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LOCAL TISSUE TOLERABILITY OF MELOXICAM, A NEW NSAID: INDICATIONS FOR PARENTERAL, DERMAL AND MUCOSAL ADMINISTRATION

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SUMMARY

Meloxicam is a new non-steroidal anti-inflammatory drug (NSAID) which has potent anti-arthritic activity and a reduced potential to induce gastric irritation in animals. The present series of animal studies investigated the local and/or systemic tolerance of meloxicam formulations: intravenous, intramuscular and subcutaneous injections, eye-drops, gel and suppositories. The concentration and formulations were as intended for therapeutic use in man. An *in vitro* haemolysis test demonstrated that the parenteral formulation of meloxicam produced only minimal haemolysis. In comparison, NSAIDs such as piroxicam, ketoprofen and indomethacin showed comparable haemolysis only after dilution. Diclofenac and ibuprofen caused considerable haemolysis even when diluted. In all studies, the local tolerance of meloxicam was good and did not differ from placebo, even when administered daily for 4 weeks. Few abnormal histopathological findings indicative of organ toxicity were observed. There were only small, transient macroscopic changes at the site of administration, with no striking histopathological changes directly attributable to meloxicam. Intramuscular piroxicam and diclofenac, however, resulted in development of an extensive, solitary necrotic area. Other formulations tested were also very well tolerated. In conclusion, all meloxicam formulations tested exhibited excellent tissue tolerability. Therefore, meloxicam appears to be suitable for parenteral, dermal and mucosal administration.

KEY WORDS: Meloxicam, Local tolerance, Eye-drops, Suppositories, Injection, Gel.

NON-STEROIDAL anti-inflammatory drugs (NSAIDs) are commonly used for the treatment of inflammation and pain associated with chronic conditions such as rheumatoid arthritis and osteoarthritis. Long-term administration of the currently available NSAIDs is predominantly via the oral route because there is a high incidence of local intolerance reactions and other adverse effects when these drugs are administered parenterally, even in the short term [1, 2].

Meloxicam is a new NSAID that has excellent oral efficacy and is well tolerated, having a low incidence of gastrointestinal side-effects [3–5]. However, given that it would be advantageous to be able to administer meloxicam by additional routes, the local tolerability of parenteral, dermal and mucosal formulations of meloxicam was investigated using animal models.

Local irritation should not be caused by either the active material or its vehicle following parenteral administration. Previous studies have shown that the ability of a substance to cause haemolysis of red blood cells is predictive of local tissue irritancy [6–8]. Therefore, prior to investigating the *in vivo* tolerance of meloxicam in animals, *in vitro* haemolysis tests were performed. Subsequently, the local tolerance of meloxicam was investigated following intravenous (i.v.), intramuscular (i.m.) and subcutaneous (s.c.) injection. Furthermore, the mucosal and dermal tolerance of meloxicam formulated as eye-drops, gel and suppositories were assessed. In all studies, the concentration and formulation of meloxicam used were those intended for therapeutic use in man. This paper is concerned only with local tissue tolerability of meloxicam; information about its general toxicologic profile will be reviewed elsewhere.

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MATERIALS AND METHODS

All studies were conducted in accordance with the Principles of Good Laboratory Practice.

Pharmaceutical development of a meloxicam solution for parenteral administration

Meloxicam (UH-AC 62 XX) has a molecular weight of 351.4 and is chemically very stable. It is non-hygroscopic. Furthermore, meloxicam is not sensitive to light.

The aqueous solubility of meloxicam is poor. Its solubility is pH dependent with a minimum at pH 4 and increasing with increasing pH. In order to obtain clinically relevant concentrations of 10–20 mg/ml, a pH of at least 8 was needed. This was considered to be inappropriate for an assessment of i.v. tolerability. However, solutions of sufficient physical stability for preclinical studies were yielded when a salt was formed *in situ* by adding molar or slightly hypermolar amounts of an appropriate base combined with an appropriate excipient to prevent hydrolysis. For the final formulation of meloxicam (15 mg/1.5 ml) for parenteral administration, meglumine was selected as the base for salt formation *in situ*. The excipients used to prevent hydrolysis were glycofurol and pluronic F68. An additional function of these co-solvents was to solubilize minimal amounts of the hydrolysed form of meloxicam.

