J. Pharm. Pharmacol. 1994, 46: 78–79
Communicated July 30, 1993

Letters to the Editor

Evaluation of rectal mucosal irritation in rabbits after sub-chronic administration of lecithin-containing suppositories

C. DE MUYNCK, C. CUVELIER*, J. P. REMON, *Laboratory of Pharmaceutical Technology, University of Gent, Harelbekestraat 72, 9000 Gent, Belgium, and *N. Goormaghtigh Institute of Pathological Anatomy, University Hospital, De Pintelaan 185, 9000 Gent, Belgium*

Previous studies have shown that triglyceride suppositories can induce rectal mucosa damage after single (van Hoogdalem et al 1990) and sub-chronic administration (De Muynck et al 1991). After sub-chronic administration it was also shown that monoglycerides and a fatty acid-fatty acid methyl ester blend reduced this irritative effect.

Lecithin is a surface active agent used in suppository formulations to improve rectal bioavailability of drugs, to obtain sustained release suppositories, or to modify the physicochemical characteristics of the suppository base. We have studied the effects of the addition of lecithin to a triglyceride suppository, on the rectal epithelium and mucosa of rabbits.

A non-lauric triglyceride (Mesuro PS, Vandemoortele N.V., Izegem, Belgium) mainly consisting of C16-C18 saturated and unsaturated glyceride fatty acids as triglycerides (97·1%, w/w) and diglycerides (2·9%, w/w) was used. To this triglyceride, 1% soybean lecithin (Vandemoortele N.V., Izegem, Belgium) was added. The most important polar components in lecithin are the phospholipids. The phospholipid composition (45%, w/w of the lecithin composition) of the soybean lecithin was 10% phosphatidyl choline, 9% phosphatidyl ethanolamine, 7% phosphatidyl inositol and 19% phosphatidic acids. From this mixture 1 g suppositories were prepared.

To male New Zealand albino rabbits, 2·5 kg, with free access to water and food, three suppositories were administered daily at 8, 16 and 24 h for fourteen days. To ensure a 30-min contact time between the base and the rectal mucosa, the anus was kept shut over this period with a clothes-peg. The lecithin suppository formulation was tested on eleven rabbits. Tissue processing, evaluation of rectal irritation and statistical evaluation were as described previously (De Muynck et al 1991). Statistical analysis was performed on the total scores and on the values obtained for hyperaemia, oedema and granulocytes. The presence of erosion, ulceration, polyps and regeneration was evaluated separately. Two nonparametric tests were used: the Kruskal-Wallis test, when more than two groups were compared and the median test when two groups were compared. A probability value of $P<0·05$ was considered to be statistically significant.

The results were evaluated against previously obtained results (De Muynck et al 1991) for two control groups (where no clothes-pegs and no suppositories were applied or where only clothes-pegs were used), placebos (non-melting parrafin suppositories) and suppositories without added triglycerides. Next, the results were compared with less irritating suppository bases where the triglyceride was additioned with 5% monoglycerides or with a mixture of 9% fatty acids and 1% fatty acid methyl esters. The mean total scores for each suppository base, control group and placebo are shown in Table 1.

The percentage of rabbits with positive scores for each pathological feature are shown in Table 2.

Hyperaemia was absent in all rabbits to which lecithin-containing suppositories were applied. Granulocytic infiltration or the presence of inflammatory cells in the lamina propria was only focally present in the lecithin-containing preparation, in all but one preparation. The suppository preparations to which lecithin was added showed a high incidence of surface injuries. Erosion, regeneration and polyp formation were observed in 72·7% of the histological sections; ulceration was found in 63·6%. This incidence was much higher than after Mesuro PS administration. For the scores obtained for erosion, ulceration, regeneration and polyp formation the lecithin-containing suppository base was significantly different from all other groups included in this study. Mesuro PS without additives was not significantly different only for polyp formation ($P=0·30$). A statistical evaluation of the total scores led to the conclusion that with the scoring system used, rectal irritation induced by Mesuro PS without additives was not significantly different from the lecithin-containing preparation ($P=0·12$). The total scores for the two control groups, the placebo group and the Mesuro PS formulations with monoglycerides or with a mixture of fatty acids and fatty acid methyl esters were significantly lower than the lecithin-containing Mesuro PS formulation.

From our experiments it seems that although lecithin has been used for several purposes in suppository formulations, care should be taken when the formulation is to be used chronically. The concentration in which lecithin was used in this study (1%) is low when compared with the concentrations used in some studies (up to 30%) with sustained release suppository formulations (Nakajima et al 1988a, b). Further experiments should be

Table 1. The mean total scores ± s.d. for each group tested.

Evaluated suppository formulation or group	Mean total score
Control group without clothes-peg	0·35 ± 0·06
Control group with clothes-peg	0·40 ± 0·90
Placebo	4·75 ± 1·21
Mesuro PS	13·80 ± 8·00
Mesuro PS + 1% lecithin	15·62 ± 7·85
Mesuro PS + 5% monoglyceride	7·00 ± 7·10
Mesuro PS + 9% fatty acid + 1% fatty acid methyl ester	3·90 ± 6·80

n = 20 except for lecithin where n = 11.

Table 2. Percentage positive scoring rabbits after administration of the placebo, Mesuro PS and Mesuro PS + 1% lecithin suppositories.

