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Research Paper

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Research Paper

Antimalarial primaquine for skin infiltration analgesia in rats

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Abstract

Objectives The purpose of this study was to estimate the ability of antimalarial medications to induce local infiltration analgesia.

Methods Using a rat model of skin infiltration anaesthesia, the effects of antimalarial medications (primaquine, chloroquine, hydroxychloroquine and amodiaquine) were compared with the application of lidocaine.

Key findings At a dose of 3 μmol, primaquine and chloroquine displayed better potency (all $P < 0.05$) and greater duration (all $P < 0.01$) of cutaneous analgesia than lidocaine, whereas the other antimalarial medications showed a similar potency and duration of cutaneous analgesia when compared with lidocaine. When a dose of 3 μmol antimalarial medication was used, primaquine was the most potent and had the longest duration of action among four antimalarial medications. The relative potency ranking (ED_{50}, 50% effective dose) has been found to be primaquine [2.10 (1.87 – 2.37) μmol] > lidocaine [6.27 (5.32 –7.39) μmol] ($P < 0.01$). Infiltration analgesia of skin with primaquine had a greater duration of action than did lidocaine on the equipotent (ED_{25}, ED_{50}, ED_{75}) basis ($P < 0.01$).

Conclusions Primaquine and chloroquine have greater potency and longer lasting skin analgesia when compared with lidocaine, while the other antimalarials display a similar potency in comparison with lidocaine.

Keywords: primaquine; lidocaine; antimalarial drugs; skin infiltration anaesthesia

Introduction

Antimalarial medications are mostly used to treat malaria.[1] For instance, primaquine is the only available drug for treatment of vivax malaria relapses.[2] Chloroquine was introduced in the market for treating malaria [3] and systemic lupus erythematosus.[4] Hydroxychloroquine, the less-toxic metabolite of chloroquine, was

used in the treatment of Sjogren's syndrome, rheumatoid arthritis, juvenile idiopathic arthritis and systemic lupus erythematosus.[5] Amodiaquine is a major drug for malaria control by combination therapies.[6] There is increasing evidence that primaquine and chloroquine blocked Na^+ channels.[7–9] Furthermore, primaquine produced dose-dependent depression of the action potential in isolated rat myocytes.[10]

Local anaesthetic drugs prevent nerve impulse generation through the inhibition of voltage-gated sodium channels, and consequently produce local anaesthesia (e.g. local infiltration analgesia).[11] However, local anaesthetics have similar chemical structures and share similar systemic toxicity and neurotoxicity.[12] There are few local anaesthetics with new structures, whereas the target compounds are mainly amide compounds in local anaesthesia. [13] For this reason, the diversity of compound structure with new structures should be considered. [13] Local infiltration analgesia with local anaesthetics was commonly used for post-surgical analgesia owing to few side effects.[14] Emerging evidence supports that antimalarial medications, such as primaquine and chloroquine, can block Na^+ channels.[7, 9] In this study, the authors determined the local analgesic effects of different antimalarial medications (primaquine, chloroquine, hydroxychloroquine and amodiaquine) in comparison with lidocaine by measuring the cutaneous trunci muscle reflexes in rats.

Materials and Methods

Animals

The investigative protocol was approved by the Institutional Animal Care and Use Committee of the China Medical University, Taiwan (IACUC Approval No: 2016–036). Male rats (Sprague–Dawley; 205–255 g) were purchased from BioLASCO Taiwan Co., Ltd, and they were housed in the climate-controlled rooms (22°C; a relative humidity of 50 %; a 12/12 h light/dark cycle) with unlimited supply of food and water.

Chemical agents

Primaquine bisphosphate, chloroquine diphosphate salt, hydroxychloroquine sulfate, amodiaquine dihydrochlororide dihydate and lidocaine HCl were obtained from Merck (formerly Sigma-Aldrich, Darmstadt, Germany). Before the start of the experiment, chemical agents were dissolved freshly in normal saline.

