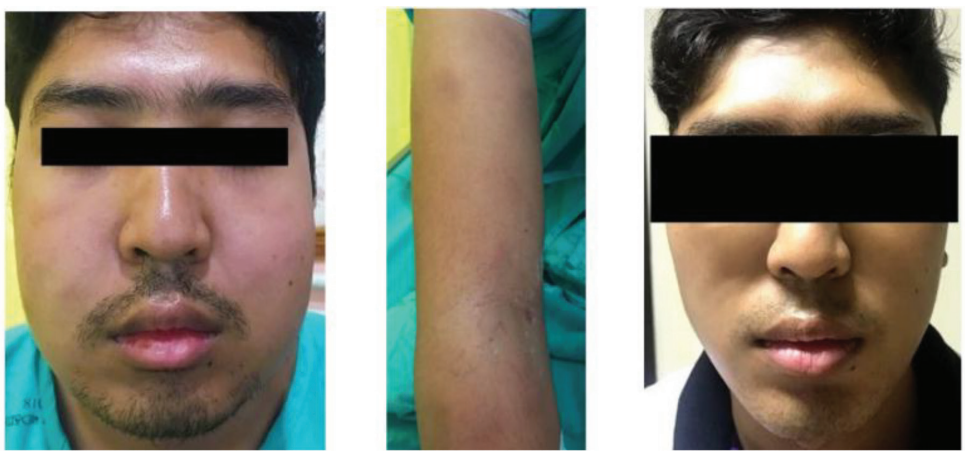
Results

Clinical features of all patients are summarized in Table 1. All patients presented with multiple painful subcutaneous nodules or indurated plaques varying in size, from 2-15 cm. in diameter on trunk and extremities, associated with fever. Facial involvement was observed in 1 patient (case 1) [Figure 1A and 1B]. Duration of disease before diagnosis varied from 4 weeks to 4 months. Three of the 4 patients presented with weight loss. Lymphadenopathy was detected in 2 patients and hepatosplenomegaly was detected in one patient. Interestingly, symmetrical polyneuropathy was observed in one case.

Table 1 Clinical features of 4 cases

	Case 1	**Case 2**	**Case 3**	**Case 4**
Sex	Male	Male	Male	Female
Age (year)	21	45	18	46
Underlying disease	No	Hypertension	No	No
Skin lesions	Multiple painless subcutaneous nodules and plaques, size 2-10 cm. on right cheek, abdomen, right arm, and back for 4 weeks	Multiple painless hyperpigmented subcutaneous nodules, size 3-5 cm. and indurated plaques size 5-10 cm. on both arms and both legs for 16 months	Multiple painless erythematous indurated plaques size 4-15 cm. on chest and all extremities for 7 weeks	Multiple, painless erythematous subcutaneous nodules size 2-5 cm. on chest abdomen and left thigh Hyperpigmented indurated plaques size 4-8 cm. on right knee and left ankle for 4 weeks
Fever	Yes	Yes	Yes	Yes
Weight loss	No	Yes	Yes	Yes
Lymphadenopathy	Right axillary lymphadenopathy	Generalized lymphadenopathy	No	No
Hepatosplenomegaly	Yes	No	No	No
Other manifestations	No	Weakness with sensory loss both legs	No	No
Hemoglobin (g/dL)	12.3	8	7.3	11.2
WBC (/ul)	6,600	4,200	3,700	2,500
Platelet (/ul)	260,000	240,000	254,000	149,000
LDH (U/L)	2,561	1,946	1,046	Not performed
BUN/Creatinine (mg/dL)	19/0.71	8/0.48	10.5/0.94	8.6/1.06
AST/ALT (IU/L)	67/108	111/41	95/50	118/52
ALP (U/L)	74	480	265	69
TB/DB (mg/dL)	2.2/0.32	0.93/0.21	0.98/0.3	0.89/0.22
CT whole abdomen	Hepatosplenomegaly	Suspected hepatic hemangioma at segment VIII	Fatty liver	Fatty liver

	Case 1	Case 2	Case 3	Case 4
Lymph node biopsy	Adipose tissue with chronic inflammation	Paracortical expansion due to polymorphous lymphoid proliferation and occasional foamy histiocytes in the sinuses	Not performed	Not performed
Hemophagocytic syndrome	No	No	No	No
Bone marrow involvement	No	No	No	No



A B C

Figure 1A Multiple erythematous indurated plaques on face in case no.1 (Before treatment)
Figure 1B Multiple erythematous and hyperpigmented subcutaneous nodules on right arm in case no.1 (Before treatment)
Figure 1C Indurated plaques resolved in case no.1 (After treatment)

For laboratory results, one patient had bicytopenia and one patient had leukopenia. LDH level were available for 3 patients, all of them were elevated. All patients had transaminitis. None of patients had hemophagocytic syndrome. CT whole abdomen revealed hepatosplenomegaly in one patient, hepatic hemangioma in one patient, and fatty liver in 2 patients. No bone marrow involvement was observed. CT brain, CSF cytology and flow cytometry in patient with polyneuropathy (case 2) was unremarkable.

Table 2 Histologic findings, immunohistochemistry, and molecular features of skin biopsies

	Case 1	**Case 2**	**Case 3**	**Case 4**
Histologic findings	Atypical lymphoid cells with hyperchromatic and irregular nuclear contours infiltrate in subcutaneous fat lobules	Large lymphoid cells with hyperchromatic nuclei with irregular nuclear contours infiltrate in subcutaneous fat lobules	Atypical cells with irregular nuclear contours, hyperchromatic nuclei, and karyorrhexis infiltrate around fat lobule in subcutaneous tissue	Medium-large lymphoid cells with hyperchromasia and irregular nuclear contours, karyorrhexis infiltrate in subcutaneous fat lobules with fat necrosis
CD3	+	+	+	+
CD4	-	-	-	-
CD8	+	+	+	+
CD20	-	-	-	-
CD30	-	-	-	-
CD56	-	-	-	-
Beta F1	+	+	+	+
TIA-1	+	No data available	-	+
Granzyme B	+	+	+	+
EBER	-	No data available	-	No data available
Ki-67	50%	40%	35%	35%
TCR gene rearrangement - Beta clonal - Gamma clonal	 + -	 + -	 + -	 + -

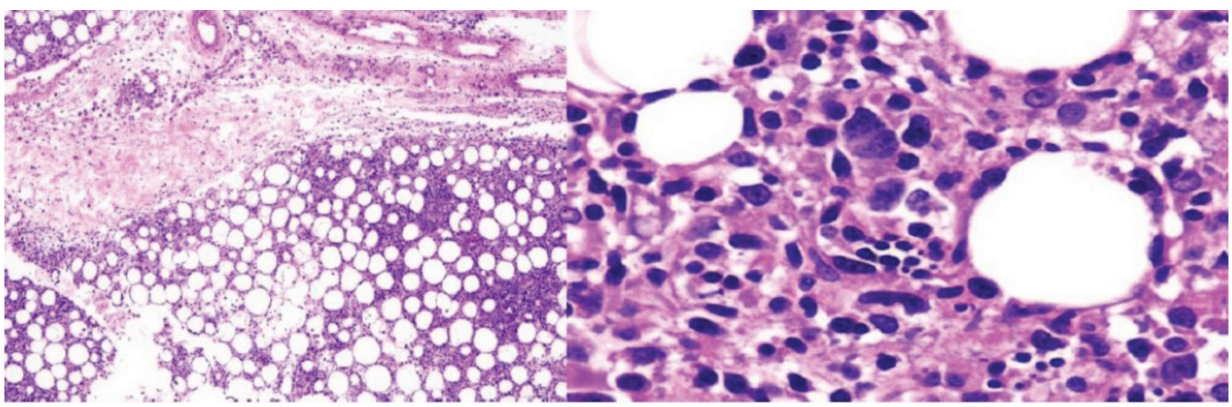


Figure 2 Histopathology of case 4 shows dense atypical lymphoid cells infiltrate in subcutaneous fat lobules

Incisional skin biopsies revealed medium to large lymphoid cells with irregular nuclear contour, hyperchromatic, and karyorrhexis infiltrate in subcutaneous fat lobules (Figure 2). Immunohistochemistry showed $CD3^+$, $CD4^-$, $CD8^+$, CD20-, and $CD56^-$. Cytotoxic molecules, including TIA-1, were positive in all patients, but granzyme B was also positive in all patients. Ki-67 was elevated (> 35%) in all patients. T-cell receptor beta rearrangement was detected in all patients (Table 2). Two patients who presented with lymphadenopathy (case 1 and 2), lymph node had biopsies performed, of the right axillary and right inguinal lymph node, respectively. The specimens showed adipose tissue with chronic inflammation (case 1) and paracortical expansion due to polymorphous lymphoid proliferation and occasional foamy histiocytes in the sinuses (case 2).

Table 3 First-line treatment and outcome

	Case 1	**Case 2**	**Case 3**	**Case 4**
Treatment protocol	Cyclosporine 300 mg/day (5 mg/kg/day) Prednisolone 60 mg/day (1 mg/kg/day)	Cyclosporine 250 mg/day (4 mg/kg/day) Prednisolone 60 mg/day (1 mg/kg/day)	Cyclosporine 300 mg/day (5 mg/kg/day) Prednisolone 60 mg/day (1 mg/kg/day)	Cyclosporine 250 mg/day (4 mg/kg/day) Prednisolone 60 mg/day (1 mg/kg/day)
Outcome	Complete remission within 8 weeks	Complete remission within 4 weeks but sensorimotor impairment partially improved	Complete remission within 4 weeks	Complete remission within 4 weeks
Status and treatment at the time of report	Alive with complete remission for 16 months On cyclosporine 100 mg/day	Alive with complete remission for 13 months duration On cyclosporine 150 mg/day + prednisolone 5 mg/day	Suspected SPTCL relapse, He presented with subcutaneous nodules and fever (biopsy not done). He died from VAP and sepsis at 5 months after initial treatment	Alive with complete remission for 4 months On cyclosporine 200 mg/day and prednisolone 60 mg/day

*VAP: Ventilator-associated pneumonia

All patients were treated with cyclosporine and prednisolone as first-line treatment. Cyclosporine 4-5 mg/kg/day and prednisolone 1 mg/kg/day were prescribed initially and gradually tapered. Cutaneous lesions, lymphadenopathy, and systemic symptoms resolved within 4-8 weeks (Figure 1C) in 3 patients and one patient (case 3) still had polyneuropathy with minimal improvement.

There was one patient in whom we suspected SPTCL relapse (case 3), He presented with multiple subcutaneous nodules and indurated plaques on his face

and abdomen with fever 4 months after treatment. He was admitted and diagnosed with sialadenitis of the left parotid and left submandibular glands. Skin biopsy was not done due to his unstable condition. Unfortunately, he died from ventilator-associated pneumonia and sepsis. Three other patients were still alive with complete remission at the time of last follow-up visit. The longest remission duration in this study was 16 months.

