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RESEARCH PAPER

Aerosol-OT Microemulsions as Transdermal Carriers of Tetracaine Hydrochloride

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ABSTRACT

The aerosol-OT (AOT)/water/isopropyl myristate microemulsion was investigated as a carrier in transdermal drug delivery of tetracaine hydrochloride. The study included in vivo analgesic studies on rats and histopathological, irritation, and oxidative stress measurements on mice. The tetracaine hydrochloride encapsulated in AOT/water/isopropyl myristate showed an eightfold enhancement in the analgesic response of drug compared to the aqueous solution of the drug as measured by the tail-flick method. The analgesic response of tetracaine hydrochloride, however, highly depended on the concentration of AOT and water of the microemulsion. The preliminary histopathological, irritation, and oxidative stress studies showed that AOT/water/isopropyl myristate microemulsion system is a safe transdermal carrier of tetracaine hydrochloride with a concentration of AOT in isopropyl myristate up to 21:79 w/w.

INTRODUCTION

For the treatment of pain, the transdermal drug delivery of local analgesic is of great important in pharmaceutical industries (1,2). Owing to poor therapeutic efficacy resulting from the inadequate penetrations of analgesic drugs through the intact skin, these drugs are seldom applied percutaneously (3). Most of the topical analgesic preparations are based on a cream or ointment and have limited transdermal potential. Recently, liposome-based analgesic formulations were developed for topical application (4,5). However, lipid-based formulations have stability limitations (6). We believed that the microemulsion could circumvent such limitations and provide formulations for the induction of local analgesic effect topically. Microemulsions, which consist of surfactant, oil, water,

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and sometimes cosurfactant, are isotropic and thermodynamically stable systems (7).

In the present study, we selected microemulsions of aerosol-OT (AOT), water, and isopropyl myristate. The local analgesic response of a microemulsion-based formulation the tetracaine hydrochloride was compared with an aqueous solution of the drug, and the efficacy of the microemulsion in transdermal drug delivery of tetracaine hydrochloride was examined. The choice of tetracaine hydrochloride was based on its potency for transdermal application (8). AOT or sodium bis(2-ethyl hexyl) sulfosuccinate is a well-known anionic and medicinal surfactant (9). AOT allows the dissolution of a considerable amount of water in oil without using cosurfactants such as alcohol, amines, and the like, and it forms a stable water-in-oil microemulsion system (10). In addition, to validate the use of a microemulsion based on AOT/isopropyl myristate for transdermal application; the dermal toxicity of such a system was evaluated. We carried out light microscopy investigations, irritancy test, and oxidative stress on mouse skin before and after the treatment with microemulsion. It could easily be discerned that a microemulsion of AOT/water/isopropyl myristate is an effective carrier of tetracaine hydrochloride for managing local pain in rats. However, the local analgesic response of the drug depended on the concentration of AOT and water in the microemulsion.

EXPERIMENTAL

Materials

AOT and tetracaine hydrochloride were procured from Sigma (St. Louis, MO), while isopropyl myristate came from Fluka (Buche, Switzerland). The chemicals were used without any further purification. Triple-distilled water was used in preparation of the AOT/water/tetracaine hydrochloride/isopropyl myristate system.

Preparation of Microemulsion

Microemulsion systems were prepared by dissolving AOT in isopropyl myristate in ratios of 5:95 w/w, 9:91 w/w, 13:87 w/w, and 21:79 w/w and adding to these solutions precise volumes of water containing tetracaine hydrochloride. Initially, addition of water produced slight turbidity; however, after sonication, water droplets containing tetracaine hydrochloride solubilized and became a transparent water-in-oil microemulsion system. The system remained stable over a period of several months. The concentration of the solution was characterized with respect to any of the three constituent components. The amount of solubilized water is expressed as a molar ratio of water to AOT, that is $W_0 = [H_2O]/[AOT]$. This ratio represents the number of water molecules added per molecule of AOT present in the solution, and it has been shown to be representative of the size of droplets (11).

Local Analgesic Assay

The therapeutic potential of tetracaine hydrochloride encapsulated in a microemulsion formulation based on AOT/water/isopropyl myristate was evaluated using the ''tail-flick'' method on a Techno Analgiseometer model ANAL-1 (Lucknow, India) (12). For each experiment, a group of six Wistar rats was used. The mean body weight of the animals was 150 g; they were kept in individual cages before the experiments. In each experiment, 50 μl of microemulsion system containing 16 μg of tetracaine hydrochloride was applied to the skin on an area approximately 1 cm^2 and about 1.2 cm from the root of the tail. Each rat's tail was excited by radiant heat (55°C ± 1°C) emitted by a hot nichrome wire. The nichrome wire was about 1/8 inch below the tail. Maximum current passed through the nichrome wire was in the range 3–4 amps. All the results obtained in the above experiments were checked for significance by Student *t* test, and a value of $p < .001$ was considered significant.