Pathological characteristic	Placebo	Mesuro PS	Mesuro PS + 1% lecithin
Hyperaemia	5	30	0
Oedema	5	50	73
Granulocytes	30	80	91
Erosion	10	15	73
Ulceration	10	20	64
Regeneration	15	20	73
Pseudopolyps	5	50	73

Correspondence: J. P. Remon, Laboratory of Pharmaceutical Technology, University of Gent, Harelbekestraat 72, 9000 Gent, Belgium.

undertaken to determine the influence of a high concentration of lecithin on the irritative effect. Whether the improved rectal bioavailability of some drugs when using lecithin is related to the rectal mucosa damage is not clear, although van Hoogdalem et al (1990) in an evaluation of the topical effects of some absorption enhancing agents (octanoate, glyceryl-1-monodecanoate and decanoate) on the rectal mucosa, could not find a direct relationship between cefoxitin bioavailability and the extent of damage.

In summary, this study demonstrated that frequent application of triglyceride suppositories induced severe rectal mucosal damage resulting in erosion, ulceration and regeneration of the rectal mucosa; no hyperaemia was seen in the lecithin group while erosion, ulceration and regeneration occurred more frequently.

References

De Muynck, C., Cuvelier, C., Van Steenkiste, D., Bonnarens, L., Remon, J. P. (1991) Rectal mucosa damage in rabbits after subchronical application of suppository bases. Pharm. Res. 8: 945–949

Nakajima, T., Takashima, Y., Furuya, A., Ozawa, Y., Kawashima, Y. (1988a) Study on slow-release of indomethacin from suppositories containing hydrogenated soybean lecithin. Chem. Pharm. Bull. 36: 3696–3701

Nakajima, T., Takashima, Y., Furuya, A., Ozawa, Y., Kawashima, Y. (1988b) Study on absorption of indomethacin from sustained-release suppositories containing hydrogenated soybean lecithin in rabbits. Chem. Pharm. Bull. 37: 3145–3147

van Hoogdalem, E. J., Vermeij-Keers, C., de Boer, A. G., Breimer, D. D. (1990) Topical effects of absorption enhancing agents on the rectal mucosa of rats in vivo. J. Pharm. Sci. 79: 866–870

J. Pharm. Pharmacol. 1994, 46: 79–80
Communicated April 30, 1993

The effects of a single dose of morphine on the concentration of substance P-like immunoreactivity in rat and guinea-pig brain

LORIS A. CHAHL, J. S. CHAHL, *Faculty of Medicine, University of Newcastle, Newcastle, NSW 2308, Australia*

Dependence in animals and man occurs following a single injection of morphine and other opioids, manifested as a withdrawal response on rapid removal of the opioid, or on administration of an opioid antagonist. Substance P is one of the neurotransmitters that plays a major role in the opioid withdrawal response in the isolated ileum (Chahl 1983) and central nervous system (CNS) (Johnston & Chahl 1991) of the guinea-pig. Therefore, it might be expected that substance P would be released from certain brain regions on opioid withdrawal. Contrary to expectation, however, it was found in in-vitro experiments that morphine itself produced release of substance P-like immunoreactivity (SP-LI) from thick saggittal slices of guinea-pig brain (Chahl 1990).

If morphine produced release of substance P in-vivo this might be reflected in a lowered tissue content. However, previous studies on rat brain have found that acute administration of morphine, 10 mg kg^{-1}, produced little or no effect on substance P levels, but that chronic administration produced increases in substance P levels (Bergstrom et al 1984). These observations in rat brain agree with the concept that opioids inhibit neurotransmitter release. In contrast, preliminary experiments on guinea-pig brain indicated that a single injection of morphine sulphate, reduced SP-LI levels (Chahl & Chahl 1989). To determine whether the effects of morphine were species-specific, we examined the effects of morphine and naloxone-induced morphine withdrawal on SP-LI concentrations in brain regions of rats and guinea-pigs.

Guinea-pigs of either sex, 410–660 g, and male rats, 160–280 g, were given either morphine sulphate (David Bull Laboratories, NSW, Australia), subcutaneously, or saline, and 2 h later morphine-treated animals were given either naloxone hydrochloride (Sigma Chemical Co. St Louis, MO), subcutaneously, or saline. Saline-treated animals were given either naloxone or a second injection of saline. Animals were killed by guillotine 30 min after the final injection. Brains were removed rapidly and dissected on an ice-cold glass plate into major regions using

Correspondence: L. A. Chahl, Faculty of Medicine, University of Newcastle, Newcastle, NSW 2308, Australia.

Table 1. Changes in substance P-like immunoreactivity (SP-LI) in guinea-pig and rat brain regions produced by morphine (15 mg kg^{-1}, s.c.) and naloxone (15 mg kg^{-1}, s.c.)-induced morphine withdrawal. Results are expressed as mean percentage differences from control ±s.e. (morphine control – saline/saline; morphine withdrawal control – saline/naloxone).

	Morphine (% of control)	Morphine withdrawal (% of control)
Cortex		
Guinea-pig	−4·6±1·8	−7·4±3·3
Rat	−17·0±4·4	−18·2±1·9
Striatum		
Guinea-pig	−17·2±6·7	−8·7±3·4
Rat	−13·7±3·0	−3·6±2·3
Diencephalon		
Guinea-pig	−23·7±2·5**	−12·1±3·3
Rat	−22·3±4·3*	−15·0±2·3
Midbrain		
Guinea-pig	−13·0±5·0	−5·3±4·9
Rat	−3·1±4·4	−16·2±3·2
Medulla/pons		
Guinea-pig	+1·2±3·6	+10·3±3·8
Rat	+14·8±6·1	+8·4±4·8
Spinal cord		
Guinea-pig	−10·7±3·0	+4·5±1·8
Rat	−6·5±3·1	−11·7±3·5

Asterisks indicate significant differences from control obtained in Bonferroni *t*-tests on SP-LI concentrations. **$0{\cdot}01 > P > 0{\cdot}001$; *$0{\cdot}05 > P > 0{\cdot}01$.