Discussion

Subcutaneous panniculitis –like T cell lymphoma (SPTCL) is a rare primary cutaneous T-cell lymphoma with good prognosis, overall, 5-year survival rates of 91% in SPTCL without HPS and 46% in SPTCL with HPS.[1] The most common manifestation in this study is multiple painless subcutaneous nodules or indurated plaques, varying in size on the trunk and extremities, especially on the lower extremities, similar to previous studies. although one patient (case 1) had no lesions on their lower extremities. None of our patients had ulcerated plaques or lipodystrophy, different from other studies (18% in GELC study by López-Lerma et al, 6% in EORTC 2008 study by Willemze et al).[1,7] Fever and weight loss were also common in our patients in line with previous studies.[1,3,12,13] There was no systemic involvement in this study. Extracutaneous involvement of SPTCL is unusual, only a few case reports show extracutaneous involvement of mesenteric fat and intraabdominal fat.[14,15] Interestingly, one patient (case 3) suffered from symmetrical polyneuropathy, which could be attributed to paraneoplastic syndrome. Unfortunately, this patient refused to undergo sural nerve biopsy, so we were unable to identify the cause of neurological deficit.

Elevated lactate dehydrogenase (LDH) and abnormal liver function (LFT) are common in SPTCL. A literature review of Ohtsuka et al reported elevated LDH in 75 % of Japanese patients, 69 % of Korean patients, whereas elevated LDH were less frequent in European patients (53 % of patients).[7,13,16] Abnormal LFT was found in 54% of Japanese patients and 46% of European patients.[1,13] HPS was not found in our study. Previous studies of Willemze et al found HPS in 17 % of patients.[1] Lee et al found HPS in 14% of Korean patients.[16] Michonneau et al and Ohtsuka et al, found a higher incidence of HPS in their studies (37% and 45% of patients, respectively).[12,13]

The histopathological findings and immunophenotype were not different to published reports. In contrast to our study, data from EORTC and GLEC studies showed polyclonal TCR gene rearrangement and gamma clonal was detected in 78% in EORTC and 64% in the GLEC study).[1,7] There was only beta clonal TCR in our case series.

Until now, current treatment protocol is controversial. There is no established standard treatment for SPTCL due to its rarity and lack of clinical trials. Various treatments are recommended including chemotherapy and immunosuppressive drugs. British guidelines point out that immunosuppressive drugs can be used in as the initial treatment in some cases.[17] Japanese guidelines and European Society for Medical Oncology (ESMO) recommend systemic steroids and/or immunosuppressive drugs for SPTCL without HPS.[18,19]

According to our study, and previous reports[3,7-11] patients treated with cyclosporine and systemic steroids achieved remission mostly within 2-4 weeks. Three of 4 patients in our study remain in long-term remission with the longest duration being 16 months. In previous literature, the longest remission duration was 9 years.[9] However, in our study SPTCL relapse was suspected in one patient about 1 month after prednisolone was stopped. He later died due to ventilator-

associated pneumonia with sepsis. Relapse is common in patients receiving immunosuppressive drugs.[18,19]

In conclusion, this study suggests that systemic steroid and cyclosporine shows benefit and could be use as first-line treatment in SPTCL patients. Further studies should be performed to confirm efficacy, dosage of cyclosporine and prednisolone, and to standardize first-line treatment for SPTCL.

Acknowledgement

I would like to express my thanks to Dr. Chadawan Dhampradit, Dr. Arnon Thaicharoen, Dr. Pattraporn Sriwichian, and Dr. Napaputch Kittipornprnpechdee of Pathology Department for their help in reviewing histopathological findings.

I would also like to extend my thanks to the technicians of the laboratory from Pathology Department for their help in preparing skin biopsy specimens.

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Implementation of Early Clinical Exposure (ECE) in Preclinical Teaching

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Received 7 October 2021 • Revised 7 November 2021 • Accepted 7 December 2021 • Published online 1 January 2022

Abstract:

Nowadays medical education for preclinical years is usually emphasizing on early exposure to clinical learning. This way of teaching can make medical students realize the importance of knowledge in the preclinical years for taking care real patients while on clinical years. This activity can lessen boring of medical students in the limited preclinical classroom and able to make correlation of preclinical and clinical. ECE can inspire medical students to pay more attention to learning in order to become physicians in the near future. ECE should have various ways, not be obligatory to medical teachers, can be blended with daily clinical service of the teachers, utilize existing hospital resources and should be modified to related curriculum in preclinical years. In this paper, the authors would like to share experience of implementing ECE in preclinical subject "Hematology and Lymphoreticular System" for medical students of Mae Fah Luang University.

Keywords: Early Clinical Exposure, Preclinical Teaching, Hematology and Lymphoreticular System

Introduction

In Thailand, it takes six years to receive medical degree (M.D.). The first three-year study consist of premedical in the first year, preclinical in the second and third year. Then they will be in the clinical year for three more years during the fourth to sixth year (externship). After graduation, they will mostly practice in the provincial hospitals for 1-3 years before coming back for specialty training 3-5 years depending on specialties and then 2 more years for subspecialty training. The life in preclinical years is quite monotonous, medical students spend their time mostly in the classroom and sometime laboratory. They have less chance to see the real patients. Some students are bored with this situation and drop out of school. Before admission in medical school, most students pledge they want to help people, patients and social. While in the medical school, many

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GMSMJ 2022; 2 (1): 25-29

students only want to make diagnosis and give the treatment. They do not realize the importance of preclinical knowledge which is the important basic for the clinical life.

ECE (early clinical exposure) is basically "A teaching and learning methodology which fosters exposure of medical students to patients (actual human contact) as early as the first year of medical college, in a social or clinical context that enhances learning of health, illness or disease, and the role of the health professional."[1] ECE sessions motivate the medical student in various ways making their academic strong, improve clinical skills, improve communication skills, and making them more confident.[2,3] So many medical schools in the present time encourage the preclinical curriculum to have ECE for many purposes. ECE will inspire medical students to pay attention to the study to become doctors in the near future, can show real life of medical teachers, residents and students in clinical years making preparedness for clinical years. ECE will moreover make conceptually correlation between preclinical and clinical knowledge.

At School of Medicine, Mae Fah Luang University, the preclinical curriculum consists of block of system, such as musculoskeletal, respiratory, gastrointestinal, reproductive, cardio-vascular, neurological, and hematology-lymphoreticular. Most of them takes 2-4 weeks depending on the credit. ECE is not an interesting issue in our medical school because of increased workload and Medical Center Hospital-Mae Fah Luang University (MCH-MFU) is not in the campus but about five kilometers from the preclinical building. Because hematology-lymphoreticular system is important and has clinical correlation teaching by paper cases. So, we would like to apply ECE in the teaching in order to make the students have chance to learn with the real patients.

Methods

Hematology-lymphoreticular system (HLS) in this semester (1/2021) was for the third year medical students and we have 30 students in this block. We had 3 weeks for HLS, which consisted of lecture, case discussion (paper), peripheral blood demonstration. We were teaching the students by onsite lecture in the first two weeks to give the knowledge about common hematologic diseases in Thailand regarding to pathophysiology and basic treatment. This year we added ECE in the third week by using half day at the hospital (MCH-MFU) and half day in the preclinical classroom.

In the morning we met the students at outpatient hematology-oncology clinic at 9 AM. We divided medical students to six groups, 5 students in each group. We prepared 6 patients who had regular appointment on that day, each patient had hematologic

Figure 1 Medical students were taking history (A, B)
Basic physical examination by teacher (C)

Figure 2 Prepare blood smear (A) and Wright staining (B) of the patients whom students had previously taken history

Figure 3 Learning laboratory operation and blood transfusion service (A, B) at Medical Center Hospital-Mae Fah Luang University

Figure 4 Presentation case the students attended in the morning (A, B, C)

Figure 5 The students explained blood smear findings (A), gave correlation (B) and differential diagnosis (C)

disease such as thalassemia, iron deficiency anemia, and plasma cell myeloma. Each group would take one hour for the medical history and basic physical examination by supervision of teacher. Then the students visited to the central laboratory learned and prepared peripheral blood smear (PBS) with Wright staining of the patients they had previously seen. Besides, they had learned the laboratory operation such as hematology, coagulogram, clinical microscopy, immunology, chemistry, and COVID-19 test. For blood transfusion service, they also learned ABO cell/serum grouping, conventional tube test and automated method. After completion of the morning activity, they went back to the preclinical classroom to interpret the abnormal findings in PBS and giving correlation with history and physical findings of the patients in the morning and finally making the initial diagnosis with explanation. During the presentation of each group, the medical students showed their capacity in gathering and formulation of clinical findings and data from peripheral blood smear and most of them were able to make a right diagnosis. In group with wrong diagnosis, the medical teacher would correct them right away and tried to make them get through to the right way of thought. For evaluation of this block, the students had a good impression with learning real patients, they also showed compassionate and wished them to do better or even to be cured. They also had some experience in taking history and basic physical examination which they expected to learn deeply in the clinical years.

Limitation

This ECE of HLS needed more clinical teachers to get high quality of teaching because we had only two in-house clinicians, one hematologist and the other one clinical pathologist. We also had limited time to do this activity. We suggest other block can do ECE depending on their topic, such as in cardiovascular system, they can take the students to the heart clinic. For musculoskeletal system they can do the similar way in orthopedics or rehabilitation clinic. Planning of ECE in real-time practices can be done at different setting with the use of appropriate resources such as log book, textbooks, notes, instruments, learning material, case record sheets, and computers.[4] We also suggest that ECE should be flexible, modifiable, and tailor-made in each system not one size fits all. We do not aim for great results but only want the students to get experience and good impression in taking care of patients and finally to have basic conceptual thinking and correlation between preclinical and clinical knowledge.

Conclusion

The authors have implemented ECE in block hematology-lymphoreticular system in preclinical year and suggest perform this activity in other block system as much as possible. The medical teachers should have this same concept to make ECE as another tool for successful medical education.

Conflict of interest

The authors have declared no conflict of interest.

Acknowledgement

The authors would like to thank medical technicians and other staffs in central laboratory, nurses and patient subjects, MCH-MFU to give knowledge and inspiration to our medical students. The authors also would like to thank Associate Professor Dr. Waraporn Eoaskoon from Medical Education Unit, School of Medicine, Mae Fah Luang University for her suggestion and comment.

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Acupuncture in Medicine

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Received 7 September 2021 • Revised 20 October 2021 • Accepted 2 December 2021 • Published online 1 January 2022

Abstract:

Traditional Chinese Medicine (TCM) consists of herb, acupuncture, and Tuina (a kind of massage). Today TCM is very popular. In Thailand there are eight colleges of TCM. Three-month course of training acupuncture for doctors was firstly opened in 1998. The course consists of basic theory of TCM e.g. Yin – Yang, Five Elements, Five Organs, Qi Blood and Essence, Twelve Meridians, Extra Meridians, Acupuncture Points, Tongue and Pulse Diagnosis, etc. After learning basic theory, the trainees have to practice needling and then treating patients with acupuncture.

