Tropical Journal of Pharmaceutical Research July 2017; 16 (7): 1481-1487
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v16i7.4

Original Research Article

Preparation and assessment of ketamine hydrogels for prolonged transdermal anaesthesia

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Sent for review: 15 December 2016 *Revised accepted: 15 June 2017*

Abstract

Purpose: *To formulate and assess thermoresponsive ketamine hydrogels for prolonged transdermal analgesia/anaesthesia.*
Methods: *Thermoresponsive ketamine hydrogels were prepared from chitosan (CTS) and poloxamer 407. Four different formulations (2 formulations of ketamine with 1 and 2 % w/w CTS and 2 formulations with 10 and 15 % w/w ploxamer 407) were assessed for pH, spreadability, drug content, viscosity, in vitro permeation/diffusion, in vivo skin irritancy, and in vivo analgesia (using the hot plate/writhing method in Wistar rats).*
Results: *The formulations had a high drug content (96.12 ± 1.24 to 98.49 ± 0.07 %) with good spreadability. They showed prolonged drug release/permeation of ketamine across the skin, ranging from 81.23 to 98.28 %, and were non-irritating to the denuded skin of Wistar rats with no erythema or oedema after 24 h. The preparation showed effective analgesia that lasted 24 to 30 h. In the writhing test, CTS hydrogels showed stronger analgesia (60.26 – 58.97 %) than those made with poloxamer-based hydrogels (56.41 and 53.85 %). Compared to the activity shown by the standard, lidocaine (which produced 62.82 % analgesia), the effect of the test formulations seem good for probable therapeutic use. Using the hot plate method, the poloxamer-based hydrogels showed more prolonged analgesia than the CTS-based hydrogels.*
Conclusion: *Ketamine hydrogels of CTS and poloxamer may be useful for prolonged analgesia in neuropathic pain and local anaesthesia in minor surgeries.*

Keywords: *Ketamine, Chitosan, Poloxamer, Thermoresponsive hydrogel, Transdermal, Skin permeation*

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INTRODUCTION

Anaesthesia is a vital requirement for surgery. Unlike major surgeries that require general anaesthesia, minor surgeries are often performed under local anaesthesia. Transdermal (i.e., through the skin) anaesthesia may be a good alternative to local blocks for minor surgeries. Transdermal anaesthesia is of potential benefit postoperatively, particularly in paediatric and elderly patients. Transdermal anaesthetic agents are available as a patch, film, gel, or cream [1–3].

Transdermal anaesthesia has been used for more than three decades in children following invasive procedures [1–3]. Transdermal anaesthetics must have a rapid onset of action with good efficacy at a low cost. Many studies have explored the transdermal administration of anaesthetic drugs in various forms that allow rapid absorption (irrespective of molecular weight and skin permeability) with consistent efficacy

[2,3]. Various polymers such as polyglycolic acid, poly l-lactic acid, polylactic copolymer, chitosan (CTS), and poloxamerhave been investigated for the development of hydrogels. In this study, CTS and poloxamer were selected due to their relative ease of conversion to thermoresponsive hydrogels [4].

Ketamine (a phencyclidine derivative) is a non-competitive antagonist of the N-methyl D-aspartic acid receptor that interacts with cholinergic opioid receptors, purinergic receptors, and adrenoreceptors yielding local anaesthetic effects [5]. It is indicated for dissociative general anaesthesia and analgesia for neuropathic pain. Although not widely used, it has several unique clinical properties that make it appropriate in certain situations. Ketamine may be administered in a topical gel for peripheral action (at opioid and sodium-potassium receptors) [6], and can also be administered intravenously, although this route is associated with side effects such as dizziness, nausea, nightmares, agitation, and hallucinations [7]. Topical and transdermal administration of ketamine have been investigated to avoid dizziness and nausea. Moreover, when the drug penetrates the skin, it acts directly on the small nerve fibres, achieving effective localised anaesthesia [8–11]. Transdermal ketamine has also been investigated as an anaesthetic for cosmetic and laser dermatology. Previous studies that investigated creams and gels of ketamine formulations, demonstrated reduced numerical pain scores of 53–100 % on a 1–10 pain intensity scale [12]. These results indicate that ketamine gel may be useful for cases of chronic neuropathic pain [12–16]. But the prior studies of topical ketamine did not compare the formulations prepared with different polymers. Therefore, this study assessed the analgaesic efficacy of ketamine hydrogels composed of two different polymers: CTS and poloxamer.

EXPERIMENTAL

Materials

Ketamine and CTS (from shrimp shells) were purchased from Sigma Aldrich (St. Louis, MO, USA). Poloxamer 407 was purchased from BASF (Ludwigshafen, Germany). The other chemicals were of analytical grade.

