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The influence of formulation on the relative bioavailability of indomethacin suppositories in dogs and rabbits

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Abstract

The bioavailability of indomethacin in dogs and rabbits after the administration of suppositories of different compositions was determined. The availability of the drug from triglyceride formulations was low but was enhanced after addition of a monoglyceride in both animal models. The absorption enhancing effect was correlated with the monoglyceride fatty acid composition and the in vitro release rate, determined in previous work. With other formulations some striking differences between both animal models were observed. The increase in indomethacin bioavailability from a formulation of a fatty acid-fatty acid methyl ester blend added to triglycerides was only seen in dogs. Two marketed formulations Indocid® and Dolcidium® showed a different availability when administered to dogs, and a similar availability when administered in rabbits. A poor correlation between bioavailability and in vitro drug release was found.

Key words: Indomethacin; Absorption enhancement; Indomethacin; Animal model; Monoglyceride; Fatty acid

1. Introduction

In previous work, the irritative effect of triglyceride suppositories in rabbits was shown to be reduced by adding 5% monoglycerides (Dimodan LS) or a mixture of 9% fatty acids and 1% fatty acid methyl esters (De Muynck et al., 1991).

Aoyagi et al. (1988) used a dialysis tubing method for the determination of the in vitro release of indomethacin triglyceride suppositories and found a good correlation with bioavailability data in rabbits and pigs. Using this in vitro method it was demonstrated that, depending on the melting point of the monoglyceride added to the triglyceride, the in vitro release of indomethacin can be modified. Our previous work (De Muynck and Remon, 1992) revealed that indomethacin in vitro release was highest from Indocid® and Dolcidium® formulations in comparison to supplemented (with monoglycerides or with a mixture of fatty acids an fatty acid methyl esters) and unsupplemented triglyceride formulations. The chemical composition and melting point of the monoglycerides added to the triglycerides influenced in

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vitro indomethacin release. No influence was observed after the addition of 5% Dimodan LS (melting point 13.35°C) while a reduction in drug release was observed after 5% Dimodan P (melting point 75.72°C) addition. A mixture of fatty acids with fatty acid methyl esters increased indomethacin release only when they were added to a lauric triglyceride (Witepsol H 15). However, the release was still significantly lower than for both commercial available formulations, Indocid® and Dolcidium® (De Muynck and Remon, 1992).

The objective of the present study was to evaluate some of the different indomethacin suppository formulations studied in vitro in rabbits and dogs in order to determine the influence of the excipient composition on the bioavailability of indomethacin, and to evaluate the possible in vivo/in vitro correlation and the bioavailailability of indomethacin from the less irritating suppository formulations containing monoglycerides or fatty acids and fatty acid methyl esters.

2. Materials and methods

2.1. Chemicals

Micronized indomethacin (< 10 μm micronized powder; European Pharmacopoeial grade; Esteve Quimica, Barcelona, Spain) was used, except in the Indocid® formulation. The internal standard for the plasma analysis was niflumic acid (Farmos Group Ltd, Turku, Finland). Methanol (Lab Scan Sciences, Lab Scan Ltd, Ireland) and tetrabutylammonium hydrogen sulfate (97% Aldrich Chemicals, Steinheim, Germany) were used as mobile phase components. Sodium hydroxide, disodium hydrogen phosphate dihydrate, citric acid monohydrate and chloroform, all pro analyse grade, were supplied by UCB Chemicals (Leuven, Belgium).

2.2. Drug formulations

Both Indocid® and Dolcidium® are marketed suppository formulations containing 100 mg indomethacin. As a reference formulation, Indocid® suppositories (Merck, Sharp and Dohme, Brussels, Belgium) using polyethylene glycols (PEG) as an excipient were selected. The excipient in Dolcidium® suppositories (Galephar, Brussels, Belgium) is a synthetic lauric triglyceride (Suppocire AP; Gattefossé, St Priest, France) mainly containing C12–C18 saturated fatty acids esterified with glycerol (85–90%) and PEG (10–15%) resulting in a mixture of triglycerides and a non-ionic surfactant (polyethylene glycol fatty acid esters).

