Antiinflammatory Activity of Tenoxicam Gel on Carrageenan-Induced Paw Oedema in Rats

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Tenoxicam is a nonsteroidal antiinflammatory drug, used in the treatment of inflammatory and degenerative disorders of the musculoskeletal system. It is from the oxicam group of nonsteroidal antiinflammatory agents. It is widely prescribed for the treatment of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, gout, extra-articular disorders, bursitis, tendonitis, and nonarticular rheumatic condition. Tenoxicam has some side effects when taken orally, viz., epigastric pain, heartburn, nausea, diarrhoea, vomiting, peptic ulcer, and hepatic impairment. The aim of this study was to formulate topical gel containing 1% of tenoxicam in 1% carbopol-940 and PEG-4000 and to evaluate it for antiinflammatory activity using carrageenan-induced paw oedema in rats. The studies were conducted on Wistar rats of either sex (160-180 g). The change in oedema volume of the rat hind paw was measured using mercury plethysmometer. The readings were measured in terms of volume displaced in millimetre using a micropipette that has mark to 10 divisions in 1 ml. The carbopol gel formulation of tenoxicam containing 15% of ethanol and 5% of sodium lauryl sulphate was significantly more effective against oedema formation than the other formulation of tenoxicam gel and compared to the marketed product of piroxicam gel. Results suggest that the 1% tenoxicam gel in carbopol-940 inhibited 52% of carrageenan-induced oedema formation as compared with the 44% inhibition obtained with marketed product of piroxicam gel.

Tenoxicam (TN)[1-5] is a thienothiazine derivative of the oxicam class of the nonsteroidal antiinflammatory drug (NSAID), which is chemically, 4-Hydroxy-2-methyl-N-2-pyridyl-2(H)-thieno[2,3-e]-l,2-thiazine-3-carboxamide-l,l-dioxide. TN exhibited potent antiinflammatory, analgesic, and antipyretic activity and is widely prescribed for the treatment of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, gout, and nonarticular rheumatic condition[5,6]. Tenoxicam has some side effects when taken orally, viz., epigastric pain, heartburn, nausea, diarrhoea, vomiting, peptic ulcer, and hepatic impairment[7]. In the present study, a 1% TN gel in 1% carbopol-940 and other gel-forming agent was evaluated for its antiinflammatory activity in rats[8-11].

MATERIALS AND METHODS

Tenoxicam (98.5 to 99.5% purity), Carbopol-940 (98.4 to 101.0% purity, and free flowing), Carrageenan were obtained as gift samples from M/s Ranbaxy Lab. Ltd., Dewas; CoreI-Pharm-Chem., Ahmedabad; and Sigma Chemicals Company, respectively. Polyethylene glycol-6000 and polyethylene glycol-200 were obtained from M/s Qualigens Fine Chemicals, Mumbai. Triethanolamine and potassium orthophosphate were obtained from Loba-Chemie, Mumbai. Methyl paraben and propyl paraben were obtained from E. Merck, Mumbai. Other chemicals used in the study were of pharmacopoeia quality (IP).

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Tenoxicam gel formulations:

The carbopol-940 and PEG gels of TN were prepared by cold method described by Schmolka[12]. Formulae of different gels of Tenoxicam formulated were as shown in Table 1. The weighed amount of carbopol was placed in a beaker, and sufficient amount of water was added to it and kept in oven at 100° for 20 min to obtain a homogenous viscous mixture and cooled to room temperature with continuous stirring. Triethanolamine was added drop-wise with constant stirring. After formation of gel, weighed amount of TN was mixed in it. The other ingredients were mixed with continuous agitation and stored at ambient temperature prior to its use. An *in vitro* diffusion study[13,14] using membrane filter showed that these formulations not only delivered a maximum permeation of TN among various formulations examined, but also exhibited desirable property and physical stability. TN was also chemically stable in these

TABLE 1: FORMULATION OF TENOXICAM GELS

Ingredients (g)	Formulations					
	F1	F2	F3	F4	F5	F6
Tenoxicam	1	1	2	3	1	3
Carbopol-940	1	1	1	1	-	-
PEG-4000	-	-	-	-	5	5
PEG-200	-	-	-	-	5	5
Ethanol	10	20	20	20	10	10
Triethanolamine	3.5	3.5	3.5	3.5	-	-
Methyl paraven	-	-	-	-	0.3	0.3
Propyl paraven	-	-	-	-	0.2	0.2
Purified water	84.5	74.5	73.5	72.5	78.5	77.5

formulations during 18-month storage at 4, 25 and 48°. The adjustments of pH of gels were necessary to achieve the maximum release rate of TN from the gels.