Preparation of hydrogels

The ketamine hydrogels were prepared from two polymers, poloxamer 407 and CTS (Table 1). To prepare the CTS hydrogel, CTS was dissolved in a 1 % acetic acid solution while mixing at 300 rpm for 15 min. Ketamine was mixed into the solution along with propylene glycol and triethanolamine until homogeneity was achieved. The poloxamer solution was prepared in pre-cooled water, and ketamine was mixed into the solution along with propylene glycol and triethanolamine until it became slightly viscous. The pH was adjusted to 6.5 to reduce its tendency to cause skin irritation, and the slightly viscous hydrogel was stored at room temperature overnight to ensure the release all of their bubbles. The hydrogels were packed into aluminium tubes that were securely closed, and stored at room temperature until used.

Table 1: Composition of ketamine hydrogel

Ingredient	Hydrogel formulation			
(% w/w)	H1	H2	H3	H4
Ketamine	5	5	5	5
Chitosan	1	2	-	-
Poloxamer 407	-	-	10	15
Polyethylene Glycol	10	10	10	10
Triethanolamine	0.1	0.1	0.1	0.1
Distilled Water	Qs	Qs	qs	qs

Physical assessment of hydrogels

The hydrogels were assessed for colour, appearance, tactile feel upon application, consistency, texture, and pH. Spreadability was also determined by sandwiching the gel between two glass plates and recording the movement of the top plate in response to a pulling force of 80 g. pH was determined using a digital pH meter.

Rheology of hydrogels

The viscosity values and rheological properties of the hydrogels were determined using a DV II + ProDigital Viscometer (Brookfield Engineering, Middleboro, MA, USA).

Drug concentration

From the vials of formulations, 1 mL of each sample was transferred to a volumetric flask using a micropipette. Methanol (up to 10 mL) was added to this sample until complete precipitation (i.e., the supernatant became mostly clear). After centrifugation (1000 rpm for 20 min), the clear supernatant was withdrawn, diluted, and analysed with a spectrophotometer at 276 nm.

Ex vivo drug diffusion studies

Ex vivo drug diffusion was assessed using the abdominal skin of rats (pre-treated to remove hair and other fatty and connective tissues) with

a Franz diffusion cell at 37 ± 1.0 °C in 12.5 mL phosphate buffer at pH 7.4. The pre-treated rat abdominal skin was placed on the bottom opening of the donor compartment in contact with the receptor medium throughout the study; both compartments were tightly fastened with clamps. The receptor medium was agitated at 200 rpm by placing the apparatus on a magnetic stirrer on a hot plate for 24 h, after which1 mL of sample was removed at various time points and replaced with the same volume of fresh phosphate buffer (pH 7.4) at the same temperature. Each sample was analysed at 276 nm in an ultraviolet–visible spectrophotometer.

In vitro release kinetic analysis

The drug release kinetics was determined by inserting the obtained data into the following model equations to check for fit:

Zero Order: C (%) = kt) ……. (1)

First order: log C [fraction unreleased] = kt/2.303…….... (2)

Higuchi: % C (%) = $Kt^{1/2}$ …….. (3)

Korsmeyer-Peppas: Log %C = Log k+ n Log t) ……... (4)

where C is drug C concentration.

In vivo study

The study was conducted using healthy male Wistar rats (200–280 g). The rats were kept in standard housing and environmental conditions. Rats were given 3–4 days to acclimatise before the *in vivo* study. The rats were given free access to drinking water and a standard diet. The *in vivo* study protocol was approved by the Animal Ethical Committee (approval reference no. 299/2015). The study was conducted in compliance with the international guidelines on animal handling [17].

Primary skin irritation studies

The primary skin irritation test was conducted to check for side effects such as discomfort, erythaema, and oedema. Animals were divided into four groups (n = 3). A 2 × 2 cmarea of dorsal skin was denuded with a razor, and then wiped with alcohol. Four hours after denuding, a non-medicated gel was applied to the skin of the rats in Group I (control group). Gel formulations H2 and H4 (with higher concentrations of CTS and poloxamer) were applied to Groups II and III. A standard irritant (0.8 % v/v aqueous solution of formaldehyde) was applied to Group IV (standard). After 24 h, the application sites were scored for erythema and oedema [18].

In vivo analgesic study

Writhing method

Acute analgesia provided by the hydrogels was assessed using the writhing method (induced by acetic acid). The rats were divided into six groups (n = 6). The first group (control) received non-medicated hydrogel, the second group received standard analgesia (topical gel of lidocaine hydrochloride, 5 µg/kg body mass), and test groups III–VI received H1, H2, H3, and H4 formulations (10 µg/kg body mass), respectively. Three hours following application, writhing was induced using 10 mL/kg acetic acid solution (0.6 % v/v) injected intraperitoneally; the number of writhes in 20 min was recorded as the percentage reduction of writhing compared to the control group.