The other suppository formulations were based on two triglyceride compositions: Witepsol H 15 (Hüls AG, Witten, Germany), a lauric triglyceride (mainly containing C12 and C14 saturated fatty acids) and Mesuro PS (Vandemoortele N.V., Izegem, Belgium), a non-lauric triglyceride (mainly containing C16–C18 saturated and unsaturated fatty acids). These triglycerides were used as such or were supplemented with monoglycerides or fatty acids and fatty acid methyl esters (FA/FAME). The monoglycerides (Dimodan® LS and P) were provided by Grindsted N.V. (Antwerpen, Belgium) and were added in a 5% concentration (w/w) to Mesuro PS (Dimodan LS and P) and Witepsol H 15 (Dimodan LS); the fatty acids and their methyl esters had an identical composition to the glyceride fatty acids of Mesuro PS (Vandemoortele, Izegem, Belgium) and were added in a 9:1% ratio (w/w) to the lauric triglycerides. The composition of the glycerides was determined by gas chromatography and is given in Table 1a and b.

2 g suppositories (100 mg micronized indomethacin in suspension) were used in the dog experiments and 1 g suppositories containing 50 mg indomethacin in the rabbit experiments. Both Indocid® and Dolcidium® suppositories were melted and remolded to obtain suppositories of approx. 1 g each containing 50 mg indomethacin. All suppositories (except 100 mg Indocid® and Dolcidium®) were made using stainless-steel suppository molds. A weight variation and a content uniformity test were performed according to the USP XXII (1989). The coefficient of variation was never higher than 4%.

The time between production and administration never exceeded 1 week. During this period the suppositories were stored at 7°C.

Table 1
Glyceride and glyceride fatty acid composition of triglycerides (a) and monoglycerides (b)

(a) Composition of triglyceride suppository bases: percent amount (w/w) of mono-, di-, triglycerides and of PEG fatty acid esters

	Mesuro PS	Witepsol H 15	Suppocire AP
Monoglycerides	–	0.3	2.5
Diglycerides	2.9	10.7	28.0
Triglycerides	97.1	89.0	55–60
PEG-fatty acid esters	–	–	10–15
Lauric/non-lauric base	non-lauric	lauric	lauric
Percent amount (w/w) of glyceride fatty acid according to its chain length			
C8:0–C10:0	–	1.4	–
C12:0	0.1	54.3	46.9
C14:0	0.8	19.7	20.0
C16:0	17.5	11.5	16.3
C18:0	6.5	12.8	16.8
C18:1	67.5	0.1	–
C18:2	6.2	–	–
C20:0–C22:0	1.4	0.2	–

(b) Composition of Dimodan® monoglycerides: percent amount (w/w) of free fatty acids and of mono- and diglycerides

	Dimodan®	LS Dimodan® P
Free fatty acids	3.35	0.34
Monoglyceride	91.00	97.50
Diglyceride	5.65	2.16
Percent amount (w/w %) of glyceride fatty acid according to its chain length		
C8:0–C15:0	2.10	2.72
C16:0	10.20	26.90
C16:1	0.12	0.13
C17	0.12	0.67
C18:0	11.50	67.50
C18:1	15.0	0.16
C18:2	59.3	–
C20:0–C22:0	1.66	1.92

2.3. Animals, drug administration and blood sampling

Six male healthy dogs (Belgian shepherd) weighing 30–32 kg were used. All formulations were tested in each dog in randomized order. From 16 h before the experiment until 8 h after suppository administration, the dogs had free access only to water. Between two experiments there was an interval of at least 1 week. Blood samples (2 ml) were withdrawn from the vena saphena at 0, 0.33, 0.66, 1, 1.33, 1.66, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 24, 36 and 48 h following drug administration. The samples were collected in heparinized glass tubes. Plasma was separated by centrifugation for 10 min at 3000 rpm and stored at −20°C until analysis. When defecation occurred during the first 3 h after administration the test was repeated (defecation occurred on three occasions and no correlation between the expulsion and the excipient could be made).

In the experiments using rabbits, in contrast to those in dogs, each animal was used for the evaluation of one suppository formulation. Six male rabbits (Witte van Dendermonde) weighing 2.4–2.6 kg were used for each formulation. Blood samples (1 ml) were withdrawn from the cephalic ear vein at 0, 0.33, 0.66, 1, 1.33, 1.66, 2, 2.5, 3, 4, 5, 6, 12, 24 and 36 h following drug administration. To prevent the suppository being expelled, the anus was kept closed during the first 3 h after administration using a clothes-peg.