The pH of the final solution formulation was 8.7.. To prevent a decrease of pH the solution was slightly buffered with glycine/sodium hydroxide. If the pH decreases below 7.8, solubility in terms of a clear solution becomes critical. Sodium chloride was present to adjust isotonicity.

In vitro haemolysis test

The haemolytic potential of the meloxicam formulation (15 mg/1.5 ml) for parenteral administration was compared with the standard commercial preparations of piroxicam (Felden® 20, Pfizer-Mack, 20 mg/ml), diclofenac (Voltaren®, Ciba Geigy, 75 mg/3 ml), ibuprofen (Imbun®, Merckle, 234 mg/3 ml), ketoprofen (Orudis®, Rhone-Poulenc, 100 mg/2 ml) and indomethacin (Vonum®, Econerica Chiesi, 50 mg/2 ml).

The test preparations (0.5 ml) were mixed with 0.5 ml citrated human blood (1 vol 3.8% sodium citrate:9 vol blood) and incubated at 37°C for 45 min. After centrifugation (1000 g for 5 min), the supernatants produced were assessed visually in comparison with 0.9% saline control solution. If there was no discoloration of the supernatant, the degree of haemolysis was defined as <2%. If the supernatant was reddish in colour, the degree of haemolysis was determined photometrically (absorbance at 578 nm). If the visible colouration indicated >10% haemolysis, the test preparation was diluted with 0.9% saline solution in the ratio 1:1 or 1:3 and tested again.

Injection tolerance—i.v. tolerance

Study in rats. Ten Chbb:THOM rats (weight range 200–250 g) of each sex per group were administered daily doses of 0.2, 0.4, 0.8 and 1.6 mg/kg meloxicam or i.v. placebo (control) for 4 weeks. A further 10 animals per sex were included in the control and high-dose groups and followed up for 8 weeks after the end of treatment.

Study in guinea-pigs. The spectrum of side effects or organotoxic effects of meloxicam were investigated in 30 Yucatan guinea-pigs (15 male, 15 female) weighing 15.5–25.8 kg. Three animals of each sex per group received daily doses either i.v. placebo (control) or meloxicam 1, 3 or 9 mg/kg for 4 weeks. A further three animals were included in the high-dose group and followed up for an additional 6-week recovery period.

Investigations. Standard laboratory safety parameters were recorded in both rats and guinea-pigs. At the end of the study animals were subjected to a full post-mortem examination. Macroscopic inspection of organs and tissues was followed by a full histopathological examination by light microscopy.

Injection tolerance—i.m. tolerance

Eighteen Chbb:HM rabbits (nine male, nine female) were divided into three groups, each consisting of three males and three females. Each group received a single i.m. injection of either a placebo solution, saline solution or meloxicam solution (5 mg in 0.5 ml) administered as a bolus of 0.5 ml into the vastus muscle of the right femur. One male and one female from each group was killed on days 2, 5 and 8 following treatment. Blood samples for determination of creatinine kinase (CK) were obtained from the marginal ear vein prior to termination. The appropriate muscle areas were examined macroscopically and then histologically.

In a second study, the tolerance of meloxicam (5 mg) was compared with piroxicam (Felden® 20; 6.6 mg) and diclofenac (Voltaren®; 25 mg) following a single i.m. injection in rabbits. Twelve Chbb:HM rabbits (six male, six female) received a single i.m. injection of the test compound or placebo (1 ml of 0.9% saline solution). Since not only the preparation but the injected volume *per se* can provoke intramuscular changes, the three NSAIDs tested were injected according to their relative volume ratio in humans (meloxicam 0.5 ml, piroxicam 0.33 ml, diclofenac 1 ml). Two rabbits (one male, one female) from each group were killed on days 2, 5 and 7, the injection sites dissected and examined histologically.