Research designs

Three designs of the research were tested (n = 8 in each group of different treatments). First, we assessed the skin analgesic effect of four antimalarial medications (primaquine, chloroquine, hydroxychloroquine and amodiaquine) and the local anaesthetic lidocaine following subcutaneous injection (0.6 ml of 5 mM). Second, we evaluated the dose-related studies of primaquine (0.6–6.0 μmol) and lidocaine (1.5–12.7 μmol) on infiltrative cutaneous analgesia. The analgesic effect of primaquine (10 mM) was compared with lidocaine (21.2 mM), while saline (0.9% NaCl) acted as the control solution. Third, we also estimated the duration of full recovery of primaquine and lidocaine based on their equipotent doses (ED_{50}s [50% effective doses], ED_{75}s and ED_{25}s).

Subcutaneous injection

Prior to the start of injection in the thoracolumbar region, the back hair (8 × 6 cm) of rats was shaved mechanically with a razor. A total volume of 600 μl drug solution through a 30-G needle, BD Ultra-Fine Insulin Syringes, was injected subcutaneously at the rat's back (thoracolumbar region). A skin wheal ~20 mm in diameter appeared following subcutaneous administration, and we marked the skin wheal with ink afterwards. For neurobehavioural examination, a rigid 18-G needle was attached to the *von* Frey style Aesthesiometer (No.15; Somedic Sales AB, Stockholm, Sweden) in order to provoke a standardised stimulation (19 ± 1 g).[15, 16] An experienced researcher, who was blinded to treatment allocation, carried out all neurobehavioural tests. Neurobehavioural examinations were always conducted between 9:00 AM and 11:00 AM.

Neurobehavioural testing

Cutaneous trunci muscle reflexes (CTMRs) in response to skin pinpricks on the back of rats were used to evaluate the cutaneous analgesic effects.[17, 18] After the CTM reflex was elicited by needle pinpricks outside the wheal, six pinpricks were applied to the wheal (marked area). Cutaneous analgesia was evaluated by the fraction of times six stimulations applied to the wheal failed to react a CTMR. Following the rule, the 50% sensory/nociceptive block (50% of possible effect, 50% PE) was defined as three CTMR reactions in response to six stimuli.

The duration of full recovery was recorded between drug administration and full recovery of the CTMRs (0% block). Area under a curve (AUC) was analysed using Kinetica software 2.0.1., 1996–2006 (MicroPharm International, USA). In addition, dose–response curves of drugs were fitted by the SAS NLIN Procedures (version 9.1, SAS Institute, Cary, NC), and the drug dose producing 50% of a maximal effect, ED_{50}, was constructed.[19, 20] Using the same computer-derived curve-fitting (SAS NLIN analysis), the ED_{25} or ED_{75} was gained.[21, 22]

Statistical analysis

Data are presented as the EDs with 95% confidence interval or mean ± SEM. All statistical analyses were performed using the statistical software packages SPSS® for Windows version 17.0 (SPSS Inc., Chicago, IL, USA). A P-value <0.05 was defined as a statistically significant result. One-way and two-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test or Student's t-test for paired comparisons was used for statistical analyses. When appropriate, Bonferroni correction was added during testing.

Results

Skin analgesic effects of antimalarial medications

Chemical structures of the local anaesthetic lidocaine and four antimalarial medications are shown in Figure 1. At the same concentration of 5 mM, lidocaine, primaquine, chloroquine, hydroxychloroquine and amodiaquine displayed 12.5, 66.7, 27.1, 12.5 and 6.3% (%MPE) of skin analgesic action (Figure 2A), with durations of action of 5.6 ± 2.7, 53.8 ± 2.6, 26.3 ± 4.9, 9.4 ± 4.2 and 6.3 ± 2.5 min, respectively (Figure 2B). Among them, primaquine is the most potent. For this reason, dose–response analgesic effects were constructed after subcutaneous injection of lidocaine and primaquine (Figure 3). The ED_{25}s, ED_{50}s and ED_{75}s are exhibited in Table 1. Based on the ED_{50}s (Table 1), the relative potency ranking was primaquine > lidocaine (P < 0.01 for the differences among medications).