2.4. Sample preparation and analytical procedure

All sample preparations were made in borosilicate glass tubes (Corning glass works, Corning, NY, U.S.A.). Niflumic acid was added in the following concentrations: 15 μg ml^{-1} in the rabbit plasma and 3.2 μg ml^{-1} in the dog plasma samples. Next, 250 μl of dog or rabbit plasma were added to the tubes and the mixture was vortexed for 1 min. After the addition of 1 ml buffer pH 3 (citric acid/disodium hydrogen phosphate) and 2.5 ml of the organic phase (chloroform-isopropanol, 90:10 v/v) the mixture was extracted by vortexing for 2 min. After centrifugation for 10 min at 3000 rpm the water layer was withdrawn and the organic phase was transferred to another tube and evaporated at 60°C under nitrogen. The samples were redissolved in 250 μl of the mobile phase and vortexed for 30 s. Next, 20 μl were analyzed using a validated HPLC method for rabbit and dog plasma samples (De Muynck and Remon, 1994). The HPLC system consisted of an isocratic HPLC pump (L-6000 Merck-Hitachi, Darmstadt, Germany), a septum-

less syringe-loaded injector loop of 20 μl (Valco Instruments Corp., Houston, U.S.A.), a reversed phase column (5 μm particles, Lichrosper RP-18; 125 mm × 4 mm, E. Merck, Darmstadt, Germany), a variable-wavelength UV-Vis detector (L-4200 Merck-Hitachi, Darmstadt, Germany) set at 320 nm and an integrator (D-2000 Merck-Hitachi, Darmstadt, Germany). The mobile phase consisted of methanol : water (62.5 : 37.5 v/v) with 5 mM tetrabutylammonium hydrogen sulfate. The pH was adjusted to 6.8 using 1 N NaOH. The flow rate was 1 ml min^{-1} and an ambient column temperature was used.

2.5. Data analysis

Areas under the plasma concentration time curve were calculated using the ABSPLOTS program (Shumaker et al., 1988). For statistical evaluation C_{max}, t_{max} and $AUC_{0-8\,h}$ were used. AUC values were only calculated from 0 to 8 h, since with both animal models plasma concentrations below the detection limit were observed after the 8 h time period. The relative bioavailabilities were calculated as $(AUC_{suppo.form.}/AUC^{®}_{Indocid}) \times 100\%$, with the subscript suppo. form. referring to the suppository formulation. Values are given as means ± S.D.

Statistical analysis of the data from the dog experiments was performed using the Wilcoxon signed ranks test. Data obtained from the rabbit experiments were analyzed using the Mann-Whitney U test. In both cases a p value of < 0.05 was considered significant.

3. Results

3.1. Bioavailability of indomethacin in dogs

C_{max}, t_{max}, $AUC_{0-8\,h}$ and the relative rectal bioavailability of indomethacin after administration of the nine different suppository bases are shown in Table 2. The mean plasma concentration time profiles are shown in Fig. 1a–c.

Comparing the Indocid® and Dolcidium® suppositories, significantly higher C_{max} and $AUC_{0-8\,h}$ values were observed for Indocid®, while a significantly greater t_{max} was seen for Dolcidium®.

The bioavailability from both triglyceride bases, Mesuro PS and Witepsol H 15, was low but not significantly different from each other.

The addition of 5% Dimodan LS monoglycerides to Mesuro PS resulted in a significant increase of the C_{max} from 4.1 ± 0.4 to 11.5 ± 0.7 μg ml^{-1} and of the $AUC_{0-8\,h}$ from 8.9 ± 1.1 to 27.0 ± 7.6 μg h ml^{-1}. The addition of this monoglyceride did not affect t_{max}. When adding 5% Dimodan P monoglycerides to Mesuro PS, C_{max} and $AUC_{0-8\,h}$ increased significantly compared to the pure triglyceride but the effect was less pronounced than that on the addition of Dimodan LS. An important and significant increase in t_{max} was observed from about 1 h for Mesuro PS and Mesuro PS + 5% Dimodan LS towards 2.2 ± 0.3 h for Mesuro PS supplemented with Dimodan P.