Injection tolerance—s.c. tolerance

To evaluate the s.c. tolerance of meloxicam, six Chbb:HM rabbits (three male, three female) received a single s.c. injection of meloxicam (5 mg in 0.5 ml) and 0.9% saline solution (0.5 ml) under the left and right dorsal skin respectively. One male and one female rabbit were killed and exsanguinated on days 2, 5 and 7 following treatment. The injection sites were examined and the skin and s.c. tissue were assessed histologically.

Mucosal and dermal tolerance

Eye-drops. The local ocular tolerance of meloxicam eye-drops administered for 4 weeks was examined in 44 (22 male, 22 female) pigmented Himalayan rabbits. The animals were randomly divided into four treatment groups: (1) 12 rabbits (six male, six female) received placebo eye-drops (50 µl), (2) 10 rabbits (five male, five female) received 25 µl 0.1% meloxicam eye-drops, (3) 10 rabbits (five male, five female) received 50 µl 0.1% meloxicam eye-drops and (4) 12 rabbits (six male, six female) received 50 µl 0.3% meloxicam eye-drops. All eye-drops were instilled into the conjunctival sac of the right eye, six times daily, at intervals of 45 min, for a period of 4 weeks. The left eye of each animal remained untreated. At the end of the 4-week treatment period, one male and one female from both the placebo group and the 0.3% meloxicam group were assigned to a 4-week recovery period. Local tolerance was assessed by ophthalmoscopy (ophthalmoscope and slit lamp) and ophthalmopathology.

Gel. The dermal tolerance of a 1% gel formulation of meloxicam (5 mg in 0.5 ml) was assessed by comparing the local reaction of clipped and depilated dorsal rabbit skin to placebo after daily occluded application for 4 weeks. The local skin reaction was scored using the Draize (1944) scoring system [9] ~1 and ~4 h after removal of the occlusive dressing. Twenty-four hours after the last gel application, the rabbits were killed, any macroscopic organ and tissue changes recorded and histological examination performed.

Suppositories. The rectal tolerance of meloxicam or placebo suppositories administered once daily for 4

TABLE I
In vitro haemolysis due to meloxicam and other NSAIDS

	Degree of haemolysis (% total saponin haemolysis)					
Dilution ratio	Meloxicam	Piroxicam	Ketoprofen	Indomethacin	Diclofenac	Ibuprofen
Undiluted	<2	>10	>10	>10	>10	>10
1:1	–	>10	>10	>10	>10	>10
1:3	–	<2	<2	<2	100	88.4

TABLE II
Abnormal histopathological findings observed at the injection site in rats (i.v.)

	No. of abnormal findings									
	Placebo		Meloxicam 0.2 mg/kg		Meloxicam 0.4 mg/kg		Meloxicam 0.8 mg/kg		Meloxicam 1.6 mg/kg	
Findings	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
No. examined	10	10	10	10	10	10	10	10	10	10
Scarring	8	7	7	8	7	7	7	6	5	11
Haemorrhage	3	5	2	2	2	2	3	1	5	5
Traumatic	1	–	–	1	–	–	1	–	–	–
Cell infiltration	–	–	–	–	1	–	–	1	–	–
Thrombus	–	–	–	–	–	1	1	–	–	2
Stenosis	–	–	–	–	–	–	2	–	–	–
Fibrosis	–	–	–	–	–	–	1	–	–	–
Necrosis	–	–	–	–	–	1	–	–	–	–

weeks to 12 Chbb:NZW rabbits (three male and three female in each treatment group) was studied. Each meloxicam suppository contained 8.76 mg of active drug. This formulation corresponded to that intended for therapeutic use in man. At the end of the 4-week treatment period, all animals were subjected to macroscopic examination at autopsy and samples of rectum and anus were examined histologically.

RESULTS

In vitro haemolysis test

Meloxicam (15 mg/1.5 ml) caused <2% haemolysis as indicated by lack of supernatant discoloration upon visual assessment. In contrast, the supernatant of undiluted and 1:1 diluted (with 0.9% sodium chloride) preparations of piroxicam, ketoprofen and indomethacin were considerably haemolysed (Table I). Only when these drugs were diluted 1:3 with 0.9% sodium chloride did the resulting supernatant appear to have no discolouration. For diclofenac and ibuprofen, even a 1:3 dilution caused haemolysis (measured photometrically) of 100 and 88.4% respectively.