Antiinflammatory activity of TN:

The studies were conducted on Wistar strain rats (six per group) of either sex, weighing 180±20 g to test the antiinflammatory activity of TN gels by the carrageenan-induced paw oedema method[15-17]. The rats receiving oral doses of Tenoxicam were fasted with free access to water for 12 h prior to the test. The change in oedema volume of the rat hind paw was measured by the method described by Winter *et al.*[18], using a mercury plethysmometer. The readings were measured in terms of the volume displaced in ml using a micropipette marked to 10 divisions in 1 ml. The TN gels (0.5 g) were applied to the planter surface of the left hind paw by gently rubbing 50 times with the index finger[19, 20]. The gel base without drug was applied in control group of rats by the same mode of application. After 2 h, the dose 0.1 ml of 1% carrageenan suspension (in sterile normal saline) was injected subplantarly into treated and control group of rats. The paw volume was immediately measured by the mercury plethysmometer. The percent swelling of the paw was determined using Eqn. l – Percent swelling = (V-Vi/Vi)×100, where V is the paw volume 3 h after the carrageenan injection, and Vi is the initial paw volume. The average paw swelling in the group of the drug-treated rats was compared with control rats and the percent of inhibition of the oedema formation was determined using Eqn. 2 – Percent inhibition = [1-percent swelling of drug-treated group/percent swelling of control group]×100. The *t*-test was used to statistically compare the difference in the antiinflammatory activity (percent inhibition) observed after the application of the different doses, or formulation of TN gels were tested.

Effect of gel base on activity of TN:

To study the effect of the gel base on percutaneous absorption and antiinflammatory activity of TN, Carbopol-940 and PEG-6000 gels were prepared according to the formula shown in Table 2. The antiinflammatory activity of 1% TN gel was also compared with 1% of ibuprofen gel commercially available in the market. An equal amount of the gel was similarly applied on rat hind paws to evaluate their antiinflammatory activities.

Duration of antiinflammatory activity of TN gels:

Schrier *et al.*[21] studied the duration of antiinflammatory action of meclofenamic acid and indomethacin topical formulations in mice. This method was not suitable for evaluating the antiinflammatory activity of a drug for >10 h due to the limited duration of the inflammatory response after carrageenan injection in mice. Another method has been suggested by Chi *et al.*[22] for the determination of duration of antiinflammatory activity in rats, wherein the extended duration of antiinflammatory activity of the new gel formulation of TN was measured by applying the gel to the paw much earlier than the carrageenan injection.

Six groups, each containing six rats, were selected and 50 mg of 1% TN gel was applied on the plantar region of their left hind paw at 0, 2, 4, 6, and 12 h prior to the injection of 0.1 ml of the 1% carrageenan solution. Three hours after the carrageenan injection, the percent swelling of the paw was measured in each rat, and the average percent inhibition of oedema formation at each dosing interval was calculated using Eqn. 2. Six rats were used as control animals, and they received the gel base alone 3 h prior to the injection of carrageenan.

The 50% effective dose (ED_{50}) of TN gel:

To determine the antiinflammatory action of 1% TN gel containing different amount of drug, 1 to 3%, formulations of 50 mg, each containing 0.5, 0.1, 1.5, 2.0, and 3.0 mg of TN per kg body weight, were applied on the plantar area of the rat hind paws 3h prior to the subplantar injection of -1.0 ml of the carrageenan solution into the treated paws.