The duration of full recovery of antimalarial medications

Subcutaneous administration of saline (vehicle) provoked no skin analgesia (Figure 4). Only three figures constructed from primaquine,

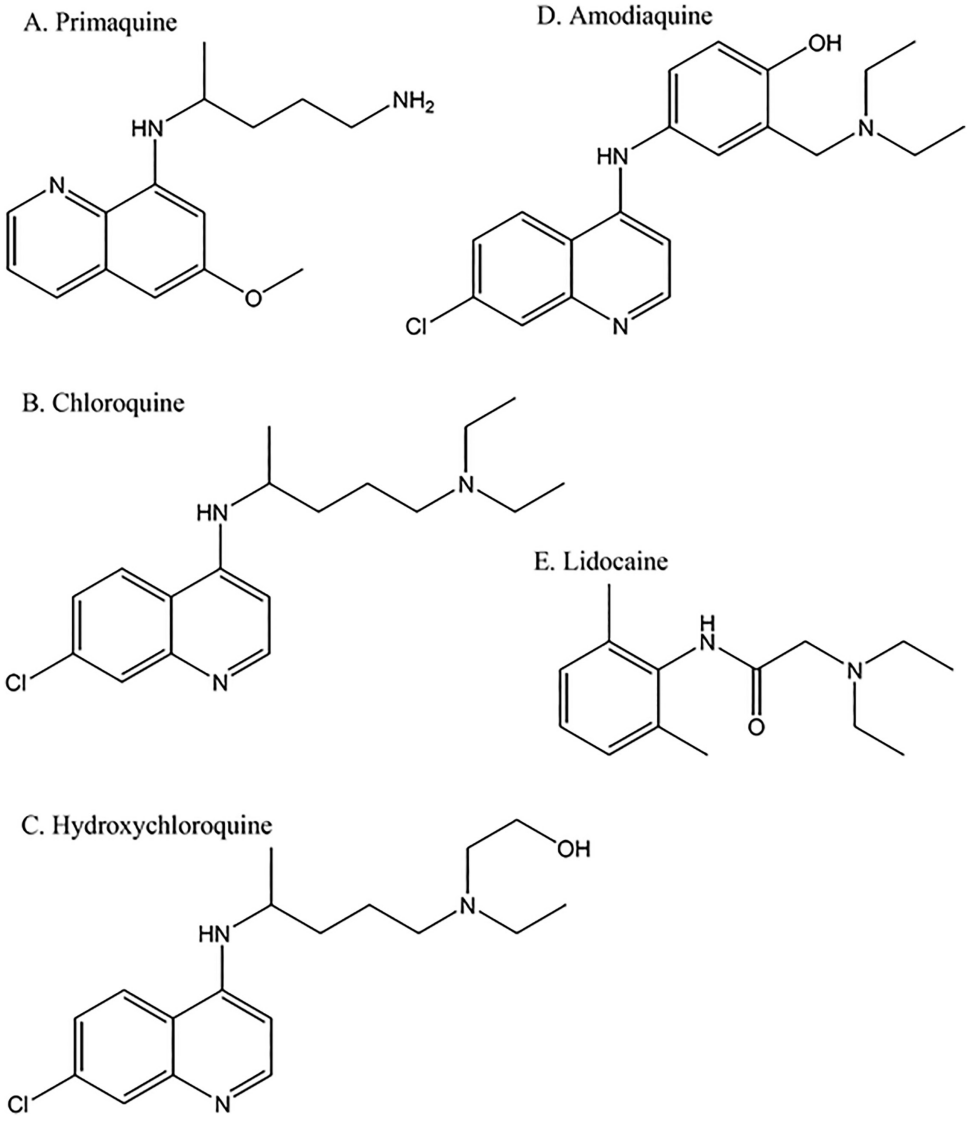

Figure 1 Chemical structures of primaquine (A), chloroquine (B), hydroxychloroquine (C), amodiaquine (D) and lidocaine (E).