Keywords: Acupuncture, Medicine, Definite treatment

Introduction

TCM has a long history more than 2500 years. Acupuncture originated in China and spread in Asia. Acupuncture spread to Europe in the early seventeenth century with skepticism of effectiveness.[1] In the USA, acupuncture has been more interesting since President Nixon's trip to China in 1972.[2] When Miriam Lee went to California USA, acupuncture was illegal. She was arrested for practicing medicine without a license in 1974. The legislators reached a compromise with Governor Reagan, and acupuncture was made an "experimental procedure" which could be carried out as research.[3] Acupuncture treatment has been proven by many research or study. In 1996, WHO reviewed the clinical practice of acupuncture, focusing on the controlled clinical trial.1 Many research and study make us understanding the mechanism and effectiveness of acupuncture more clearly.[1,2] In Thailand there are eight colleges of TCM. Three-month course of training acupuncture for doctors was firstly opened in 1998. Some of army doctors attended the three-month course of training acupuncture. The course consists of basic theory of TCM e.g., Yin – Yang, Five Elements, Five Organs, Qi Blood and Essence, Twelve Meridians, Extra Meridians, Acupuncture Points, Tongue and Pulse Diagnosis, etc. After learning basic theory, the trainees have to practice needling and then treating patients with acupuncture. Royal Thai Army Medical Department (RTAMD) has opened the three-month

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GMSMJ 2022; 2 (1): 31-34

course of training acupuncture for army doctors since 2007, and continues the training till now.

Conditions for acupuncture

The clinical conditions have been classified into 4 categories:[1]

1. The clinical conditions for which acupuncture has been proved to be an effective treatment: adverse reactions to radiotherapy and/or chemotherapy, allergic rhinitis (including hay fever), biliary colic, depression (including depressive neurosis and depression following stroke), dysentery (acute bacillary), dysmenorrhea (primary), acute epigastralgia (including peptic ulcer, acute and chronic gastritis, and gastro-spasm), facial pain (including craniomandibular disorders), headache, hypertension (essential), hypotension (primary), induction of labor, knee pain, leukopenia, low back pain, malposition of fetus (correction of), morning sickness, nausea and vomiting, neck pain, pain in dentistry (including dental pain and temporomandibular dysfunction), periarthritis of shoulder, postoperative pain, renal colic, rheumatoid arthritis, sciatica, sprain, stroke, and tennis elbow.

2. The clinical conditions for which acupuncture has shown the therapeutic effect but additional controlled studies are needed: abdominal pain (in acute gastroenteritis or due to gastrointestinal spasm), acne vulgaris, alcohol dependence and detoxification, Bell's palsy, bronchial asthma, cancer pain, cardiac neurosis, cholecystitis (chronic, with acute exacerbation), cholelithiasis, competition stress syndrome, craniocerebral injury (closed), diabetes mellitus (non-insulin dependent), earache, epidemic hemorrhagic fever, epistaxis (simple, without generalized or local disease), eye pain due to subconjunctival injection, female infertility, facial spasm, female urethral syndrome, fibromyalgia and fasciitis, gastro-kinetic disturbance, gouty arthritis, hepatitis B virus carrier status, varicella-zoster virus *(human alphaherpes-virus 3)*, hyperlipemia, hypo-ovarianism, insomnia, labor pain, lactation (deficiency), male sexual dysfunction (non-organic), Ménière disease, neuralgia (post-herpetic), neurodermatitis, obesity, opium, cocaine and heroin dependence, osteoarthritis, pain due to endoscopic examination, pain in thromboangiitis obliterans, polycystic ovary syndrome (Stein–Leventhal syndrome), postextubation in children, postoperative convalescence, premenstrual syndrome, prostatitis (chronic), pruritus, radicular and pseudoradicular pain syndrome, Raynaud syndrome (primary), recurrent lower urinary tract infection, reflex sympathetic dystrophy, retention of urine (traumatic), schizophrenia, sialism (drug-induced), Sjögren syndrome, sore throat (including tonsillitis), spine pain (acute), stiff neck, temporomandibular joint dysfunction, Tietze syndrome, tobacco dependence, Tourette syndrome, ulcerative colitis (chronic), urolithiasis, vascular dementia, and whooping cough (pertussis).

3. The clinical conditions for which there are only individual controlled trials reporting some therapeutic effects, but for which acupuncture is worth trying because conventional treatment and other therapy is difficult: chloasma, choroidopathy (central serous), color blindness, deafness, hypophrenia, irritable colon syndrome, neuropathic bladder in spinal cord injury, pulmonary heart disease (chronic), and small airway obstruction.

4. The clinical conditions in which acupuncture may be tried provided the practitioner has special modern medical knowledge and adequate monitoring equipment: breathlessness in chronic obstructive pulmonary disease, coma, convulsions in infants, coronary heart disease (angina pectoris), diarrhea in infants and young children, encephalitis (viral, in children, late stage), and paralysis (progressive bulbar and pseudobulbar).

Acupuncture technique

The technique of acupuncture is simple and safe. The result is instant.[4] When I started treatment with acupuncture, I found that most patients came to the acupuncture clinic with the problem of pain and stroke sequelae. Pain and stroke are the condition that acupuncture is effective treatment, but the course of treatment and the result depend on the duration of the illness, age, and physical status of the patient. The onset of stroke within three months is good for acupuncture. If the patient is late, the result will be less. If the patient is young or physical status is good, the healing will be better. There are many types of acupuncture: eye acupuncture, scalp acupuncture, hand acupuncture, abdominal acupuncture, wrist and ankle acupuncture, ear or auricular acupuncture, tongue acupuncture, Tung's style acupuncture, balance method acupuncture, and etc. The acupuncturist can combine two or three types of acupuncture for better result.

Limitation

The limitation of acupuncture is the experience of the acupuncturist. If the diagnosis is correct, the treatment will be effective. For me the TCM diagnosis is hard to understand. I have to read the TCM book again and again and compare it to the modern medicine because I have to simplify the knowledge for the medical students taking elective course of acupuncture. The technique of needling is also important. The acupuncturist should know the depth of insertion, how to reinforce or how to reduce, duration to retain the needle, and the frequency of treatment. Usually the action of acupuncture pertains for two to three days, so the optimum frequency is two to three times per week.

Effects of acupuncture

Acupuncture by the well-trained person is safe and effective. The action of acupuncture is bidirectional. For example, when needling at Neiguan which effects the heart rate, it can adjust the heart rate to be normal either fast or slow.[5] The needle used in acupuncture is sterile and disposable. The diameter of the needle is 0.16–0.3 millimeter, smaller than the injection needle, so it is hard to be injured. When the patient is needled, the patient will feel dull or electrical or sharping sensation that means good. This feeling is called De Qi which means the energy is arrived. The result of acupuncture is dramatic. Many patients were relieved from shoulder pain by the first time of acupuncture. Besides treating pain, acupuncture regulates physiological function, such as asthma, arrhythmia, allergic rhinitis, vertigo, etc.[1,2] The duration and frequency of treatment which may be 2-3 times per week for 4 weeks makes a course of treatment. Usually when one course of treatment is done, the patient has to rest for 1-2 weeks, before starting the next course of treatment.

Preparation before acupuncture

The treatment is not done by the doctor only, multidisciplinary team makes holistic treatment and the patient's duty is to adjust the bad habits. The patient should do these things: relax the mind and body, optimum healthy meal, optimal exercise or physical therapy appropriate to age and clinical condition, seven-hour of sleeping. The medical team should coach the patient. The patient and medical team should plan and set a goal, check and adjust to get it. The combination of acupuncture with modern medicine, is considered to be integrative medicine.

Conclusion

Acupuncture is proved to be the definite treatment for some diseases and can be the alternative treatment. For better result of treatment, combined acupuncture with modern medicine to make holistic approach. I hope that the integrative medicine with acupuncture is widely accepted.

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In Silico Prediction of the Action of Ivermectin-like Compounds on Binding Sites of the SARS-CoV-2 Spike Protein and Receptor-binding Domain of ACE2

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Received 13 August 2021 • Revised 22 August 2021 • Accepted 30 August 2021 • Published online 1 January 2022

Abstract:

Background: Ivermectin (IVM), a macrocyclic lactone anthelmintic drug, is a promising lead compound that may disrupt the binding interface of the SARS-CoV-2 spike protein with the protein-binding domain of angiotensin-converting enzyme 2 (ACE2), and so could present an opportunity for further drug development of anti-COVID-19 medication.

Objective: This study aimed to determine and predict the most effective IVM-based analogs against the SARS-CoV-2 spike protein and human angiotensin-converting enzyme 2 by using computational analysis.

Method: This study performed a rational *in silico* study to screen ivermectin-like compounds with a similarity score less than 0.70 and then screened these for acceptable pharmacokinetic properties, to further examine molecular docking analysis of SARS-CoV-2 spike protein and protein-binding domain of angiotensin-converting enzyme 2.

Result: The results showed that compound **14**, with a similar score of 0.722, exerted the most binding affinity with both targets, with a binding energy of -8.32 and -7.98 kcal/mol to the SARS-CoV-2 spike protein and the protein-binding domain of angiotensin-converting enzyme 2 respectively, showing better values than that of ivermectin.

Conclusion: Our study confirms the possibility that the ivermectin-like compound **14** may be a most promising candidate drug, acting on the SARS-CoV-2 spike protein and angiotensin-converting enzyme 2, so should be studied further as part of a drug discovery and development process.

Keywords: Ivermectin, *in silico* analysis, COVID-19, SARS-CoV-2 Spike protein, Angiotensin-converting enzyme 2

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GMSMJ 2022; 2 (1): 35-42

Introduction

Since 2019, the world has suffered from the emergence of the coronavirus disease 2019 (COVID-19) outbreak, which is a major public health issue and a cause of high levels of morbidity and mortality.[1] It has affected more than 205 million people worldwide, including 4 million deaths.[2] To reduce the harmful sequelae of COVID-19 infection, such as respiratory failure, or multi-organ dysfunction, treatment involving antiviral agents is one of the promising therapeutic approaches for this emerging infectious disease.[1]

Of the ongoing drugs in the COVID-19 pipeline, ivermectin (IVM) is a most interesting compound because it exhibits a broad spectrum of antiviral activity *in vitro* apart from its well documented anti-parasitic activities.[3-7] We believe that IVM could be a potential anti-COVID-19 lead candidate for further drug development because it has been reported that IVM inhibits the SARS-CoV-2 virus *in vitro*[3-4] and *in vivo*.[8] Furthermore clinical studies have also revealed that IVM is associated with a lower mortality rate in hospitalized COVID-19 patients.[9-11] One of the postulations of the mechanism of action of IVM toward SARS-CoV-2 virus is inhibition and disruption of the binding of the SARS-CoV-2 spike protein to the angiotensin-converting enzyme 2 (ACE2) receptor.[12] An *in-silico* analysis demonstrated that IVM disrupted the binding interface between the Leu91 of SARS-CoV-2 spike protein and the His378 of host cell ACE2.[13-14]

This finding inspired our idea that chemistry containing structural moieties similarly to IVM, in terms of IVM-like analogs, could be possible compounds with efficacy and safety for COVID-19 pharmacotherapy. The objectives of this study were to determine the most effective IVM-based analogs and their favorable pharmacokinetic properties, by using computational analysis of the SARS-CoV-2 spike protein and human ACE2 receptors.