TABLE 2: PERCENT INHIBITION OF OEDEMA FORMULATION BY TENOXICAM GELS

Formulation	Dose (mg/paw)	Number of rats	Percent swelling	Percent inhibition
Control	0	5	29.25±3.25	-
F2	50	5	13.90±1.85	52.45
F4	50	5	17.10±2.65	41.50
F6	50	6	17.90±2.28	38.75
MF	50	6	16.10±2.13	44.50

MF: Marketed product

The 50% effective dose (ED_{50}), calculated as the dose required to produce 50% inhibition of the carrageenan-induced oedema formation. To compare the effectiveness of TN from the topical and oral routes, the oral ED_{50} was determined in rats of the same batch used for the topical dose. An appropriate amount of TN was suspended in physiological normal saline solution containing 0.5% tragacanth, and 1 ml of this suspension was administered in rats by animal-feeding needles prior to the carrageenan injection. One ml of 0.5% tragacanth saline solution was used as control. The oral dose administered was of 2, 4, 8, and 10 mg of TN per kg body weight. The relative equiponderal availability (REA) of the topical gel and oral ED_{50} value was calculated using Eqn.3 – REA(%) = (ED_{50} of oral TN / ED_{50} of topical TN)×100.

RESULTS AND DISCUSSION

The percent inhibition of carrageenan-induced oedema formation by 1% TN gels in carbopol-940 (Formulation F2) and PEG base (Formulation-5) are shown in Table 2. The 1% TN in carbopol base inhibited 61.4% of the carrageenan-induced oedema formation, while the PEG base exhibited a 44.4% reduction in swelling. On the basis of these results, it can be concluded that at least 1.5 times greater antiinflammatory activity was achieved with the carbopol gel than the PEG base. The percent oedema inhibition with carbopol gel was close to maximum for a topical application. The antiinflammatory activity of 1% TN gel in carbopol-940 was compared with the other NSAID topical gel preparation of ibuprofen.

Table 3 shows the percent inhibition of the oedema formation by TN gel prepared using carbopol-940 and PEG-6000. The ibuprofen gel showed a 50.32% inhibition of oedema formation, which was similar to that previously reported[23-26]. The carbopol gel containing 1% of TN showed 62.5% inhibition of oedema. It was similar to that reported by Prakash *et al.*[27], who measured the antiinflammatory activity of TN gel and compared the antiinflammatory activity with other NSAIDs.

TABLE 3: DURATION OF ANTIINFLAMMATORY EFFECT OF 1% TENOXICAM GEL

Time (h)	Dose (mg/paw)	Number of rats	Percent swelling	Percent inhibition
Control	0	7	31.50±2.14	-
0	50	5	24.90±1.75	20.95
2	50	5	18.35±1.95	41.75
4	50	6	17.20±2.65	45.40
6	50	6	15.70±4.12	50.15
8	50	6	15.05±2.35	52.22
12	50	7	14.45±1.95	54.12
16	50	6	15.85±3.25	49.68
24	50	6	18.72±2.75	40.55
30	50	5	21.75±1.95	30.95
36	50	6	24.50±2.38	22.22

TABLE 4: ED_{50} VALUES OF 1% TENOXICAM GEL AND ORAL DOSE OF TENOXICAM

Route	Dose (mg)	Number of rats	Percent swelling	Percent inhibition	ED_{50}
Topical	0.0	6	38.50±3.32	-	1.90
	0.5	5	33.15±2.40	13.89	
	1.0	6	24.24±3.30	37.14	
	1.5	7	21.65±4.32	43.76	
	2.0	6	18.25±2.36	52.59	
	3.0	6	16.46±4.25	57.27	
Oral	0.0	6	37.25±3.38	-	7.60
	4.0	6	27.25±2.62	26.30	
	6.0	6	21.50±3.06	42.28	
	8.0	5	17.15±4.55	52.61	
	10.0	7	15.25±2.99	59.06	

In the present study, the antiinflammatory activity was determined in Formulation 2, containing 1% of TN. Table 3 shows the percent inhibition of oedema formation by TN after the topical application of 1% TN gel at various times between 0-24 h prior to the carrageenan injection. When the gel was applied on the rat paw between 2-12 h prior to the carrageenan injection, maximum inhibition was observed at 3 h after the carrageenan injection. When the gel was applied 6 or 8 h prior to the carrageenan injection, marked reduction in inhibition was observed. These results clearly indicate the prolonged antiinflammatory activity of 1% TN gel prepared.