Table 1 The ED_{50}s (50% effective doses), ED_{25}s and ED_{75}s

Drug	EDs (95% Confidence Interval)		
	ED_{25}	ED_{50}	ED_{75}
Primaquine	1.42 (1.31 – 1.57)	2.10 (1.87 – 2.37)	3.11 (2.80 – 3.49)
Lidocaine	4.35 (3.65 – 5.16)	6.27 (5.32 –7.39)	9.03 (7.88 – 10.38)

The EDs (μmol) were constructed from Figure 3. The drug's potency (ED_{50}) has been found to be primaquine > lidocaine ($P < 0.01$) by using the one-way ANOVA followed by pairwise Tukey HSD test.

lidocaine and saline are displayed because of similarities between the time course studies (Figure 4). Primaquine (10 mM) and lidocaine (21.2 mM) caused 100 ± 0 and 100 ± 0% nociceptive/sensory blockade with duration of skin analgesia of 206.3 ± 8.9 and 50.6 ± 4.7 min, respectively (Table 2). The value of AUCs, complete block time or full recovery time of lidocaine (21.2 mM) was smaller than that of primaquine (10 mM) ($P < 0.001$; Table 2). On the basis of ED_{25}s, ED_{50}s and ED_{75}s (Figure 5), the duration of full recovery of cutaneous analgesia with primaquine was greater than lidocaine's ($P < 0.01$ for the differences among medications).

Discussion

In this study, antimalarial medications (primaquine, hydroxychloroquine, chloroquine and amodiaquine) had the effects of local anaesthesia on infiltrative skin analgesia. At a concentration of 5 mM, primaquine and chloroquine was more potent than lidocaine, while hydroxychloroquine and amodiaquine had a similar potency when compared with lidocaine. A similar result was obtained with that lidocaine is almost one-fourth of potency of bupivacaine in spinal anaesthesia clinically.[23, 24] Among the antimalarial medications, primaquine is the most potent. Action of primaquine or lidocaine is dose dependent to the skin analgesic effect, and primaquine is more potent than lidocaine based on an equipotent (ED_{50}) basis. Furthermore, the duration of skin analgesia of primaquine is greater than lidocaine's at ED_{25}, ED_{50} and ED_{75} equipotent doses.

Because local anaesthetics block voltage-gated sodium channels, they produce infiltrative skin analgesia, spinal anaesthesia and