Injection tolerance—i.v. tolerance

Study in rats. At the injection site, haemorrhage and scarring were the most common findings observed. However, the incidence was similar between treated and control groups, suggesting the local injection tolerance of meloxicam was very good (Table II). Post-mortem examination revealed histological changes in the stomach and kidney that were characteristic of NSAID toxicity. In the stomach, erosions (3/10 males, 2/10 females) and ulcers (1/10 male, 1/10 female) were evident in the high-dose (1.6 mg/kg) group. Small erosions were also observed in 2/10 males of the 0.4 mg/kg group. No lesions characteristic of NSAID toxicity were found in the 0.8 or 0.2 mg/kg treatment groups. Various abnormal findings were also found in the kidney. These did not appear to be dose related and, indeed, were most commonly observed in females of the control group. In animals assigned to the recovery period, there were no striking differences observed between those receiving high-dose meloxicam and those receiving placebo.

TABLE III
Macroscopic findings at injection site (i.m. injection)

	Day 2	Day 5	Day 8
Placebo	Focal haemorrhage or normal	Focal erythema or normal	Slight erythema or normal
Meloxicam	Focal haemorrhage	Normal	Normal
Saline	Focal haemorrhage or normal	Focal erythema or normal	Normal

Study in guinea-pigs. There was no evidence of local injection site intolerance or systemic drug-related toxicity to daily i.v. administration of 1, 3 or 9 mg/kg meloxicam for 4 weeks. No evidence of delayed toxicity of local reaction was evident in animals retained free of treatment for 6 weeks.

Injection tolerance—i.m. tolerance

Study 1. There were no changes in CK activity in either the control groups or those receiving meloxicam. Focal haemorrhages were macroscopically evident in all treatment groups on day 2 after injection. Erythema in

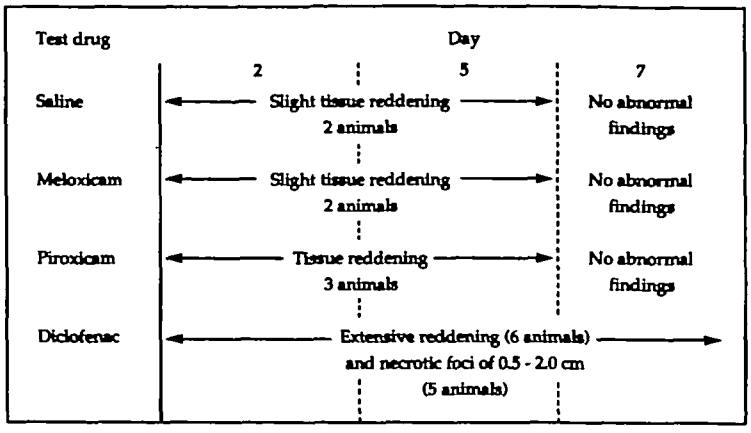

FIG. 1.—Macroscopic examination of injection site.

the area of the injection site was seen on day 5 in both the placebo and saline-treated rabbits and on day 8 in the rabbit from the placebo group (Table III). Histological examination revealed traumatic muscle lesions at the site of injection with subsequent healing. There was slight focal epithelial necrosis of the skin, focal to disseminated necrosis of muscle fibres and slight haemorrhage in all groups. There were signs of regeneration with increasing time; decomposition of the debris by macrophages and granulocytes, presence of myoblasts and immature muscle cells. Muscle regeneration appeared to be nearly complete by day 8. Therefore, there were no differences between control groups and meloxicam-treated animals with respect to macroscopic or microscopic findings. No differences between the groups were observed with respect to stimulation of CK. The muscle lesions were considered to be traumatic in origin and healed rapidly.