Table 3 shows the percent swelling of the rat paw measured 3 h after the injection of the carrageenan solution and the percent inhibition of swelling by the application of 1% TN gel in doses of 0, 0.5, 1, and 3 mg of TN per kg body weight 3 h prior to the injection of carrageenan. Table 4 exhibits the antiinflammatory activity of TN after oral administration in doses of 0, 2, 4, 8, and 10 mg per kg body weight in rats. The topical ED_{50} of 1.90 mg/kg and oral ED_{50} of 7.6 mg/kg of TN gave an equiponderal availability of 4% for 1% TN gel calculated by the Eqn. 3. It was found that the equiponderal availability would be a better indication of the efficacy of the topical antiinflammatory application as compared to oral activity preparation.

REFERENCES

1. Davis, R. and Brogden, R.N., **Drugs,** 1994, 48, 431
2. Magni, E**., Drugs,** 1993, 46 (Suppl. 1), 10
3. Bevilacqua, M. and Magni, E., **Drugs,** 1993, 46, 40
4. Singh, S.. Sharda, N. and MahaJan, L., **Int. J. Pharm.,** 1999, 176, 261.

5. Vavia, P. R. and Adhage, N. A., **Drug Develop. Ind. Pharm.,** 1999, 25, 543.
6. Velpandian, T., Mathur, P. and Sengupta, S., **Phrmacology,** 1998, 56, 137.
7. Perpigana, G., Bogliodo, A. and Puccetti, L. **Int. J. Clin. Pharmacol. Res.,** 1994, 14, 230.
8. Heintz, R.C., **Drug Saf.**, 1995, 12, 110.
9. Slaven. G.M., Walter, R.J., Zacharias, M., Fawcett. J.P., Hodgron, B.F. and Aust. N.Z. J**. Med.**,1998,28,772.
10. Ulugol, A., Unalan,H., Dokmeci, F. and Geisslinger, **G.. Drug Metab. Dispor.,** 1998, 24, 1107.
11. Cordero, J.A., Alarcon, I., Escribano, E., Obach, R. and Domenech, J., **J. Pharm. Sci,** 1997,86, 1054.
12. Schmolka, I.S., **J. Biomed. Maater. Res.,** 1972, 6, 571.
13. Chi, S.C. and Jun, H.W, **J. Pharm. Sc.,** 1991, 80, 280.
14. Morimono, Y., **Therapeutic Rec.,** 1990, 11, 662.
15. Sproviera, S., Scala, G., Ferrara, A.M., Raucci, G. and AItucci, P., **Recerti Kaucci, Prog. Med..** 1992,83,567.
16. Itzkouitch, D., Ginsperoz, F., Icon, M., Bernard, V. and Appelboom, T., **Clin. Rheumatol,** 1996, 15,604.
17. Roleofse, J.A., Vander. B.P. and Joubert, J.J., **Anesth. Prog.,** 1996, 43, 103.
18. Winter,C.A., Risley, E.A. and Nuss, G.W., **Proc. Soc. Exp. Biol. Med.,** 1962, 111, 544.
19. Sarcerdote, P. and Panercci, A.E., **Int. J. Tissue React.,** 1993, 15, 175.
20. Troconiz, I.F., Lopez-Bustamante, L.G. and Fos, D.,, **J. Pharm. Sci.,** 1995, 84, 1482.
21. Schier, D.S., Moniot, S., Gluckman, M.I. and Gilbertsen, R.B., **J. Pharm. Pharmacol**, 1986,39,57.
22. Chi, S.C. and Jun, H.W., **J. Pharm. Sci.,** 1990, 79, 974.
23. Walker, J.L., Bak, A., McKnight, W., Asfaha, H., Sharkey, K.A., and McNaughton, W.K., **Gastrointesology,** 1998, 115, 101.
24. Wallaces, J.L., Chaapman, K., and McKnight. W, **Brit. J. Phrmacol.,** 1999, 126, 1200.
25. Sengupta, S., Velpandian, T., Sapra, P., Mathur, P. and Gupta, S.K., **Skin Pharmacol. Appl. Skin Physiol.,** 1998, 11, 273.
26. Harad, Y., Kawamura, M., Hataraka. K., Saito, M., Ogino, M-, Ohno, T., Ogino, K. and Yang, Q., **Prostaglandins Other Lipid Medial..** 1098, 55, 345.
27. Prakash, J., Gupta, S.K., Awar, L., Joshi, S., Velpandian, T. and Sengupta. S., **Inflam. R. Res.,** 1996,45,580.

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