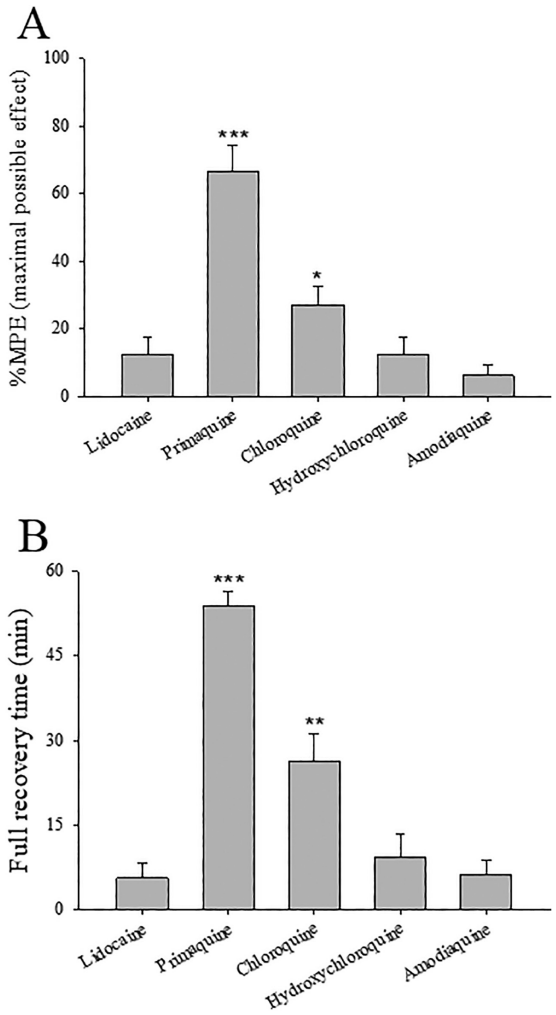

Figure 2 The percent of maximal possible effect (%MPE) (A) and the duration of full recovery (B) of cutaneous analgesia with lidocaine and four antimalarial medications at 5 mM (n = 8 rats in each group of different treatments). Data are shown as mean ± SEM. Symbols (*, **, ***) indicate $P < 0.05$, 0.01 and 0.001, respectively, when compared with lidocaine by using the one-way ANOVA followed by pairwise Tukey HSD test.

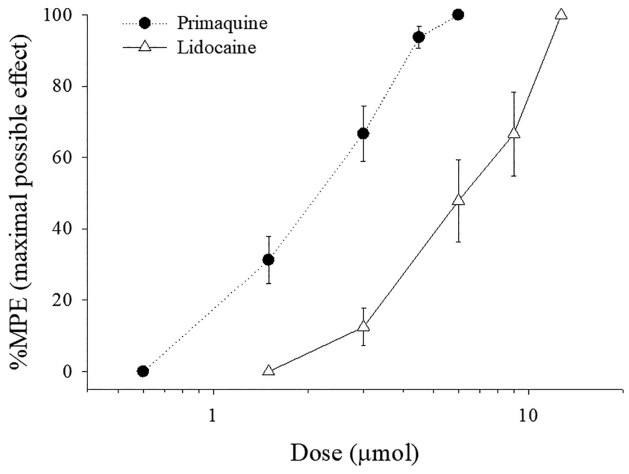

Figure 3 Dose-dependent analgesic effects of the local anaesthetic lidocaine and antimalarial primaquine. For each testing point of the dose-response curve, n = 8 rats. Data are presented as mean ± SEM.

Table 2 The percent of maximal possible effect (%MPE), duration of action and area under the curves (AUCs)

Drug	%MPE	Duration (min)		AUCs (% × min)
		Complete block time	Full recovery time	
Primaquine (10 mM)	100 ± 0	66.0 ± 9.2[a]	206.3 ± 8.9[a]	13,236 ± 666[a]
Lidocaine (21.2 mM)	100 ± 0	18.0 ± 2.9	50.6 ± 4.7	3,373 ± 388
Saline	–	–	–	–

Data (mean ± SEM) were constructed from Figure 4. n = 8 rats in each group. [a]$P < 0.001$ when compared with lidocaine (21.2 mM) by using the one-way ANOVA followed by pairwise Tukey HSD test.

peripheral neural block.[11, 25] According to the distribution of different gene sequences and organs, there are 10 different Nav channels (Nav 1.9 to Nav 1.1 and Na_x), including Nav 1.9 to 1.6 and Na_x distribute in the peripheral nervous system, Nav 1.5 distributes in the heart, Nav 1.4 distributes in the muscle and Nav 1.3 to Nav 1.1 distribute in the brain.[11, 25] Interestingly, local anaesthetics block all of the subunits of the sodium channel.[11, 25] Moreover, combination therapy with chloroquine was useful for treating sciatica and low back pain [26] or interstitial cystitis.[27]