Methodology

1. Selection and preparation of IVM analogs.

IVM was submitted in the Simplified Molecular Input Line Entry System (SMILES) format, to calculate similarity scores, by using SwissSimilarity, a free web tool that can compute the similarity of all compounds that are available in the Sigma Aldrich library.[15] The top compounds that had a similarity score of more than 0.70 were included and these IVM analogs were submitted into SwissADME (http://www.swissadme.ch/index.php), to compute their physicochemical and pharmacokinetic properties.[16] All compounds that were judged to be orally active drugs with favorable pharmacokinetic properties were included in this analysis.[17] Structures of IVM and selected IVM-like analogs were initially constructed using ChemDraw Professional 16.0, followed by three-dimensional (3D) structure transformation, using Chem3D Professional 10.0.

2. Preparation of structure of SARS-CoV-2 Spike protein and human ACE2.

A Crystal structure of a SARS-CoV-2 spike receptor-binding domain, bound with ACE2 (PDB ID: 6M0J),[18] was prepared by removing all water molecules, any solvent, and the ligand.

3. Molecular docking analysis.

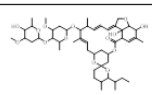
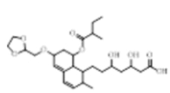
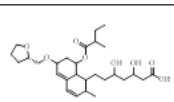
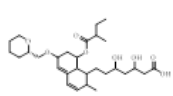
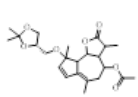
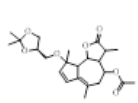
The binding free energy and inhibitory constant of IVM and its analogs were docked and then analyzed by using AutoDock 4.2.6 software.[19] Each energy-minimized IVM and its analogs were submitted into the well-prepared targets with default parameters of docking procedures. The binding site sphere for IVM and its analogs interaction

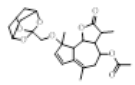
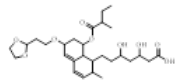
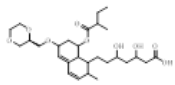
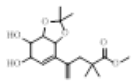
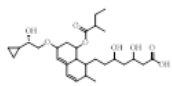
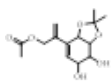
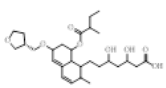
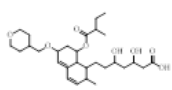
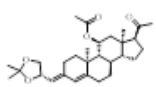
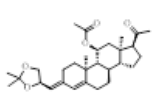
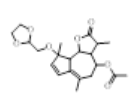
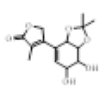
was defined according to the previous studies. The molecular docking protocol was obtained from the active site of the SARS-CoV-2 spike protein and ACE2 receptor with a molecular grid at 0.375 Å grid spacing. Docking results of all analogs with SARS-CoV-2 spike protein and ACE2 receptor were evaluated using the best binding free energy (BE, kcal/mol) and inhibitory constant from all clusters of each conformational structure. Virtual analysis of the best results was then viewed and analyzed by using UCSF Chimera.[20]

Results and discussion

The SwissSimilarity results showed 75 compounds from the Sigma Aldrich library having similarity scores more than 0.7 (the range of 0.71-0.83), which were further submitted to SwissADME. All of the pre-selected IVM-like compounds belonged within Lipinski's rule of five criteria.[17] Serious neurological adverse drug reactions of IVM have been documented and there is a need to avoid harm to patients in situations of overdose. The mechanisms of this adverse drug reaction are unclear but might be due to IVM inhibiting the P-glycoprotein drug pump (MDR-1) of the BBB (blood brain barrier) causing CNS toxicity.[21] We then excluded 9 of the pre-selected IVM-preselected compounds exhibiting increased BBB permeability and 36 other selected compounds that were classified as P_{gp} substrate. Finally, we obtained 17 lead compounds that showed favorable physiochemical and pharmacokinetic properties as shown in Table 1.

Table 1 Physiochemical properties of IVM and IVM-based compounds

Compound	Chemical structure	Similarity	MW	HBA	HBD	*c*LogP
IVM		1.0	875.09	14	3	4.37
1		0.772	510.62	9	3	2.72
2		0.768	508.64	8	3	3.3
3		0.768	522.67	8	3	3.56
4		0.765	420.5	7	0	2.64
5		0.765	420.5	7	0	2.68

Compound	Chemical structure	Similarity	MW	HBA	HBD	*c*LogP
6		0.765	460.52	8	0	2.51
7		0.762	524.64	9	3	3
8		0.748	524.64	9	3	2.71
9		0.742	326.38	6	2	1.6
10		0.738	508.64	8	4	3.1
11		0.729	284.31	6	2	0.59
12		0.728	508.64	8	3	3.22
13		0.728	522.67	8	3	3.49
14		0.722	470.64	5	0	4.83
15		0.722	470.64	5	0	4.84
16		0.715	392.44	7	0	2.09
17		0.711	282.29	6	2	0.4
Required parameters[a]	-	-	< 500	< 10	< 5	2-5

[a] Required parameters necessary to fulfill appropriate physiochemical properties as judged appropriate according to Lipinski's rules.[17]

IVM was docked with the SARS-CoV-2 spike protein and ACE2 in the region of the receptor-protein binding interface. The binding energy of IVM to SARS-CoV-2 spike protein and ACE2 were -6.60 and -4.84 kcal/mol, with an estimated inhibition constant (Ki) of 14.54 and 283.49 μM, respectively. It was noted that IVM favored binding to the SARS-CoV-2 spike protein compared with ACE2. According to molecular docking analysis, the compounds that exerted binding free energy greater than that of the IVM towards SARS-CoV-2 spike protein were compound **4-6, 14,** and **15,** while binding with ACE2 were compounds **4-6, 11, 14-15,** and **16-17** (Table 2). The compound with the best binding affinity toward both SARS-CoV-2 spike protein and ACE2 was compound **14** which provided binding energies of -8.32 and -7.98 kcal/mol respectively.

Table 2 Molecular docking analysis of IVM and IVM-based compounds toward SARS-CoV-2 spike protein and ACE2

Compound	SARS-CoV-2 spike protein		ACE2	
	BE (kcal/mol)[a]	Inhibition Constant (μM)[a]	BE (kcal/mol)[a]	Inhibition Constant (μM)[a]
IVM	-6.60	14.54	-4.84	283.49
1	-4.09	999.74	-4.73	341.18
2	-4.96	230.62	-4.02	1.13 mM
3	-4.39	603.20	-3.63	2.19 mM
4	-6.70	12.20	-7.08	6.43
5	-6.63	13.86	-6.61	14.27
6	-6.57	15.38	-6.92	8.46
7	-4.22	805.28	-3.80	1.63 mM
8	-3.76	1.77 mM	-3.23	4.31 mM
9	-5.69	67.99	-4.69	362.60
10	-3.51	2.65 mM	-2.73	10.04 mM
11	-5.40	110.21	-5.33	123.87
12	-3.99	1.19 mM	-3.62	2.22 mM
13	-4.27	737.17	-4.60	422.19
14	-8.32	0.79	-7.98	1.40
15	-7.62	2.60	-7.60	2.67
16	-6.37	21.36	-6.70	12.23
17	-6.23	27.04	-5.88	48.88

[a] Binding free energy and inhibitory constant results were obtained from AutoDock 4.2.6 software.[19]

For binding mode analysis of compound **14** with ACE2, the heteroatoms of compound **14** interact via H-bonding interaction with the H-bond donor amino acid including Arg403, Tyr453, and Ser494; and by hydrophobic interaction with Tyr449, Leu452, Leu455, Phe490, Leu492, Gln493, Tyr495, Gly496, and Tyr505 (Figure 2).

The interaction of SARS-CoV-2 spike protein, both IVM and compound **14** were found to interact via H-bonding interaction with Lys26, and Gln96 but the bond distance in the case of IVM was 2.85, and 2.95 Å, respectively, whereas in the case of compound **14** it was 2.74, and 3.02 Å, respectively (Figure 2). The hydrophobic interactions between SARS-CoV-2 spike protein and IVM were found to interact with Glu22, Asp30, Asn33, Asn90, Val193, and Pro389, whereas the hydrophobic interactions of compound **14** were found to interact with Glu23, Thr27, Asp30, Asn33, and Pro389 (Figure 2).

Compound **14**, a triterpene analog, appears to be a promising compound that effectively binds to both the SARS-CoV-2 spike protein and to ACE2. This compound could have a potential role in inhibiting viral entry, so may be considered as a possible antiviral agent to fight SARS-CoV-2 infection. The results demonstrate that this rationale of *in silico* prediction of IVM-based compounds is one of the approaches that can be used to screen and design drug candidates, which is less time consuming and provides essential information to prioritize drug discovery and development processes in the ongoing COVID-19 situation. However, this computational analysis still requires experimental studies to further confirm this *in silico* hypothesis.

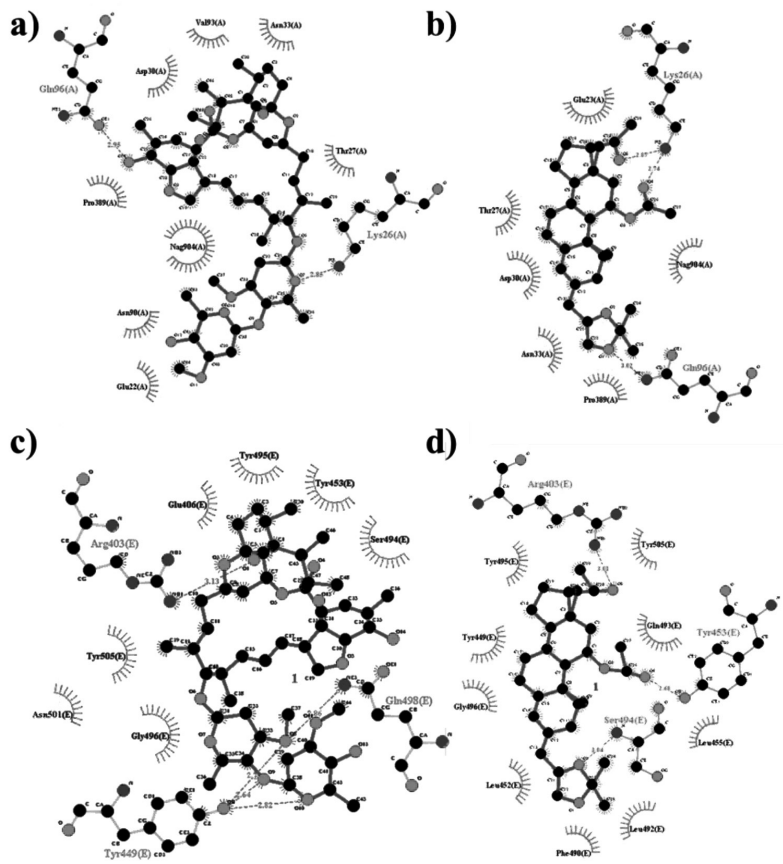


Figure 1 Binding mode results of IVM (a) and compound **14** (b) toward SARS-CoV-2 spike protein (6M0J); IVM (c) and compound **14** (d) toward ACE2 receptor. The green dashed line denoted H-bonding interaction.