Study 2. There were no clinical signs or abnormal alterations or masses observed at the site of injection for meloxicam (Fig. 1). Macroscopic examination revealed only slight tissue reddening on days 2 and 5, but not on day 7, following meloxicam or saline injection. Piroxicam administration produced tissue reddening on days 2 and 5, whereas on day 7 no abnormal findings were detected. In contrast, i.m. injection of diclofenac resulted in extensive reddening and necrotic foci of 0.5–2 cm diameter, which were still visible on day 7. Histopathological examination revealed small disseminated foci of necrotic muscle cells in rabbits treated with i.m. meloxicam (Fig. 2). In comparison, i.m. administration of piroxicam or diclofenac (Fig. 3) resulted in the development of an extensive, solitary necrotic area in the muscle tissue.

Injection tolerance—s.c. tolerance

Subcutaneous injection of meloxicam (5 mg in 0.5 ml) in rabbits resulted in minor macroscopic and microscopic changes. These included partly spotted reddening (3 cm diameter) in the musculature, disseminated small focal haemorrhage in subcutis, necrosis and degradation of single myofibres of cutaneous muscle and slight inflammatory changes. A similar spectrum of findings were evident for saline control sites indicating that meloxicam is well tolerated when administered s.c.

Mucosal and dermal tolerance

Eye-drops. No ocular changes indicative of poor ocular tolerance were evident following 4 weeks of treatment with meloxicam (50 µl, doses up to 0.3% meloxicam).

Gel. No drug-induced changes of in-life parameters were observed. With the exception of one female rabbit receiving placebo, no necrotic or atrophic changes of the epidermis, particularly of the stratum germinativum, were detected histologically. The hairs between the bulb and the stratum corneum of the epidermis were also unchanged. No degenerative or inflammatory processes occurred in the corium or the subcutis.

Suppositories. Histological examination revealed only slight and non-specific inflammatory changes in the anus and rectum of rabbits in both groups. These changes were characteristic of the animal species following administration of suppositories. No drug-associated changes were observed.

DISCUSSION

Parenteral administration of NSAIDs for the short-term relief of acute pain is increasing, but remains unsuitable for the long-term treatment of rheumatic conditions. Compared to the oral route of administration, injectable forms of NSAIDs may increase compliance, act more rapidly by reaching peak concentrations more quickly, and may increase tolerability by avoidance of direct contact of the drug with the gastric mucosa. Rarely, injectable intramuscular formulations may allow treatment of patients in whom the administration of oral or rectal formulations is not possible. However, the local tissue tolerance of the currently available NSAIDs is very limited following parenteral injection. A high incidence