Hot plate method

The four test groups of rats were each administered an individual formulation. The control group received non-medicated gel and the standard group received lidocaine (topical 2 % gel, 5 µg/kg). After 30 min, the rats were placed on an analgesiometer at 50 °C. The duration and maximum response were noted for each group.

Statistical analysis

All of the data are presented as mean ± standard deviation (SD). One-way analysis of variance was used for statistical analysis with Origin 5 as the software. $P < 0.05$ was considered statistically significant.

RESULTS

The ketamine hydrogels were thermoresponsive and gelled after heating. The gels showed good spreadability and were clear and transparent. The surface pH ranged from 6.5 ± 0.01 to 6.9 ± 0.02 (Table 2).

Drug content was relatively high, ranging from 96.12 ± 1.24 to 98.49 ± 0.07 % for all the hydrogel formulations.

Rheological properties

The viscosity of the ketamine hydrogels showed pseudo-plastic rheology. The shear rate increased in direct proportion to the angular

velocity in the pre-gelled formulations. A change in viscosity with angular velocity (shear stress) was observed (Figure 1).

Table 2: Physiochemical characteristics of ketamine hydrogels

Formulation code	pH	Drug content (%)
H1	6.5 ± 0.01	97.14 ± 1.06
H2	6.5 ± 0.03	96.12 ± 1.24
H3	6.9 ± 0.01	98.02 ± 0.42
H4	6.9 ± 0.02	98.49 ± 0.07

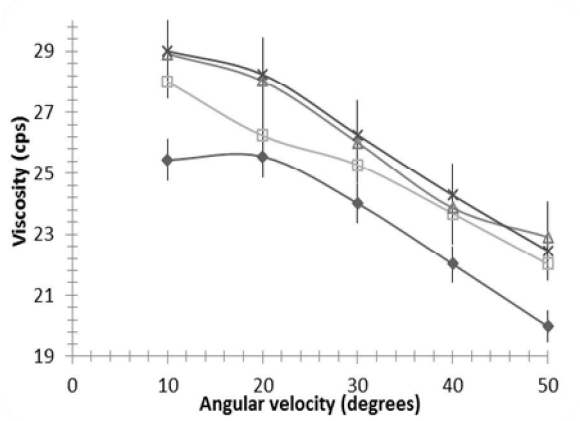

Figure 1: Viscosity of ketamine hydrogel formulations H1 (♦), H2 (□), H3 (Δ) and H4 (×)

At the angular velocity of 50 °C, the viscosities of the hydrogels were 19.99, 22.01, 22.91, and 22.45 cps for H1, H2, H3, and H4, respectively.

In vitro diffusion/permeation

The drug flux was constant and slow across the biomembrane. The hydrogels H3 and H4 showed the highest *in vitro* drug permeations across the membrane (Fig. 2).

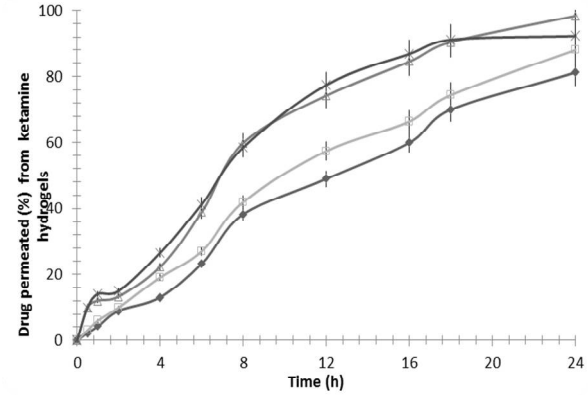

Figure 2: *In vitro* drug permeation of ketamine from hydrogels across rat abdominal skin. ***Note:*** H1 (♦), H2 (□), H3 (Δ) and H4 (×)

After 24 h, drug permeation was 81.23, 88.04, 98.28 and 92.28 % for H1, H2, H3, and H4, respectively. On subjecting the data to various pharmacokinetic models, all the hydrogels demonstrated Higuchi-type permeation. Matrix diffusion was seemed to be the main mechanism involved.

Primary skin irritation

No formulation showed any observable skin irritation. No side effects such as swelling or erythema were observed at the application site after 24 h (Table 3).

Table 3: Primary skin irritation test results for ketamine hydrogels

Animal Group	Mean ±SD	
	Erythema*	***Oedema*****
I Control	0.67±0.58[a]	0.34±0.58[a]
II Test 1 (H2)	0.67±0.58[a]	0.34±0.58[a]
III Test 2 (H4)	0.67±0.58[a]	0.34±0.58[a]
IV Standard Irritant	3 ± 0	1.67±0.50

[a]*P < 0.05, significant compared toformalin; *erythema scale: 0, none; 1, slight; 2, well defined; 3, moderate; 4, scar formation. **Oedema scale: 0, none; 1, slight; 2, well defined; 3, moderate; 4, severe*

In vivo analgesia

The writhing results demonstrated that H1 and H2 had good analgesia with a percentage of 62.82 and 60.26 %, respectively (Table 4).