Conclusion

This computational analysis revealed that compound **14** shown the best binding affinity towards both the SARS-CoV-2 spike protein and ACE2 receptor, with higher values than IVM, whilst also exhibiting acceptable physicochemical characteristics and pharmacokinetic properties. This result suggests that *in silico* analysis has proved to be an advantageous tool for drug design, reducing the time required to ratify rational strategies for anti-COVID-19 drug development. However, preclinical studies are required to further evaluate its efficacy and toxicity.

Acknowledgments

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. English language support was kindly provided by Dr. Roger Timothy Callaghan (MB, ChB), School of Medicine, Mae Fah Luang University.

Conflicts of Interest

The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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GMSMJ Greater Mekong Subregion Medical Journal

Okra Jelly Affecting Self-Perceived Xerostomia and Oral Health Related Quality of Life in The Elderly: A Preliminary Study

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Received 7 September 2021 • Revised 22 September 2021 • Accepted 22 October 2021 • Published online 1 January 2022

Abstract:

Background: The prevalence of xerostomia, the subjective sensation of dry mouth, is quite high in elder people. Okra contains mucilaginous substance which has moisturizing and lubricating properties similar to human natural saliva.

Objective: This study aimed to investigate if okra jelly can affect self-perceived xerostomia and oral health related quality of life in the elderly.

Methods: We used an experimental study. There were 12 participants. We allocated 2 groups: the experimental group used okra jelly and the control group used jelly without okra. Participants received jelly twice daily, between breakfast-lunch and lunch-dinner for 24 days. Self-reported visual analogue scale (VAS) for dry mouth and the Thai version of Oral Health Impact Profile-14 (OHIP-14-Th) had been done at before intervention, Day 12, and Day 24. Two-sample Wilcoxon rank-sum (Mann-Whitney) test, multilevel mixed-effects logistic regression and GEE population-averaged model were used for analyzing the differences between tests and controls at different studied times with p-value < 0.05 was considered to be significant.

Results: After adjusting baseline VAS and OHIP-14-Th score, age and gender, the results showed that every 12-day, the VAS score of the experimental group decreased significantly ($p < 0.01$) and the control group decreased insignificantly. Comparing the two groups, it was found that the experimental group had a greater score reduction significantly ($p < 0.01$). The results of OHIP-14-Th score every 12-day showed that both the experimental and control groups decreased significantly ($p < 0.01$). Comparing the two groups, it was found that the experimental group had a greater score reduction, however, insignificantly.

Conclusion: Okra jelly seems to have promising results on the reduction of self-perceived xerostomia and oral health related quality of life in the elderly.

Keywords: Elderly, Okra, Quality of life, Xerostomia

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GMSMJ 2022; 2 (1): 43-53

Introduction

The elderly has a higher probability of suffering from many conditions of health, both physical and mental functions, which can decrease quality of life. Among these, dryness of some organs is undergoing extensive physiological changes i.e. eyes, nose, skin and mucous membrane.[1] Oral health status is considered to be one of the important contributing factors to health and well-being in the elderly. The prevalence of xerostomia, the subjective sensation of oral dryness, is quite high in elder people ranging from 17% to 40% among community-dwelling elders and 20% to 72% in the institutionalized elders.[2,3] Severity of dry mouth symptoms ranges from mild oral discomfort to significant oral diseases. This condition causes trouble in chewing, swallowing, tasting, speaking and difficulties with denture wearing. Thereafter, it makes the higher risk for pathological oral manifestations such as dental caries, mucositis, candidiasis, and periodontal disease.[1-3] Many studies have been reported the association of xerostomia and poorer quality of life in elderly.[4-6] The study of the quality of life in patients with type II diabetes mellitus was also conducted and found that elderly patients with xerostomia, compared to healthy individuals, had a worse quality of life, in which the complications of xerostomia were the main determinants of quality of life in these patients.[7] Management of alleviating xerostomia depends on the causes, other than physiological changes, which may include salivary gland hypofunction, some types of systemic diseases and medication.[8-10] No matter what the causes of dry mouth, intraoral topical agents are among the most common recommended treatments for the management of xerostomia. These include oral sprays, sugar-free chewing gums and candies, lozenges, saliva stimulants and substitutes in the forms of gel, mouthwash, and toothpaste.[9-11] The main purpose is to moisten and lubricate the mouth, and in the form that need chewing, it stimulates the natural production of saliva.

Okra *(Abelmoschus esculentus)*, originated from the northeastern Africa, is presently grown in many countries throughout tropical and warm temperate regions.[12] Various parts of okra have been studied to carry several therapeutic potentials i.e., antidiabetic, anticancer, antioxidant, antiulcerogenic, gastroprotective, and diuretic.[13,14] The edible green seed pod, known as Lady's finger, is popularly used as a vegetable in Asian cuisine (Figure 1). It contains many nutritional constituents such as carbohydrate, protein, fiber, vitamins, calcium, potassium, iron, and folate.[12-14] Okra seed pods is well known for its mucilaginous, the characteristic of goo or slime. Plant mucilages are used as adjuvant in pharmaceutical preparations such as thickening, binding, suspending, emulsifying, stabilizing, and gelling agents.[13,14] Other than okra, several plants contain mucilage which can be used as thickening, moisturizing, and lubricating agents in artificial saliva formulations.[15] Recently, this research group indicated high biological activity and safety of the developed formulation containing mucilage from Ceylon Spinach and concluded the potential to be used as artificial saliva for xerostomia patients.[16]

Figure 1 Okra *(Abelmoschus esculentus)* is called lady's finger in England, gumbo in the United States of America, guino-gombo in Spanish, guibeiro in Portuguse, bhindi in India[12,13]

The term "quality of life" (QoL) is described as a wellness resulting from a combination of physical, functional, emotional and social factors. Oral Health Related Quality of Life (OHRQoL) is defined as an individual´s assessment of how functional factors, psychological factors, social factors and experience of pain/discomfort in relation to orofacial concerns affect their well-being.[4,6] At the present time, the Oral Health Impact Profile-14 (OHIP-14) has been mostly used questionnaire to assess OHRQoL.[17-18] The Thai version of the Oral Health Impact Profile-14 (OHIP-14-Th) has been demonstrated good construct validity and acceptable reliability.[19] The author concluded that it can be used to measure the OHRQoL in Thai elderly populations. In addition, it has been reported for a valid and reliable instrument for the measures of social impact of oral diseases or disorders on individuals in community settings.[20]

Considering the growing number of elderly and the association of xerostomia in age population, the aim of this preliminary study was to investigate if okra, in the preparation form of jelly, can affect self-perceived xerostomia and oral health related quality of life in the elderly by using self-reported Visual Analogue Scales (VAS) and the Thai version of the Oral Health Impact Profile-14 (OHIP-14-Th).

Materials and Methods

Okra jelly preparation

Fresh okra 1.5 kg were washed thoroughly with running tap water, cut into small pieces and steamed for 30 minutes. The steamed okra was mixed with 1,500 ml drinking water in the blender, then filtered with straining cloth. The filtrated okra was stored at 4°C until use.

Carrageenan 340 g was slowly mixed with 680 ml stevia syrup at 70°C. Then 820 ml filtrated okra was added into the mixture with constant stirring until the mixture appeared homogeneously before adding flavoring agent (Mint, Winner's®). The mixture was poured into the 25 ml plastic cup, let it cool down and harden at room temperature, the jelly then refrigerated at 4°C until use. Jelly without okra was prepared with the same formula but used drinking water instead of filtrated okra and added coloring agent (Green, Adinop®). Both test and control jelly were fresh prepared every 6 days and gave to the volunteers to keep in the refrigerator at 4°C.

Dry mouth screening questionnaire

Eighty elderly who were the members of Seniors' School at Tha sut, Chiang Rai responded to the 2-sections screening questionnaire. Section I was general information about age, gender, systemic-related diseases, current drug taken, smoking, daily food and life style, the need for caregiver. Section II was the 14 questions regarding general dryness of organs/ body, for examples, dryness of eyes, nose, lips, the need to sip water during chewing, difficulty in swallowing dry food. The rating scores of how frequent of the problems they had been experienced were rated as 1 (Never), 2 (Hardly ever), 3 (Occasionally), 4 (Fairly often), 5 (Very often). The elderly who had a sum score more than 25 and met the inclusion criteria were the subjects in the study. The inclusion criteria were identified as the persons who regularly brush the teeth twice daily, none of current oral lesions, non-smoking, no regular jelly consumption and be able to communicate. Whereas the exclusion criteria were the handicapped, the persons who had cognitive impairment and allergic to tested materials i.e. okra, carrageenan, gluten, mint and soy.

Self-perceived xerostomia and oral health-related quality of life (OHRQoL)

The self-reported Visual Analogue Scales (VAS) and the Thai version of the Oral Health Impact Profile-14 (OHIP-14-Th)[19,20] was used to conduct self-perceived xerostomia and oral health related quality of life, respectively. The volunteers made a mark on the line of VAS record form to reflect the feeling of severity of dry mouth as 0 mm (not at all severe: no dryness) to 100 mm (extremely severe: worst imaginable dryness). The OHIP-14-Th consisted of 14 questions representing seven domains i.e. functional limitation (difficult to pronounce words, worsened taste), physical pain (pain, uncomfortable to eat), psychological discomfort (self-conscious, feel tensed), physical disability (diet unsatisfactory, interrupted meals), psychological disability (difficult to relax, embarrassed), social disability (irritable, difficult to do jobs), handicap (life less satisfying, totally unable to function). The volunteers selected the score related to the frequency of impact they had been experienced as 0 (never), 1 (hardly ever), 2 (occasionally), 3 (fairly often) and 4 (very often). The sum of the scores of the 14 questions ranged from 0 to 56 and the sum of each domain ranges from 0 to 8. Thus, a higher score indicated poorer OHRQoL.

Study design

Twelve of eighty screening volunteers met the criteria and participated in this experimental study. The volunteers were randomly divided into 2 groups; the test group received okra jelly and the control group received jelly without okra. VAS and OHIP-14-Th were recorded for baseline.