Antimalarial primaquine blocks four isoforms (Nav 1.2, 1.4, 1.5 and 1.7) of voltage-gated sodium channels.[7, 10] In addition, chloroquine at a concentration of 10 μm inhibits cardiac Na^+ channels (Nav 1.5) using isolated cat ventricular myocytes.[8] Owing to similar chemical structures, it can be expected that antimalarial medications may have the local anaesthetic effects by blocking the other sodium channels. For this reason, infiltration analgesia of skin with antimalarial medications was investigated. Our data, as we expected, indicated that four antimalarial medications (primaquine, chloroquine, hydroxychloroquine and amodiaquine) produced the local anaesthetic effects on infiltrative cutaneous analgesia. In agreement with a previous study that subcutaneous injection of chloroquine produced skin analgesia in a dose-dependent fashion in rats.[28] Moreover, primaquine displayed a strongest potency with a longest duration of analgesia; chloroquine had an intermediate effect with an intermediate-duration; hydroxychloroquine and amodiaquine, a weak potency with a shortest duration of analgesia (Figure 2). The durations of anaesthesia are associated with the amount of plasma protein binding data for drugs[29] and the drugs with higher lipophilicity and lower permeation efficiency.[30]

In accordance with their chemical structures, antimalarial medications can be separated into secondary and tertiary amines (Figure 1). In our present experiment, we revealed that, in general, infiltration analgesia of skin with tertiary amine medications is not as strong as secondary amine medications in comparison with lidocaine. After subcutaneous injection, the tertiary amine medications such as hydroxychloroquine, amodiaquine and chloroquine demonstrated a similar potency of cutaneous analgesia when compared with lidocaine, whereas the secondary amine medications (i.e. primaquine) demonstrated a higher efficacy of cutaneous analgesia than did lidocaine (Figure 2). In addition, primaquine (a secondary amine medication) induced a greater duration of cutaneous analgesia than did lidocaine (Figures 4 and 5). Our resulting data can be beneficial in subjects who need longer duration analgesic therapy.

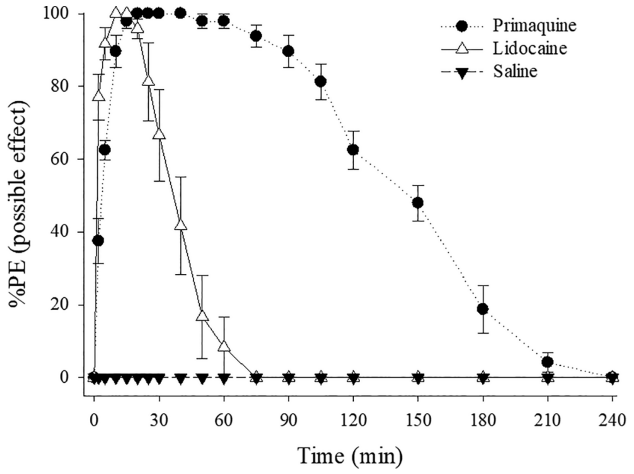

Figure 4 Time courses of cutaneous analgesia (%PE) of primaquine (10 mM) and lidocaine (21.2 mM) in rats (n = 8 in each group). Saline was used as a vehicle control. Data are shown as mean ± SEM. Neurological evaluation was carried out before, and 2, 5, 10, 15, 20, 25, 30, 40, 50, 60 min afterwards, and then again at 15–30 min interval until 4 h.