Addition of 9% FA and 1% FAME to Mesuro PS resulted in an increase in t_{max} although it was

Table 2
Pharmacokinetic parameters after administration of 100 mg indomethacin in different suppository bases to dogs (mean ± S.D.; $n = 6$)

	C_{max} (μg ml^{-1})	t_{max} (h)	$AUC_{0-8\,h}$ (μg h ml^{-1})	Relative bioavailability (%)
Indocid®	9.7 (±0.8)	1.0 (±0.1)	23.6 (±0.9)	100
Dolcidium®	5.1 (±1.1) [a]	1.5 (±0.2) [a]	18.6 (±4.2) [a]	78.5 (±15.9) [a]
Mesuro PS	4.1 (±0.4) [a]	1.0 (±0.3)	8.9 (±1.1) [a]	37.7 (±4.7) [a]
Mesuro PS + 5% Dimodan LS	11.5 (±0.7) [a]	0.9 (±0.1)	27.0 (±7.6)	115.6 (±35.0)
Mesuro PS + 5% Dimodan P	5.2 (±0.3) [a]	2.2 (±0.3) [a]	13.8 (±0.5) [a]	58.5 (±0.8) [a]
Mesuro PS + 9% FA/1% FAME	16.1 (±0.7) [a]	1.4 (±0.1) [a]	54.6 (±1.5) [a]	232.2 (±10.5) [a]
Witepsol H 15	3.8 (±1.5) [a]	0.8 (±0.2)	7.8 (±3.0) [a]	33.2 (±12.9) [a]
Witepsol H 15 + 5% Dimodan LS	9.8 (±1.4)	1.0 (±0.4)	29.1 (±6.9)	123.6 (±28.3)
Witepsol H 15 + 9% FA/1% FAME	15.4 (±3.6) [a]	1.3 (±0.2)	44.5 (±7.9) [a]	189.5 (±37.3) [a]

[a] Significantly different from Indocid®.

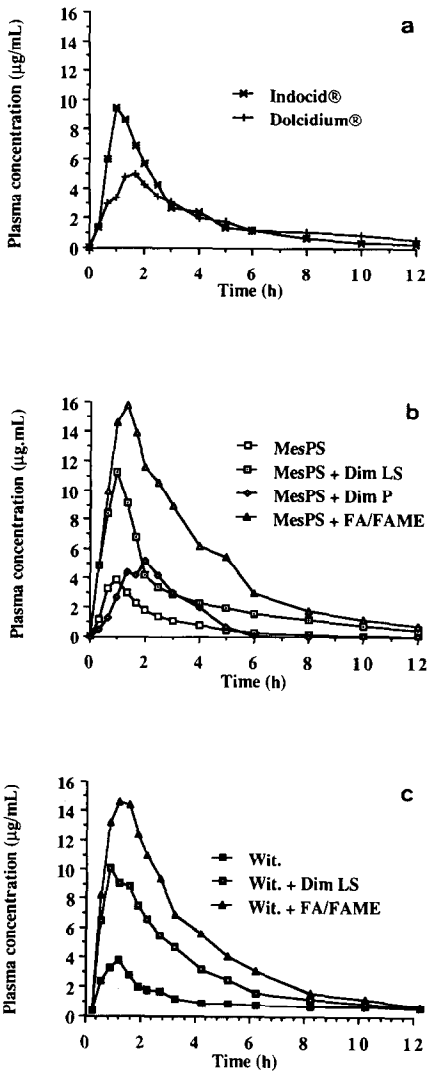

Fig. 1. Mean plasma concentration time profiles after administration of 100 mg indomethacin in different suppository bases to dogs ($n = 6$). (a) Commercial formulations; (b) Mesuro PS (MesPS) formulations; (c) Witepsol H 15 (Wit.) formulations. Dim: Dimodan monoglyceride and FA/FAME fatty acid-fatty acid methyl esters.

less important than with the Dimodan P supplemented suppository. However, the t_{max} was significantly higher than with the unsupplemented triglyceride (Mesuro PS) or with the Dimodan LS supplemented formulation. The most striking result with this formulation was the dramatic increase in C_{max} and $AUC_{0-8\,h}$ in comparison to all other Mesuro PS formulations, yielding values of 16.1 ± 0.7 $\mu g\ ml^{-1}$ and 54.6 ± 1.5 $\mu g\ h\ ml^{-1}$, respectively.