The volunteers received 2 cups of their assigned jelly per day. They were instructed to have a cup of jelly in the morning (between breakfast and lunch) and another cup in the afternoon (between lunch and dinner). The jelly was scooped out 5-6 times per cup, the volunteers slowly chewed jelly into small pieces before swallowing. Every 12-day having the jellies, at Day 12 and Day 24, the volunteers responded to self-perceived VAS and OHIP-14-Th questionnaire.

The study protocol was approved by The Ethical committee at Mae Fah Luang University (COA: 146/2020, EC 19293-22). A signed informed consent was obtained from all participants.

Statistical analysis

Statistical analyses were performed using a commercially available software program. Two-sample Wilcoxon rank-sum (Mann-Whitney) test was used to compare

the difference of VAS score and total OHIP-14-Th score between the first period (Day 1 to Day 12) and the second period (Day 13 to Day 24). Comparison the overall VAS score and total OHIP-14-Th score between the two jellies was analyzed using multilevel mixed-effects logistic regression which adjusted by baseline score, age and gender. And, to compare the reduction of OHIP-14-Th scores every 12-day in each aspect between control and test group with GEE population-averaged model; adjusted by baseline score, age and gender. p-value < 0.05 was considered to indicate statistical significance.

Results

From an initial population of 80 elderly who responded the screening questionnaire, only 12 individuals got sum score more than 25 and passed the inclusion criteria. After the experiment had been conducted for a week, there was the announcement of COVID-19 affected area to home restriction, 4 volunteers dropped out and 8 volunteers completed the experiment. All of the volunteers denied any systemic diseases and received medication more than 6 months. Table 1 shows median of age, gender distribution, baseline VAS of the severity of dry mouth and baseline total OHIP-14-Th score of the volunteers. The statistical difference was found on baseline VAS of the severity of dry mouth between the test and the control groups ($p = 0.02$).

Table 1 Comparison of volunteers' characteristics, VAS of the severity of dry mouth and total OHIP-14-Th scores between the test and control group at baseline.

Characteristic	Test group	Control group	*p*-value
Female	2	1	
Male	2	3	
Median of age, years (range)	69.5 (63-81)	75 (60-80)	0.77
VAS of the severity of dry mouth	64.5 (59-100)	23 (9-31)	0.02*
Total OHIP-14-Th scores	31 (29-37)	32.5 (16-38)	0.66

*Statistical significance p-value < 0.05 by Two-sample Wilcoxon rank-sum (Mann-Whitney) test

Test group = received okra jelly; Control group = received jelly without okra; VAS = Visual analog scale; OHIP-14-Th = Oral Health Impact Profile - Thai

VAS score of the severity of dry mouth

The results demonstrated the reduction of VAS score for dry mouth during the first period (Day 1 – Day 12) and the second period (Day 13 – Day 24) of the test group at 51.5-point and 13-point and the control group at 12-point and 1-point, respectively. This reduction, however, was not statistically significant in both groups. Two-sample Wilcoxon rank-sum (Mann-Whitney) test was showed in Table 2

Table 2 Comparison of the VAS dry mouth reduction between the first period (Day 1 – Day 12) and the second period (Day 13 – Day 24) of the test and control group

Group	Time		*p*-value
	Day 1 to Day 12	Day 13 to Day 24	
Test	51.5 (9-100)	13 [(-2)-50]	0.27
Control	12 [(-23)-24]	1 [(-40)-44]	0.88

*Statistically significant p-value < 0.05 by Two-sample Wilcoxon rank-sum (Mann-Whitney) test
Test group = received okra jelly; Control group = received jelly without okra; VAS = Visual analog scale

After adjusting baseline VAS score, age and gender by using multilevel mixed-effects logistic regression, it was found that every 12 days, the test group had a 3.30 score reduction significantly ($p < 0.01$) and the control group had a 0.58 score reduction insignificantly ($p = 0.23$). Comparing the two groups, it was found that the test group had a greater score reduction significantly ($p < 0.01$). (Figure 2)

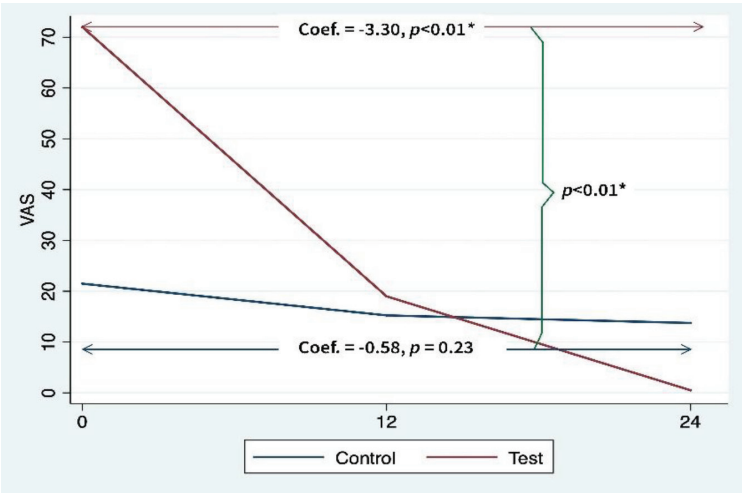

Figure 2 Comparison of the VAS dry mouth reduction at the first period (Day 1 – Day 12) and the second period (Day 13 – Day 24) between the test and control group after adjusting baseline VAS score, age and gender
*Statistically significant p-value < 0.05 (Multilevel mixed-effects logistic regression)
Test group = received okra jelly; Control group = received jelly without okra; VAS = Visual analog scale

OHIP-14-Thai score

The results demonstrated the reduction of total OHIP-14-Th scores during the first period (Day 1 – Day 12) and the second period (Day 13 – Day 24) of the test group at 13.5-point and 2.5-point and the control group at 6-point and 3.5-point, respectively. This reduction, however, was not statistically significant in both groups. Two-sample Wilcoxon rank-sum (Mann-Whitney) test was showed in Table 3.

Table 3 Comparison of total OHIP-14-Th score between the first period (Day 1 – Day 12) and the second period (Day 13 – Day 24) of the test and control group

Group	Time		*p*-value
	Day 1 to Day 12	Day 13 to Day 24	
Test	13.5 (10-23)	2.5 (0-7)	0.68
Control	6 [(-3)-24]	3.5 (0-19)	0.72

*Statistically significant *p*-value < 0.05 by Two-sample Wilcoxon rank-sum (Mann-Whitney) test
Test group = received okra jelly; Control group = received jelly without okra; OHIP-14-Th = Oral Health Impact Profile - Thai

After adjusting baseline total OHIP-14-Th score, age and gender by using multilevel mixed-effects logistic regression, it was found that every 12 days, the test group had a 1.36 score reduction significantly ($p < 0.01$) and the control group had a 0.60 score reduction significantly ($p < 0.01$). Comparing the two groups, it was found that the test group had a greater score reduction, however, insignificantly ($p = 0.24$). (Figure 3)

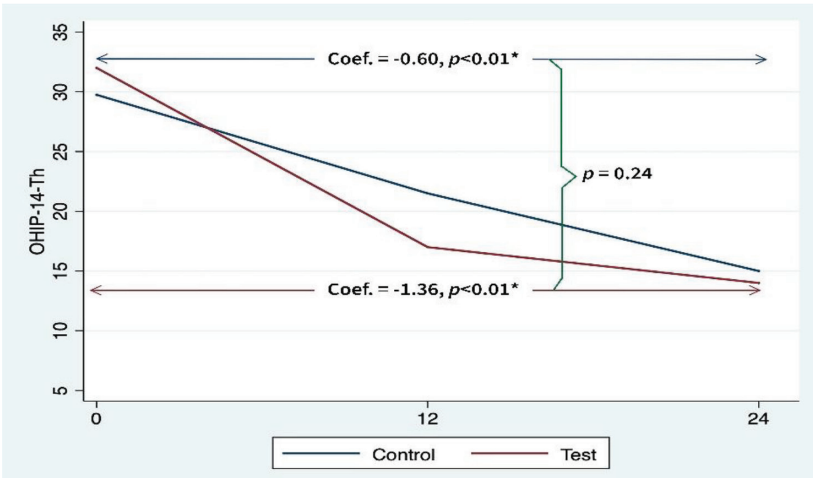

Figure 3 Comparison of the OHIP-14-Th reduction at the first period (Day 1 – Day 12) and the second period (Day 13 – Day 24) between the test and control group after adjusting baseline VAS score, age and gender
*Statistically significant *p*-value < 0.05 (Multilevel mixed-effects logistic regression)
Test group = received okra jelly; Control group = received jelly without okra; OHIP-14-Th = Oral Health Impact Profile - Thai

After adjusting baseline score, age and gender by using GEE population-averaged model, it was found that the reduction of total OHIP-14-Th scores in every 12-day in the test group was more than the control group in all domains, however, insignificantly. In the test group, it was found that psychological discomfort scores were lowered the most, followed by functional limitation and psychological disability, respectively. The social disability aspect was the least reduction in score every 12-day. In the control group,

the reduction of scores in the functional limitation aspect was the highest, followed by psychological disability and physical disability, respectively. Social disability score was least reduced, the same as the test group. (Table 4)

Table 4 Comparison of the reduction of OHIP-14-Th scores every 12-day in each domain between the test and control group

Domain	OHIP-14-Th score reduction (p-value)[a]		*p*-value[b]
	Control group	Test group	
Functional limitation	0.094 (p = 0.01)*	0.230 (p < 0.01)*	0.12
Physical pain	0.058 (p = 0.02)*	0.142 (p < 0.01)*	0.40
Psychological discomfort	0.037 (p = 0.15)	0.607 (p = 0.03)*	0.50
Physical disability	0.069 (p = 0.13)	0.148 (p = 0.08)	0.80
Psychological disability	0.090 (p < 0.01)*	0.203 (p < 0.01)*	0.34
Social disability	0.003 (p = 0.67)	0.006 (p = 0.67)	1.00
Handicap	0.029 (p = 0.21)	0.077 (p = 0.41)	0.48

*Statistically significant *p*-value < 0.05 (GEE population-averaged model)
p-value [a] = Intragroup comparison of OHIP-14-Th scores every 12-day
p-value [b] = Intergroup comparison of OHIP-14-Th scores every 12-day
Test group = received okra jelly; Control group = received jelly without okra; OHIP-14-Th = Oral Health Impact Profile - Thai

Satisfaction of the studied jelly

The results of the satisfaction questionnaire demonstrated that the volunteers rated more satisfied with color and odor of okra jelly than jelly without okra. Meanwhile, they rated less satisfied with taste and consistency of okra jelly than jelly without okra. In overall, the volunteers' satisfaction was almost no different between both jellies. (Figure 4)

Figure 4 Satisfaction of okra jelly and jelly without okra after completing the 24-days experiment

Discussion

Xerostomia can compromise the elder's health, both physical and mental functions, so it should be diagnosed correctly in order to treat its negative effects and to achieve a higher quality of life. This preliminary study demonstrated the promising results from okra jelly on self-perceived xerostomia and oral health related quality of life in the elderly who denied systemic diseases and medication.