Histopathological Studies

Swiss albino female mice were used for finding out the dermal toxic effect of AOT microemulsion. Skin treated topically with 200 μl of AOT-based formulation was collected in 10% phosphate buffer formalin (pH = 7.4) and processed for histopathology studies. A tissue section of 3 μm was cut, stained by the routine hematoxylin/eosin method, and photographed through an Olympus light microscope (Venox-S, AH-2, Tokyo, Japan).

Irritancy Test

For the irritancy test, a single dose of 10 μl of microemulsions at various concentrations of AOT and water was applied to the left ear of the mouse, with the right ear considered a control. The development of erythema was monitored daily for 6 days using the method of Utelly and Van Abbe (13).

Estimation of Glutathione

The skin glutathione (GSH) was determined by the method of Jollow et al. (14), with 1 ml of postmitochon-

drial supernatant (PMS; 10% w/v) precipitated with 1 ml of sulfosalicylic acid (4%). The samples were kept at 4°C for 1 hr and centrifuged at 1200*g* for 15 min at 4°C. The assay mixture contained 0.1 ml of filtrate supernatant and 2.7 ml of sodium phosphate buffer (0.1 M, pH = 7.4), and 0.2 ml of dithionitrobenzoic acid (stock = 100 mM sodium phosphate buffer, pH = 7.4) was added subsequently. The development of a yellow color was monitored immediately at 412 nm on a Beckman spectrophotometer (California).

Lipid Peroxidation

The assay of microsomal lipid peroxidation was determined using the method of Wright et al. (15). The 1 ml of reaction mixture contained 0.58 ml 0.1 M phosphate buffer, 0.2 ml microsomes, 0.2 ml ascorbic acid (100 mM), and 0.02 ml ferric chloride (100 mM). The reaction mixture was incubated at 37°C in a water bath and was shaken for 1 hr. The reaction was stopped by the addition of 1 ml of thioacetic acid (10%). Following the addition of 1 ml 0.67% thiobarbituric acid, all the tubes were placed in a boiling water bath for 20 min, cooled, and then centrifuged at 2500*g* for 10 min. The amount of substances reactive to thiobarbituric acid was determined in each of the samples by measuring the optical density of the supernatant at 535 nm against a reagent as a blank. The results are expressed as nanomole MDA (malone dialdehyde) equivalent formed per hour per gram of tissue at 37°C using a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

Estimation of Catalase

The change in optical density per minute at 230 nm of the reaction mixture of 0.02 ml of microsomes, 1.9 ml 0.1 M phosphate buffer, and 1 ml hydrogen peroxide (0.34%) was measured on a Beckman spectrophotometer (16). The results are expressed in nanomoles of hydrogen peroxide consumed per milligram per minute.

RESULTS AND DISCUSSION

Analgesic Response

An increase in the response time after applying a microemulsion system containing tetracaine hydrochloride was considered an analgesic response of the drug. The mean local response time of tetracaine hydrochloride at various water concentrations in the AOT/water/isopropylmyristate microemulsion is shown in Fig. 1. The heat-sensitive response of an untreated tail was in the range 25 ± 5 sec. The lower curve of Fig. 1 depicts the analgesic response time of a topically applied aqueous solution of tetracaine hydrochloride that was considered a control, while other three curves represent the microemulsion-based formulations containing tetracaine hydrochloride. The response of the drug starts soon after the application of the microemulsion-based formulation. Maximum effect was attained after 10 min of the application and then reduced gradually. However, a marked anesthetic effect is noticeable even after 180 min of application of microemulsion systems containing tetracaine hydrochloride compared to the control. The analgesic response of an aqueous solution of tetracaine hydrochloride is in the range 27–32 sec, and it increases up to 155–158 sec for AOT/water/isopropyl myristate microemulsion at W_0 = 15 after 10 min of application of the formulation to the tail of the rat. It is also clear from Fig. 1 that the analgesic response of drug increases as the concentration of water increases in the microemulsion system. This is due to an increase in hydration of the stratum corneum as the water concentration in the AOT microemulsion is increased