After the addition of 5% Dimodan LS to Witepsol H 15 a significant increase in C_{max} and in $AUC_{0-8\,h}$ was observed compared to the pure triglyceride base, while the t_{max} was not affected. When these results were compared with those obtained for the Dimodan LS supplemented formulation to the other triglyceride base, Mesuro PS, only the C_{max} was significantly higher when using Mesuro PS.

Addition of FA/FAME to Witepsol H 15 resulted in a significant enhancement in C_{max} and $AUC_{0-8\,h}$ compared to the values obtained for the unsupplemented triglyceride and the Dimodan LS supplemented formulation. The results were comparable to those for the FA/FAME supplemented Mesuro PS formulation except that the AUC was significantly higher when using the Mesuro PS base.

The relative bioavailabilities were calculated taking Indocid® as the reference formulation (100% value). For the other commercially available formulation, Dolcidium®, the bioavailability was $78.5 \pm 15.9\%$. The use of both triglyceride formulations without additives resulted in low bioavailabilities ($\pm 35\%$). This was increased to some extent after addition of Dimodan P to Mesuro PS ($58.5 \pm 0.8\%$) but still remained significantly lower than for the Indocid® formulation. With the addition of Dimodan LS to both bases a significant difference with the Indocid® formulation was no longer observed. For Mesuro PS and Witepsol H 15 supplemented with FA/FAME significant higher bioavailabilities of 232.2 ± 10.5 and $189.5 \pm 37.3\%$, respectively, were observed in comparison to Indocid®.

3.2. Bioavailability of indomethacin in rabbits

C_{max} and t_{max} values for the nine suppository bases are listed in Table 3. Due to the presence of enterohepatic recycling AUCs were not calculated for this animal model. An example of this enterohepatic recycling is shown in Fig. 3. Because enterohepatic recycling was not observed in all rabbits its presence is not clear from the mean

Table 3
Pharmacokinetic parameters after administration of 50 mg indomethacin in different suppository bases to rabbits (mean ± S.D.; $n = 6$)

	C_{max} ($\mu g\ ml^{-1}$)	t_{max} (h)
Indocid®	23.9 (±8.3)	0.7 (±0)
Dolcidium®	28.1 (±9.0)	0.7 (±0)
Mesuro PS	12.8 (±1.2) [a]	1.4 (±0.3) [a]
Mesuro PS + 5% Dimodan LS	37.8 (±10.6) [a]	0.8 (±0.2)
Mesuro PS + 5% Dimodan P	13.6 (±6.2) [a]	1.2 (±0.7)
Mesuro PS + 9% FA/ 1% FAME	11.9 (±4.5) [a]	0.9 (±0.6)
Witepsol H 15	13.2 (±1.7) [a]	1.2 (±0.3) [a]
Witepsol H 15 + 5% Dimodan LS	40.6 (±19.0)	0.6 (±0.2)
Witepsol H 15 + 9% FA/1% FAME	18.6 (±6.0)	0.8 (±0.2)

[a] Significantly different from Indocid®.

plasma concentration time profiles shown in Fig. 2a–c.

No significant difference in C_{max} and t_{max} was observed between Indocid® and Dolcidium® suppositories. This was also the case when C_{max} and t_{max} of Mesuro PS were compared to the pharmacokinetic parameters of Witepsol H 15, Mesuro PS supplemented with 5% Dimodan P and Mesuro PS supplemented with 9% FA and 1% FAME. However, for these three formulations lower C_{max} values were seen in comparison to Indocid® and Dolcidium®.

The addition of 9% FA and 1% FAME to the Mesuro PS and Witepsol H 15 formulations did not result in a significant increase in C_{max} as compared to the pure triglycerides. No significant decrease in t_{max} was observed in comparison with the pure triglycerides.