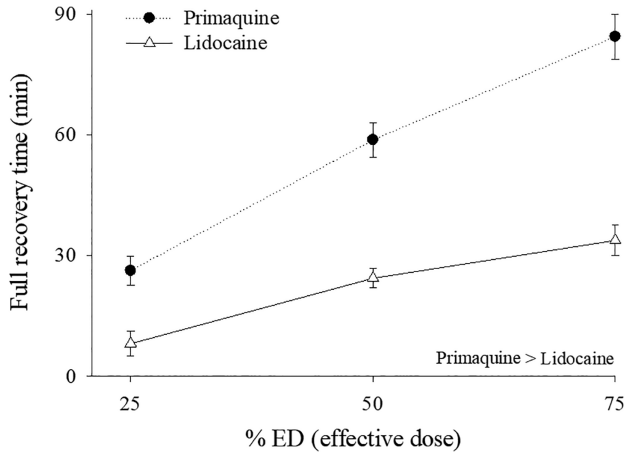

Figure 5 The duration of full recovery of cutaneous analgesia by lidocaine and primaquine on the basis of ED_{50} (50% effective dose), ED_{25} and ED_{75} (n = 8 rats in each group of different treatments). Data are presented as mean ± SEM. The differences in duration of full recovery were evaluated by the two-way ANOVA followed by pairwise Tukey HSD test.

Subcutaneous infiltration of local anaesthetics is an acceptable technique for the management of postoperative pain or surgical anaesthesia.[31] The local anaesthetic lidocaine and bupivacaine are mostly recommended in this field.[31] We found that antimalarial primaquine, with the potency ranging between that of lidocaine and bupivacaine, provoked the cutaneous analgesic effect. Local anaesthetics shared similar central nervous system and cardiovascular system toxicity [12] due to their similar chemical structures. However, antimalarial primaquine and chloroquine have quite different chemical structures from traditional local anaesthetics. Chloroquine exerts vascular protection during the application of Tf-CRM107 as brain tumour therapy.[32] Besides, antimalarial medications may generate acceptable central nervous system adverse effects because antimalarial medications produce therapeutic effects in brain disorders.[33, 34] For instance, chloroquine has neuroprotective effects in traumatic brain injury,[34] and hydroxychloroquine inhibits foetal brain abnormalities.[33] Antimalarial medications may be an alternative to conventional local anaesthetics for skin infiltration anaesthesia.

There are limitations in this study. Because of poorly water-soluble antimalarial medications, only four antimalarial medications (primaquine, chloroquine, hydroxychloroquine and amodiaquine) were tested and compared. Because antimalarial medications are used clinically, they may display expected adverse effects.[35] However, it is unclear if antimalarial medications could cause local neurotoxicity and if repeated infiltration of primaquine or primaquine could result in neurotoxicity when compared with lidocaine. Systemic and central nervous system toxicity and local neurotoxicity should be considered before its application in clinical practice.

Conclusions

The preclinical data showed that primaquine and chloroquine had a better potency of skin analgesic effect when compared with lidocaine, whereas hydroxychloroquine or amodiaquine had a similar potency in comparison with lidocaine. Among four antimalarial medications (a concentration of 5 mM), primaquine showed the most potent and the longest duration of skin analgesia. Based on the equipotent doses, the analgesic quality and duration of primaquine were greater than lidocaine's. The analgesic effect of primaquine has not been mentioned before, and toxicity must be tested in the future before the possible use of primaquine as an analgesic in clinical practice.

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Authors' Contributions

Conception and design of research: Ying-Jen Chang, Yu-Wen Chen, Ching-Hsia Hung; Conduct experiments: Ying-Jen Chang, Kuo-Sheng Liu, Jhi-Joung Wang, Yu-Wen Chen; Interpret results of experiments: Ying-Jen Chang, Kuo-Sheng Liu, Yu-Wen Chen, Ching-Hsia Hung; Prepare figures: Ying-Jen Chang, Kuo-Sheng Liu, Jhi-Joung Wang; Draft manuscript: Ying-Jen Chang, Kuo-Sheng Liu, Yu-Wen Chen, Ching-Hsia Hung, Jhi-Joung Wang; Approve final version of manuscript: Ying-Jen Chang, Kuo-Sheng Liu, Jhi-Joung Wang, Yu-Wen Chen, Ching-Hsia Hung; Edit and revise manuscript: Yu-Wen Chen, Ching-Hsia Hung.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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