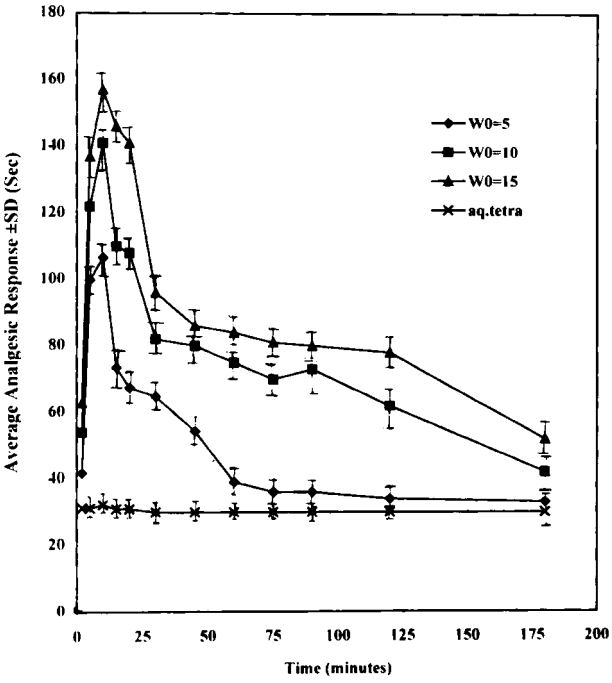

Figure 1. Analgesic action of topically applied tetracaine hydrochloride encapsulated in AOT/H_2O/isopropylmyristate microemulsion system (AOT:isopropyl myristate = 5:95 w/w) at various water contents as measured with the rat tail-flick assay.

(17–19). Furthermore, at lower W_0, most of the water molecules in the water pool of AOT microemulsion systems are bound to the surfactant (14); therefore, tetracaine hydrochloride molecules remain entrapped and are not available as free molecules as at higher W_0 for partitioning into the skin. But, as the W_0 increases, the fraction of free water molecules in AOT microemulsion systems increases, which makes the tetracaine hydrochloride free for transportation to the skin.

Figure 2 depicts the effect of AOT concentration in the AOT/water/isopropylmyristate microemulsion to the mean local analgesic response of drug at $W_0 = 15$. It shows that the local analgesic effect of tetracaine hydrochloride using a microemulsion as a carrier is always greater than that of the control. The better analgesic response of the tetracaine hydrochloride using AOT microemulsion could be explained on the basis of the anionic nature of AOT. The hydrophobic part of the AOT interacts with the keratinous proteins and induces additional anionic sites on the membrane cells, which results in the softening of the stratum corneum (20–22). On increasing the weight ratio of AOT to isopropyl myristate in microemulsion systems from 5:95 w/w to 13:87 w/w, the mean local analgesic response time increased. However, the analgesic response of drug using a microemulsion with 21:79 w/w of AOT:isopropyl myristate decreased compared to microemulsions having low concentrations of the AOT.

This is an important observation for developing an optimized formulation based on AOT microemulsion for tetracaine hydrochloride in transdermal drug delivery. The reason is not yet clear, but could be explained on the basis of random motion of the droplets in microemulsions. The droplets of microemulsions collide each other and are subjected to interactions among themselves (11). It could be assumed that, in the case of a microemulsion having an AOT:isopropyl myristate concentration of 21:79 w/w, the interdroplet collisions initially are inelastic because of the rigid structure of the interface of droplets; as a result, tetracaine hydrochloride could not come out from the droplets. With the passage of time, the microemulsion system took water from the skin, interdroplet collisions became elastic, and then drug from the droplets permeated the skin. Thus, one has to be careful to maintain the concentration of the AOT in the microemulsion to obtain the maximum analgesic response of the tetracaine hydrochloride.

Histopathology

Figure 3A represents untreated skin (control), while Fig. 3B represents skin that was treated for 48 hr with

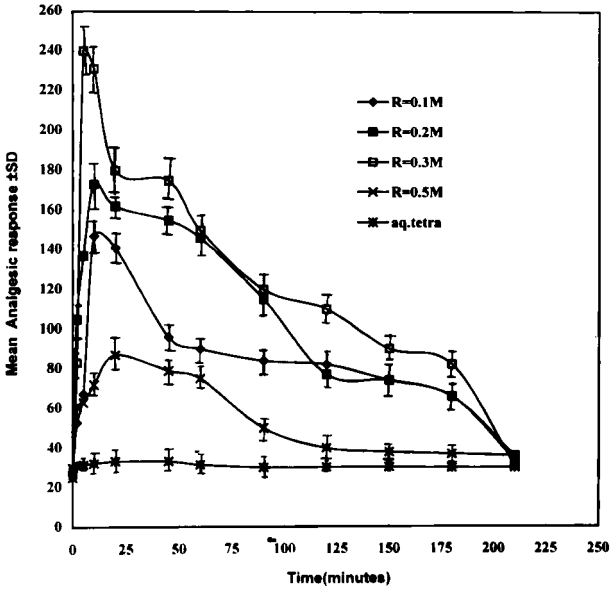

Figure 2. Analgesic action of topically applied tetracaine hydrochloride encapsulated in AOT/H_2O/isopropylmyristate microemulsion system ($W_0 = 15$) at various concentrations of AOT as measured with rat tail-flick assay.