After addition of 5% monoglycerides (Dimodan LS) to Mesuro PS a significant increase in C_{max} from 12.8 ± 1.2 to $37.8 \pm 10.6\ \mu g\ ml^{-1}$ was seen. The observed C_{max} was also significantly higher in comparison to the FA/FAME supplemented formulation.

A similar increase in C_{max} from 13.2 ± 1.7 to $40.6 \pm 19.0\ \mu g\ ml^{-1}$ was seen after 5% Dimodan LS addition to Witepsol H 15, while the t_{max} was reduced on coadministration of the additive. No significant differences in C_{max} and t_{max} were observed between both triglyceride bases supplemented with Dimodan LS.

Using Indocid® as a reference formulation, both triglyceride formulations without additives and Mesuro PS supplemented with FA/FAME or 5% Dimodan P resulted in significantly lower C_{max} values. With the addition of 5% Dimodan LS or 9% FA and 1% FAME to Witepsol H 15 the C_{max} value was not significanty different from

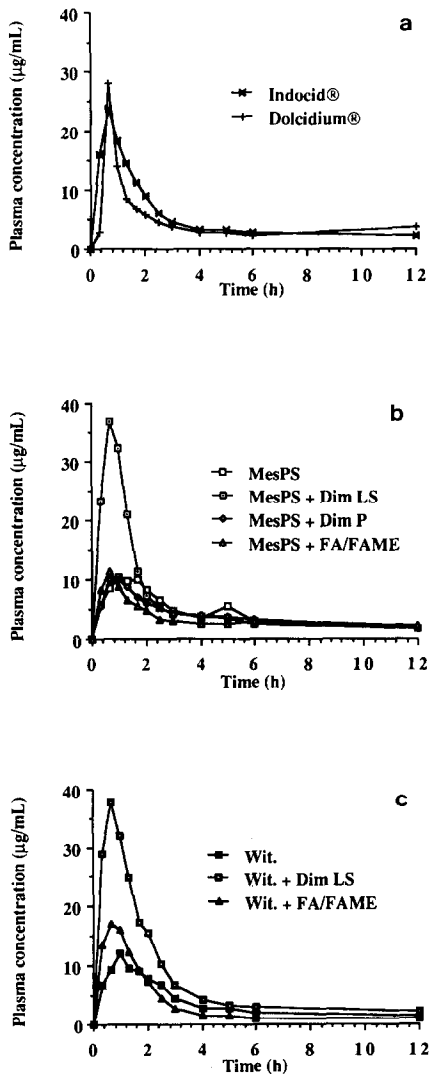

Fig. 2. Mean plasma concentration time profiles after administration of 50 mg indomethacin in different suppository bases to rabbits ($n = 6$). (a) Commercial formulations; (b) Mesuro PS (MesPS) formulations; (c) Witepsol H 15 (Wit.) formulations. Dim: Dimodan monoglyceride and FA/FAME fatty acid-fatty acid methyl esters.

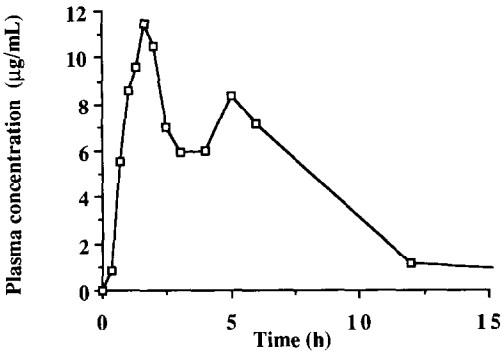

Fig. 3. Plasma concentration time profiles after administration of 50 mg indomethacin in Mesuro PS to a rabbit.

that of the Indocid® formulation. The addition of 5% Dimodan LS to Mesuro PS resulted in a significantly higher C_{max} compared to Indocid® while the addition of the FA/FAME blend resulted in a significant lower value (Table 3).

4. Discussion

In this study dogs and rabbits were used to assess the relative bioavailability of indomethacin from suppository formulations with a particular interest for the less irritative formulations containing monoglycerides and a fatty acid-fatty acid methyl ester mixture. Moreover, an in vitro-in vivo correlation was performed using previously reported in vitro release data (De Muynck and Remon, 1992). In order to maintain the ratio of active substance to excipient in both animal models, comparative doses of 100 mg in dogs, using 2 g suppositories, and 50 mg drug in rabbits, using 1 g suppositories, were used.