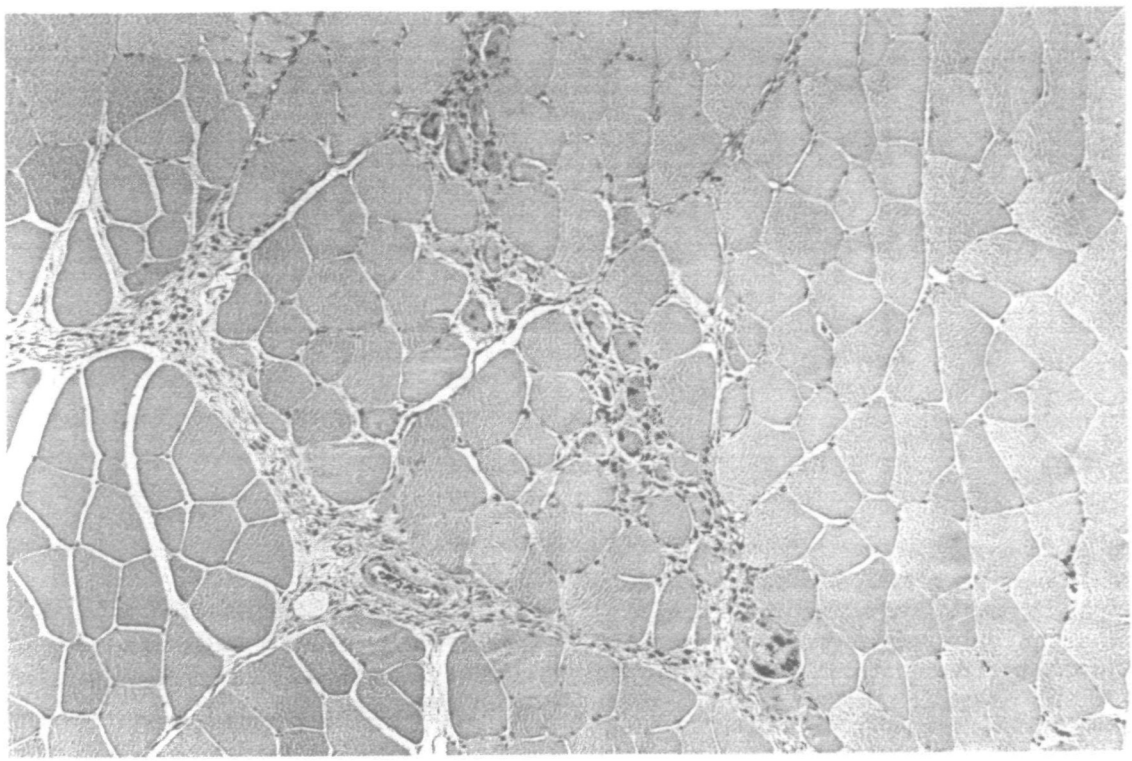

FIG. 2.—Seven days after i.m. administration of meloxicam. Disseminated foci of necrotic muscle cells.

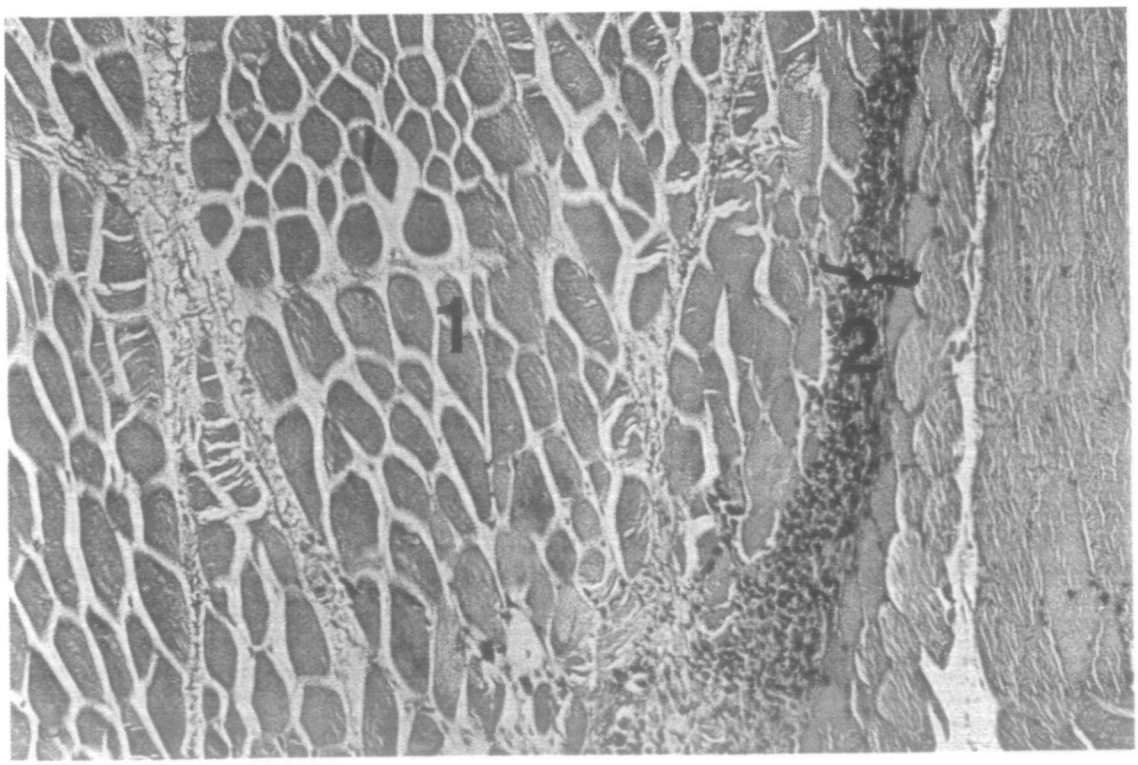

FIG. 3.—Seven days after i.m. administration of diclofenac. Development of an extensive, solitary necrotic area (1), with peripheral zone of disintegration (2).