Most managements of xerostomia aim to reduce patients' symptoms and/or increase salivary flow.[9,10] In this study, we developed okra jelly under the assumption that the mucilage of okra has moisturizing and lubricating properties similar to human saliva, meanwhile, the moisturizing with semi-rigid texture and syneresis of jelly would make the elder easily to chew and swallow, and help lubricate mouth and throat. Within limit of a short period of studied time, the results showed trend of the reduction of VAS score of severity of xerostomia and OHIP-14-Th scores, both in the test group who received okra jelly and the control group who received jelly without okra. This is in agreement with the previous study which reported that gelatinous substitutes of saliva showed a significant reduction of the dryness-related complaints in patients suffering from severe xerostomia.[21] However, the VAS score of severity of xerostomia in okra jelly group showed greater reduction than the control group significantly as well as the OHIP-14-Th score showed pattern of the great volume of reduction, though insignificantly, suggests that the mucilaginous property of okra may have the potential in alleviating xerostomia and lead to better quality of life.

For the elderly, it is advised to have smaller meals and snacks more frequently. Though this is unfamiliar Thai culture, having snack like jelly in this study was a pleasurable experience for the volunteers. Based on the fact that salivary flow increases in response to both gustatory (taste) and mechanical (chewing) stimuli.[22] The more mechanical exercise, the more salivation. Thus, chewing jelly between meals may also play a role in stimulating salivary flow. Besides, okra is well-known for its rich in various nutritional values which provides incremental health benefits.[13] The consumption of sugar-free, low-calorie okra jelly in this study, therefore, supports the dietary medicine/nutraceutical perspective of okra.[14]

This study has potential limitations. The study conducted in the rural northern part of Thailand with the small numbers of subjects, this may not be the representative of elderly population. Further study should evaluate the efficacy of okra jelly on a greater population sample size, longer period of trials with relevant objective clinical parameters. Since the term dry mouth has been used to describe both xerostomias, the subjective sensation of oral dryness, and hyposalivation, the objective symptoms of a reduced salivary flow, the future researches should include the clinical examination and actual measurements of salivary flow rate with both stimulated and unstimulated conditions. While the most frequent cause of hyposalivation is the use of certain medications such as anticoagulants, antidepressants, antihypertensives, therefore, individuals with systemic diseases and medications should be included.[3,8] It should be noted that the impact of xerostomia on quality of life has been well emphasized,[5-7] by any means to help solve the problems has become a matter of focus.

Conclusion

Okra jelly seems to have promising results on the reduction of self-perceived xerostomia and oral health-related quality of life in the elderly.

Acknowledgements

We wish to express our special thanks to Assistant Professor Dr. Chutamat Niwat, School of Agro-Industry, Mae Fah Luang

University, who shared her knowledge and experience on the formula of okra jelly preparation. We also would like to thank Dr.Paween Tangchitphisut, School of Medicine, Mae Fah Luang University, who shared his expertise and advice on statistical analysis. Our special thanks are extended to the elderly who are members of Seniors' School at Tha sut, Chiang Rai, participants of this study, for their cooperation.

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Case Report

GMSMJ Greater Mekong Subregion Medical Journal

CMV Enteritis and Guillain-Barré Syndrome after Stem Cell Transplantation for Lymphoma

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Received 11 June 2021 • Revised 27 October 2021 • Accepted 30 November 2021 • Published online 1 January 2022

Abstract:

We report a 31-year-old male from Kuwait, diagnosis of advanced diffuse large B-cell lymphoma stage IV presented with extradural mass and spinal cord compression at T6 level. After T7-T8 laminectomy with 4 cycles of rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone (R-CHOP) chemotherapy and high dose methotrexate (MTX) only one time then followed with 4 cycles of rituximab, cyclophosphamide, vincristine, doxorubicin, and dexamethasone (R-Hyper CVAD)/high dose MTX and cytarabine (Ara-C). The non-myeloablative stem cell transplantation (NMSCT) was performed because of morbid obesity (body weight 135 kg). The conditioning regimen was thiotepa, fludarabine and cyclophosphamide. The graft versus host disease (GVHD) prophylaxis was short-course methotrexate and tacrolimus. The patient developed chronic diarrhea with abdominal pain caused by CMV colitis on day 57 post-transplant and was treated with ganciclovir. Subsequently he developed Guillain-Barré syndrome manifested with progressive weakness of lower extremity which successful treatment with intravenous immunoglobulin (IVIg) 2 g/kg. The recovery of motor power was starting 2 days later. By the same period, patient developed pancytopenia from stem cell rejection. The 0.95 x 10^6/kg of stem cell was re-infused on day 72 post-transplant and reachieved engraftment 13 days later. The motor power was recovered from grade I to grade IV and he was able to walk with walker support after 25 days treatment of IVIg

Keywords: Lymphoma, Stem cell transplantation, Guillain-Barré syndrome

Introduction

The management of advanced or refractory diffuse large B-cell lymphoma (DLBCL) with CNS and bone marrow involvement should consider high dose chemotherapy with allogeneic stem cell transplantation (SCT). The cytomegalovirus (CMV) infection during immunosuppression is common. Guillain-Barré Syndrome (GBS), manifests as acute inflammatory demyelinating polyneuropathy, can be triggered by viral infections which CMV is the second most common reported pathogen

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GMSMJ 2022; 2 (1): 55-60

preceding GBS. The aforementioned Dutch GBS study found CMV to be present in 13% of patients.[1] The graft rejection is also possible if the patient has serious infection. We report a case of advanced DLBCL post allogeneic stem cell transplantation developed CMV enteritis with GBS and graft rejection after that.

Case Presentation

A 31-year-old Kuwaiti male was referred for lymphoma treatment. In March 2020, he was diagnosed with stage IV diffuse large B-cell lymphoma (DLBCL) involving scapular and T6 vertebral body with spinal cord compression from extradural mass. In Kuwait, he was treated with T7-8 laminectomy and 4 cycles of R-CHOP (rituximab, cyclophosphamide, vincristine, prednisolone) chemotherapy followed by one course of high dose methotrexate (MTX) and 2 doses of intrathecal MTX. Then, he was referred for further treatment in Thailand.

During that period, there was a pandemic spread of COVID-19 and he had to be in hospital quarantine for 14 days. The disease evaluation was done by PET/CT scan compared to the previous one in his country showed no significant change of residual left paravertebral mass adjacent to T7, 8 vertebral bodies, 1 cm in diameter, SUVmax = 2.86 (previous 1 cm SUVmax= 2.6, initially 1.8 cm thick, SUVmax= 15.9) which was determined as complete metabolic response, scoreII. Osteoblastic lesion in the T8 vertebral body showed no FDG avidity, SUVmax=3.64, same as normal vertebra; post treatment change of bone involvement (complete metabolic response) (Figure 1). The bone marrow study showed no lymphoma cell involvement.

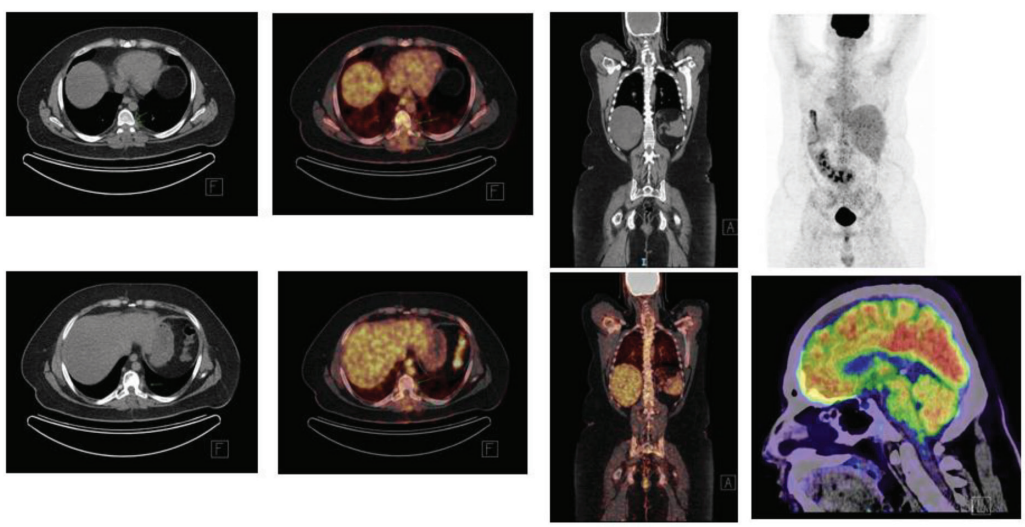

Figure 1 PET/CT scan study on 1 September 2020 showed no significant change of residual left paravertebral mass adjacent to T7, 8 vertebral bodies, 1 cm in diameter, SUVmax = 2.86 (previous 1 cm SUVmax=2.6, initially 1.8 cm thick, SUVmax= 15.9) which was determined as complete metabolic response, score II.

By that time, we determined this case as an advanced DLBCL which was likely to involve spinal cord and had some residual paravertebral mass with SUVmax only 2.86 and determined as complete metabolic response score II after treatment from Kuwait. We decided to continue treatment with second line salvage immuno-chemotherapy followed by high dose chemotherapy with stem cell transplantation.

The salvage chemotherapy R-Hyper CVAD (rituximab/cyclophosphamide/vincrisine/doxorubicin/dexamethasone) alternating with high dose MTX and cytosine arabinoside (Ara-C) had been given for 4 cycles. The lymphoma involved bone and vertebra which was bone marrow space. The patient was morbid obesity (body weight 135 kg) and poor performance status, so we considered reduce-dose chemotherapy of non-myeloablative stem cell transplantation (NMSCT) from his sister which had matched related HLA. The conditioning regimen was thiotepa/fludarabine/cyclophosphamide (TFC) which the dose was reduced by using adjusted body weight. The PET/CT scan was evaluated on 7 Jan 2021 after NMSCT (D0=25 Jan 2021) which showed no significant change of residual left paravertebral mass adjacent to T7, 8 vertebral bodies, 0.9 cm in diameter, SUVmax =3.03 (previous 1 cm, SUVmax = 2.86, initially 1.8 cm thick, SUV max = 15.9); complete metabolic response, Score II and osteoblastic lesion in the T8 vertebral body shows no FDG avidity, SUV max = 2.24(previous SUVmax=3.64), same as normal vertebra; post treatment change of bone involvement (complete metabolic response) (Figure 2)