The hot plate method demonstrated that H3 and H4 (poloxamer-based hydrogels) yielded prolonged analgesia compared to that of H1 and H2 (Table 5).

Table 5: *In vivo* analgesic activity of ketamine hydrogels with the hot plate method

Formulation	MR (s)[a]	TMR (h)	DA (h)
H1	6.5±1.02	1.12	9
H1	7.5±1.20	1.25	>9
H3	9.5±1.0	2.50	>12
H4	10.5±1.2	1.75	>12

[a]*Data are expressed as mean ± SEM (n = 6); MR = maximum analgesic response; TMR = time of maximum analgesic response; DA = duration of analgesic action*

Table 4: *In vivo*analgesic activity of ketamine hydrogels based on writhing method

Drug	Dose (µg/kg)	Analgesicactivity	
		No. of writhes [a]	Analgesia (%)
Control (blank films)	-	78±1	-
lidocaine (Standard)	5	29±2 [b]	62.82
H1	10	31±3 [b]	60.26
H2	10	32±3 [b]	58.97
H3	10	34±3 [b]	56.41
H4	10	36±3 [b]	53.85

[a] *Mean ± standard error of the mean (SEM), n = 6;* [b] *p < 0.05 vs. control.*

DISCUSSION

In a previous small scale clinical study, ketamine was administered to humans at a dose range of 0.093 – 9.33 mg/kg) [12].Initial application provided significant pain relief to all of the patients. The average pain score decreased from 8.8 (pre-application) to 1.6 post-application (mean, 15 min) [12]. The ketamine hydrogels investigated in this study were thermoresponsive, had good viscosity, and spread easily on the skin surface. The viscosity should be optimum for a gel in the sense that it should neither be too high nor be too low. The higher viscosity may lead to the stiff gel which would not be applicable properly onto the skin. On the other hand, the less viscous gel shall flow and not be retained onto the skin. In both the cases the drug release may get affected. In this aspect the prepared formulations showed desired viscosity with easy to spread property.

Poloxamer hydrogels yielded more effective analgesia than CTS-based hydrogels. In a previous study the phase transition temperature (Tsol-gel) and rheological properties of poloxamer 407 gel were evaluated [19]. Tsol-gel reduced (without compromising the gel strength) and pseudoplastic rheology was observed upon the addition of microparticles (or drug). Thus, a thermoreversible gel was obtained with a rheology suitable for application. In the present study, the easy sol gel transition might have contributed to the improved drug delivery by poloxamer based hydrogels. The pH was satisfactory for all of the formulations, but the H2 and H4 formulations (with higher concentrations of CTS and poloxamer) had the best permeation across the skin. Moreover, drug permeation across the skin followed the Higuchi kinetics which confirms that the drug diffused via the matrix diffusion process.

The poloxamer hydrogels showed better drug release across the skin in the present study. The nature of polymers governs the release profile. Unlike chitosan (a hydrophilic polymer), Poloxamer 407® is an amphiphilic synthetic copolymer consisting of a hydrophobic poly (oxypropylene) (POP) block between two hydrophilic poly(oxyethylene) (POE) blocks. Due to their amphiphilic nature, poloxamer molecules can readily self-assemble to form micelles depending on the concentration and temperature, and the drug release can be improved from these micelles. The micellization makes it easy for the drug to pass through across the skin. The amphiphilic polymers (like poloxamer) pass better across the skin and hence show the better drug permeation across the skin.

The results also showed that different concentrations of polymers may differ in efficacy and duration of action. This might be due to the formation of different degrees of micellization with different concentration in case of poloxamers. On the other hand, in case of chitosan, the thickness of diffusional barrier of polymeric network varies with the concentration resulting in change in drug release or duration of action. These results indicate that the structure of the gel functioned as an increasingly resistant barrier to drug release as the concentration of chitosan increased [20]. Therefore, the concentration of polymers may be chosen or may be optimized for desired drug release.

The *in vivo* results showed that thermoresponsive hydrogels provided effective analgesia. CTS hydrogel formulations had quicker onset times but shorter durations of action compared to the poloxamer hydrogels. Topical and transdermal routes have also been investigated for the administration of other anaesthetic agents [21–25].

CONCLUSION

The findings of this study demonstrated that thermoresponsive ketamine hydrogels prepared using CTS and poloxamer 407 may provide effective analgesia in minor surgery and neuropathic pain. Furthermore, the concentration of polymer in the hydrogel may affect transdermal absorption of ketamine.

DECLARATIONS

Acknowledgement

The authors would like to thank Fuzhou General Hospital for providing the necessary facilities to conduct this research.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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