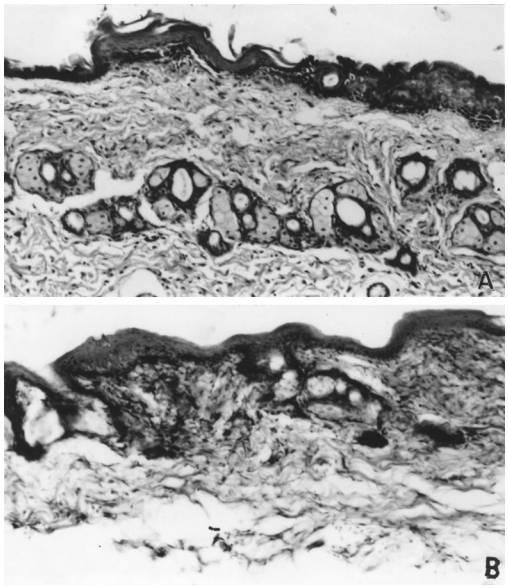

Figure 3. (A) Untreated mouse skin (control, ×400 magnification); (B) 48-hr treated with aerosol-OT/water/isopropyl myristate microemulsion (×400 magnification).

Table 1

Erythema Indices Value for AOT Microemulsion

Group (n = 6)	Systems	Dose (μl)	Erythema Indices Value[a]
1	AOT/water/IPM/tetracaine hydrochloride (AOT:IPM = 2.5:97.5 w/w)	10	0
2	AOT/water/IPM/tetracaine hydrochloride (AOT:IPM = 5:95 w/w)	10	0
3	AOT/water/IPM/tetracaine hydrochloride (AOT:IPM = 9:91 w/w)	10	1
4	AOT/water/IPM/tetracaine hydrochloride (AOT:IPM = 13:87 w/w)	10	2.5
5	AOT/water/IPM/tetracaine hydrochloride (AOT:IPM = 21:79 w/w)	10	3.3
6	AOT/water/IPM/tetracaine hydrochloride (AOT:IPM = 33:67 w/w)	10	4
7	AOT/water/IPM/tetracaine hydrochloride (AOT:IPM = 49:51 w/w)	10	8.6
8	AOT/water/IPM/tetracaine hydrochloride (AOT:IPM = 75:25 w/w)	10	12

IPM = isopropyl myristate.
[a] Mean of six experiments.

the microemulsion having a concentration of AOT at W_0 = 15 (i.e., AOT:isopropyl myristate 21:79 w/w). Compared with the control, no significant alteration was apparent with the treatment of the AOT microemulsion-based formulation to the skin. In particular, it is clear from the figure that the stratum corneum remained intact. But, compared to the control, an increase in vacuole size of the cell in the spinous layer of the epidermis is observed in treated skin.

Irritancy Test

The values of the resultant indices are shown in Table 1. As per the test of Utely and Van Abbe, if the indices

Table 2

Effect of Pretreatment of Mice with AOT/Water/Isopropyl Myristate Microemulsion, Saline, and Isopropyl Myristate on Induced Skin Glutathione, Lipid Peroxidation, and Catalase

Systems	Glutathione (mmol/g of tissue) ± SD	Lipid peroxidation (nmol MDA/hr/g of tissue) ± SD	Catalase (nmole H_2O_2 consumed/mg/min) ± SD
Saline	0.079 ± 0.0041	23.61 ± 0.11	84.55 ± 1.23
Isopropyl myristate	0.078 ± 0.005	23.52 ± 0.099	85.50 ± 1.17
AOT/water/IPM (AOT:IPM = 5:95 w/w)	0.76 ± 0.004	23.11 ± 0.098	82.31 ± 1.14
AOT/water/IPM (AOT:IPM = 21:79 w/w)	0.76 ± 0.0035	22.65 ± 0.21	88.66 ± 1.13
AOT/water/IPM (AOT:IPM = 49:51 w/w)	0.074 ± 0.003	22.87 ± 0.24	89.30 ± 1.15

Each value in the table represents the mean ± standard deviation of six experiments.
MDA = malone-dialdehyde. IPM = isopropyl myristate.

value lies between 0 and 9, it indicates that the applied dose probably would not irritate human skin (13). However, 10 μl solution of AOT:isopropyl myristate having a concentration equal to 75:25 w/w has a value of 12, indicating that this solution may be an irritant to the human skin. Thus, a AOT/water/isopropyl myristate microemulsion having a concentration of AOT in isopropyl myristate up to 49:51 w/w is safe for developing the formulation for transdermal drug delivery.