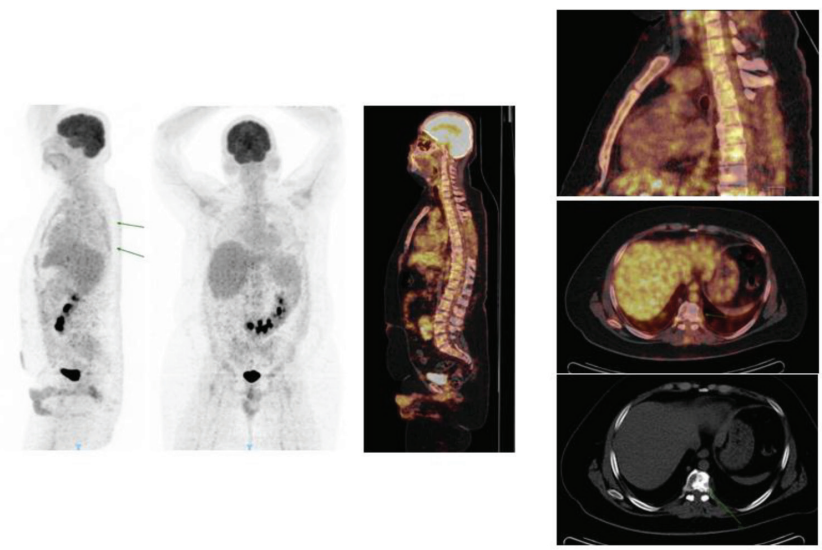

Figure 2 PET/CT scan on 7 Jan 2021 after Hyper CVAD chemotherapy and non-myeloablative stem cell transplantation (NMSCT) showed no significant change of residual left paravertebral mass adjacent to T7, 8 vertebral bodies, 0.9 cm in diameter, SUVmax =3.03 (previous 1 cm SUVmax=2.86, initially 1.8 cm thick, SUVmax= 15.9); complete metabolic response, Score II and osteoblastic lesion in the T8 vertebral body shows no FDG avidity, SUVmax= 2.24 (previous SUVmax=3.64), same as normal vertebra; post treatment change of bone involvement (complete metabolic response)

On day 40 after NMSCT, the patient developed skin rash on his face and both ears as a manifestation of graft versus host reaction (GVHD). The tacrolimus level was 2.3 ng/mL (therapeutic range 10-20). The reaction was well controlled after increased dosage of tacrolimus with steroid combination. On day 57, the patient was admitted because of chronic diarrhea with abdominal pain. Again, the GVHD was suspected. The blood tests showed hemoglobin 14 g/dL, WBC 6.0 x 10^9/L, platelet 97.0 x 10^9/L, BUN 55.4 mg/dL, creatinine 2.79 mg/dL, AST 86 U/L, ALT 198 U/L, total bilirubin 1.1 mg/dL. The tacrolimus level was 25.7 ng/mL. The endoscope had been done and showed only moderate antral gastritis, gastric ulcer and duodenitis. The whole abdominal

CT-scan showed diffuse bowel wall and mural thickening of small bowel and colon with surrounding perirectal and mesenteric stranding possibly due to infectious or inflammatory process. (Figure 3).

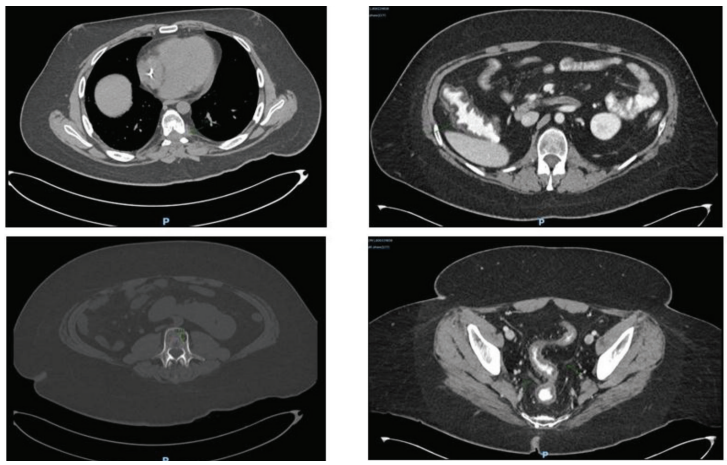

Figure 3 The abdominal CT scan showed bowel wall thickening

The investigations of infectious cause had been done and demonstrated CMV in the stool by PCR test but the serum CMV viral load was undetected and the patient never had fever. The antiviral, ganciclovir, was started with his abdominal symptoms gradually improved. By that period, the overdose of tacrolimus was diagnosed and hold with intravenous hydration. The blood level was down and also the serum creatinine level and liver function returned to normal.

By day 68 of NMSCT, the patient developed progressive pancytopenia with hemoglobin 7.1 g/dL, WBC 2.7 x 10^9/L, platelet 17.0 x 10^9/L, AST 31 U/L, ALT 34 U/L, and total bilirubin 4.9 mg/dL. The pancytopenia may cause from CMV infection, ganciclovir or graft rejection. The cause of hyperbilirubinemia might be from infection or medication. The bone marrow evaluation had been done and showed severe hypocellular marrow without viral inclusion bodies or definite lymphoma involvement. The stem cell 0.95 x 10^6 /kg was reinfused on day 72. The recovery of the blood was achieved thirteen days after (hemoglobin 10.5 g/dL, WBC 17.2 x 10^9/L, and platelet 2.0 x 10^9/L). By the same period (day 71), the patient developed progressive weakness of both legs. The MRI of T-spine and PET/CT scan did not show any new tumor mass or spinal cord compression. (Figure 4)

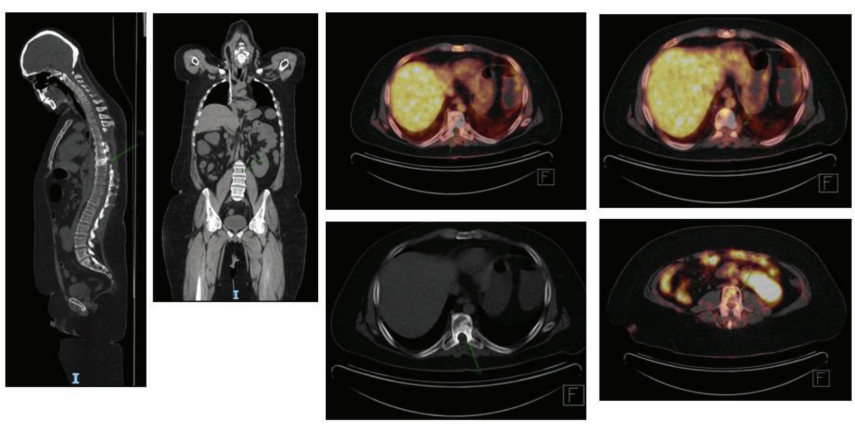

Figure 4 PET/CT scan on 9 April 2021 showed stable small residual left paravertebral mass adjacent to T7, 8 vertebral bodies, 1 cm in diameter, SUVmax =2.74 (previous 0.9 cm SUVmax=3.03, Score 2, complete metabolic response.

The neurological examination demonstrated: awake, alert, intact cranial nerve, intact ocular movement, intact vision, cooperation and followed commands, no facial palsy, generalized weakness (upper arm grade 2/5 and hand grip grade 3-4/5), paraparesis with weakness both of legs grade 1/5 (more on proximal), no abnormal movement. Other neurological tests, bilateral flexor response of plantar reflex, impaired pinprick sensation, temperature and proprioception both feet, negative straight leg raising test and absence of all deep tendon reflexes. The conclusion was symmetrical proximal weakness of arms and legs which suspicious of acute inflammatory demyelinating polyneuropathy (AIDP e.g., Guillain-Barré syndrome). The nerve conduction study confirmed acute acquired demyelinating process. The lumbar puncture was not done because of low platelet number. The patient was treated with intravenous immunoglobulin (IVIg) 2 g/kg body weight. The motor power was gradually gained 2 days after infusion and reached more than grade 3/5 after 10 days.

Discussion

This patient was 31-year-old Kuwaiti man came with advanced DLBCL with spinal cord compression from paravertebral mass and vertebral bone involvement. After treatment from Kuwait with laminectomy and R-CHOP chemotherapy with high dose methotrexate, he still had residual tumor even through the SUVmax from PET/CT scan did not increase (score II). We considered to treat with second line chemotherapy followed by NMSCT. This technique was used because of vertebral bone involvement and morbid obesity. The conditioning regimen was Thiotepa-based (TFC) which the dose was reduced by using adjusted body weight. Thiotepa is a cytotoxic agent of the polyfunctional type related to nitrogen mustard. The radiomimetic action of thiotepa is believed to occur through the release of ethylenimine radicals which, like radiation, disrupt the bonds of DNA. Both thiotepa and its active metabolite, TEPA, efficiently cross the blood-brain barrier. After intravenous administration, the cerebrospinal fluid concentration achieved are nearly identical to those in plasma.2,3 The acute graft versus host disease (aGVHD) was very mild and well controlled. On day 57 of NMSCT, the patient developed sudden onset of diarrhea with abdominal pain on and off. The GVHD was ruled out because the serum tacrolimus level was high and endoscope with biopsy had been done. The CMV enteritis and colitis were diagnosed because of positive CMV in stool by PCR and abdominal CT scan. The GI symptoms were improved after ganciclovir treatment.

Unfortunately, the patient developed graft rejection which might be triggered by CMV infection or antiviral medicine. CMV infection is associated with an increased expression of MHC class II on multiple cell types. Since recognition of non self MHC antigens is the major determinant of allograft rejection, an upregulation of these molecules could contribute to graft failure.[4,5] Ganciclovir triphosphate is a competitive inhibitor of deoxyguanosine triphosphate incorporation into DNA and preferentially inhibits viral DNA polymerase more than cellular DNA polymerase.[6] This effect will be associated with bone marrow suppression, particularly leukopenia.[7] In fact, disseminated CMV per se characteristically suppresses bone marrow production, but antiviral therapy usually results in improvement of hematologic parameters. The bone marrow of the patient had been engrafted after 13 days of stem cell re-infusion. By the same period around day 68 of NMSCT, the patient developed progressive weakness of both legs and showed symmetrical proximal weakness of arms and legs which suspicious of acute inflammatory demyelinating polyneuropathy (AIDP e.g., Guillain-Barré syndrome). Infection with

Campylobacter jejuni, which causes diarrhea, is one of the most common risk factors for GBS. People can also develop GBS after some other infections, such as influenza virus, cytomegalovirus, Epstein-Barr virus, and Zika virus. CMV is the second most common reported pathogen preceding GBS. The aforementioned Dutch GBS study found CMV to be present in 13% of patients.[1] IVIg is a proven effective treatment for GBS (class 1 evidence). However, about 25% of patients need artificial ventilation and 20% are still unable to walk unaided after 6 months. Important clinical factors associated with poor outcome were age, presence of preceding diarrhea and the severity of disability in the early course of disease.[8] A second IVIg course should not be considered for treatment of Guillain-Barré syndrome because of a poor prognosis.[9] Fortunately, our patient had good respond after only one course of treatment.

Conclusion

The authors report a case of advanced DLBCL post allogeneic NMSCT, developed CMV enteritis with GBS which successful treatment with ganciclovir and IVIg. The patient had graft rejection which engrafted again by reinfusion of the donor stem cell.

Conflict of interest

The authors have declared no conflict of interest.

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