of local side-effects has led the German regulatory authorities to allow only a single, initial parenteral administration of a NSAID. As a consequence of the excellent therapeutic profile of meloxicam in rats, the local tolerance of non-enteral administration of meloxicam formulations has been investigated in the present series of studies.

The *in vitro* haemolysis test is a model to assess i.v. use of a drug. This test does not really reflect the clinical situation of an i.v. injection since the administered injection solution is quickly diluted by blood. However, it was demonstrated clearly that the parenteral formulation of meloxicam intended for therapeutic use caused less haemolysis than all other NSAIDs tested. The relevance of the *in vitro* haemolysis test for i.m. use is under discussion. Studies have described the use of *in vitro* haemolysis tests to predict i.m. irritancy of a formulation [6, 8]. The *in vivo* i.v. tolerance studies described here showed no drug-related clinical signs in the general condition of rats and guinea-pigs. Histopathological findings at the site of injection were not related to administration and dosage of meloxicam. Thus, the drug appears to cause no local intolerance.

It is widely believed that serum levels of CK increase as a consequence of skeletal muscle injury and are a very reliable index of tissue damage [10, 11]. In the present study i.m. injection of meloxicam in rabbits produced no changes in CK activity and less severe pathological changes than either piroxicam or diclofenac. Histopathological examination revealed small disseminated regions of necrosis following i.m. injection of meloxicam. Consequently, phagocytosis of dead myocytes, with subsequent myofibre regeneration, could lead to an almost complete muscle regeneration within a short time. There was no clear histopathological difference between piroxicam and diclofenac despite a 3-fold higher injection volume with diclofenac. Moreover, the muscle changes produced by meloxicam were much milder compared with piroxicam, despite a greater injected volume. Both piroxicam and diclofenac administration resulted in the development of large areas of necrosis in muscle tissue. Since the phagocytic phase of healing by macrophages occurs faster when there are small disseminated foci of muscle necrosis rather than single large areas, phagocytic degradation and regeneration of the musculature can be completed much earlier with meloxicam than with piroxicam or diclofenac. Because of the relatively mild alterations in the musculature and the potential for rapid regeneration, the i.m. tolerance of meloxicam was superior to piroxicam and diclofenac and meloxicam was considered to be well tolerated.

Previous studies of the single and multiple instillation for 3 days of meloxicam (100 µl of 0.1%, 0.3% and 0.5%) or placebo eye-drops into the conjunctival sac of rabbit eyes resulted in no macroscopic changes in any part of the eyes (data on file, Boehringer Ingelheim). This indicated that meloxicam eye-drops were well tolerated locally. Another study has also demonstrated that instillation of meloxicam eye-drops (100 µl of a 0.01%, 0.1% and 0.3% solution) into the conjunctival sac of rabbit eyes had no local anaesthetic effect (data on file, Boehringer Ingelheim.). The present study confirmed these observations by histopathological examination. No drug-related pathological changes were detected.

No appreciable drug-related changes were observed in animals treated with either meloxicam gel or suppositories, indicating that these formulations are well tolerated and suitable for mucosal administration.

CONCLUSIONS

As a component of the full safety assessment of the new NSAID meloxicam, the local tolerance of this enolic acid compound was examined following i.v., i.m. and s.c. injection in a range of animal models. The rectal, dermal and conjunctival tolerance of meloxicam was also investigated. In all studies, the formulations of meloxicam that are intended for therapeutic use in man were used. The results of these tissue tolerability assessments, and of the *in vitro* haemolysis tests, indicated that the formulations tested exhibited excellent tissue tolerance. Meloxicam therefore appears to be an NSAID that will also be suitable for parenteral, dermal or mucosal administration.

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