Oxidative Stress Test

Table 2 shows the glutathione, catalase, and lipid peroxidation levels of the skin treated with the formulation and with saline as a control. It is clear that there is no significant change in glutathione, catalase, and lipid peroxidation levels of the mouse skin; therefore, the AOT/water/isopropyl myristate system did not induce oxidative damage in the mouse skin.

CONCLUSION

In vivo results showed that the AOT/water/isopropyl myristate microemulsion acts as a safe transdermal carrier of the tetracaine hydrochloride. The local analgesic response time of tetracaine hydrochloride was dependent on the composition of the microemulsion. The local analgesic responses of tetracaine hydrochloride increased as the weight percentage of AOT and water increased up to a certain concentration in the microemulsion. The optimum composition for obtaining maximum analgesic response time from the transdermal delivery of the tetracaine hydrochloride in the microemulsion having a weight ratio of 9.4:6.9:83.7 w/w of AOT, water, and isopropyl myristate, respectively. The optimal formulation shows eightfold enhancement in local analgesic response of the tetracaine hydrochloride compared to the aqueous solution. Furthermore, preliminary irritation tests, histopathology, and oxidative stress studies confirm that the AOT/water/isopropyl myristate microemulsion system is a safe transdermal carrier of tetracaine hydrochloride.

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REFERENCES

1. D. F. McCaffery, A. D. Woolfsonk, H. McCelland, and V. Boston, Br. J. Anesth., 60, 64 (1988).
2. R. Worz (Ed.), *Pharmakotherapia bei Schmerz*, VCV Verlag, Weinheim, 1986.
3. M. E. Planas, P. Gonzalez, L. Rodriguez, S. Sanchez, and G. Cevc, Anesth. Analg.,75, 615 (1992).
4. W. J. Albery and J. Hadgraft, J. Pharm. Pharmacol., 31, 140 (1979).
5. A. Gesztes and M. Mezei, Anesth. Analg., 67, 1079 (1988).
6. L. Arendt-Nielsen and P. Bjerring, Anesth. Analg., 67, 115 (1988).
7. T. K. Day and A. Maitra, Adv. Colloid Interface Sci., 59, 95 (1995).
8. M. Foldvari, A. Gesztes, M. Mezei, L. Cardinal, I. Kowalczyk, and M. Behl, Drug Dev. Ind. Pharm., 19, 2499 (1993).
9. S. N. Malik, D. H. Canaham, and M. W. Gouda, J. Pharm. Sci., 64, 987 (1975).
10. D. W. Osborne, A. J. I. Ward, and K. J. O'Neill, J. Pharm. Pharmacol., 43, 451 (1990).
11. T. K. Jain, M. Varshney, and A. Maitra, J. Phys. Chem., 93, 7409 (1989).
12. F. E. D. Amour and D. L. Smith, Br. J. Pharm. Exp. Thre., 72, 74 (1941).
13. M. Utely and N. J. Van Abbe, J. Soc. Cosmet. Chem., 24, 217 (1973).
14. D. J. Jollow, R. R. Mitchell, N. Zanpaglione, and J. R. Gillete, Pharmacology, 11, 151 (1974).
15. J. R. Wright, H. D. Colbey, and P. R. Miles, Arch. Biochem. Biophys., 206, 296 (1981).
16. A. Caliborn, Catalase activity, in *CRC Handbook of Methods of Oxygen Radical Research* (R. A. Greenwood, Ed.), CRC Press, Boca Raton, FL, 1985, p. 283.
17. T. K. Ghose and A. K. Banga, Pharm. Tech., 17, 72 (1993).
18. C. Ackermann and G. L. Flynn, Int. J. Pharm., 36, 61 (1987).
19. M. Varshney, T. Khann, and M. Changez, Colloids Surf., B, 13, 1 (1999).
20. W. T. Gibson and M. R. Teall, Food Chem. Toxicol., J. Soc. Cosmet. Chem., 37, 199 (1986).
21. L. D. Rheien, C. R. Robbins, K. Fernce, and R. Cantore, J. Soc. Cosmet. Chem., 37, 125 (1986).
22. H. J. C. Blake, D. Scala, L. D. Rhein, and C. R. Robbins, J. Soc. Cosmet. Chem., 37, 199 (1986).