In a study on the absorption of antibiotics, rectal absorption in dogs was described as being similar to that in humans (Toya et al., 1983). Several studies on rectal availability of indomethacin from suppositories using rabbits as an animal model have been reported (Vidras et al., 1982; Kuroda et al., 1983; Ohnishi et al., 1987; Aoyagi et al., 1988). The problem encountered during this bioavailability study with rabbits was the occurrence of a variable enterohepatic recirculation of indomethacin which was also observed previously after i.v. administration of indomethacin to rabbits (De Muynck and Remon, 1994). Therefore, AUCs and relative bioavailabilities were not calculated and the formulations were compared through their C_{max} values since in dogs, pigs (Aoyagi et al., 1988) and humans (Fredj et al., 1983; Möller, 1984; Aiache et al., 1987) a good correlation was reported between the C_{max} values and AUCs.

When administered to dogs, C_{max} and $AUC_{0-8\ h}$ were greater for the Indocid® formulation than for the Dolcidium® formulation. This contrasts with previously reported in vitro results, using the dialysis tubing method, where a rapid and not significantly different in vitro indomethacin release rate from Indocid® and Dolcidium® formulations was seen (De Muynck and Remon, 1992). The good rectal bioavailability of indomethacin from PEG suppository bases (as in Indocid®) has already been described in the literature (Kerckhoffs and Huizinga, 1967; Gueurten et al., 1983; Ohnishi et al., 1987). Ohnishi et al. (1987) found the bioavailability not to be influenced by the molecular mass of the PEG (between 1000 and 4000 Da) and attributed this to the rapid dissolution of indomethacin in vitro as well as in vivo. Therefore, the exact qualitative and quantitative excipient composition of the Indocid® formulation, in relation to the PEG mixture that is used, is of minor importance. Although the bioavailability of Dolcidium®, in dogs, was significantly lower than for Indocid®, it was significantly higher compared to the triglyceride bases without additives. For both pure triglyceride bases, Mesuro PS and Witepsol H 15, very low bioavailabilities were obtained. These bioavailabilities were not dependent on the glyceride fatty acid composition, diglyceride content (both formulations) or the presence of unsaturated fatty acids (Mesuro PS). Literature data on experiments in other species confirm the low bioavailability of indomethacin from triglyceride bases (Kerckhoffs and Huizinga, 1967; Gueurten et al., 1983; Möes, 1983; Möller, 1984). This low bioavailability was attributed to the low percentage of di- and monoglycerides in the fatty suppository formulations (Möller, 1984).

The greater rectal indomethacin absorption from Dolcidium® as compared with pure triglyc-

erides can be explained on the basis of the action that non-ionic surfactants of the PEG fatty acid ester type exert on the rectal membrane. A lowering of the resistance of the unstirred water layer and mucus barrier and increased permeability of both the paracellular and transcellular routes of the epithelial cell layer were attributed to these surfactants (Morrow and Anderson, 1986; Sakai et al., 1986). The hydrophilic-lipophilic balance value and the reduction in surface tension due to non-ionic surfactants do not have an influence on these effects (Morrow and Anderson, 1986). An optimum in enhancing effect was obtained for surfactants with a C12 hydrocarbon chain, the chain length predominant in the Dolcidium® suppository base (Ishikawa et al., 1980). Another factor that might contribute to the greater bioavailability was the faster release rate from Dolcidium® in comparison to other triglyceride formulations, as observed in vitro (De Muynck and Remon, 1992).

The addition of the monoglyceride Dimodan LS to the triglycerides resulted in a significant increase in C_{max} and $AUC_{0-8\,h}$. The poor correlation with the in vitro release, not being significantly different from the unsupplemented formulations (De Muynck and Remon, 1992), is an indication of the effect that monoglycerides exert on biological membranes and by which indomethacin absorption was enhanced. An increase in phenol red absorption in all segments of the gastrointestinal tract of rats (Higaki et al., 1987) was related to the absorption and presence of medium chain glyceride components in the epithelial cell bilayer (Higaki et al., 1990). Glyceryl monooctanoate was found to be the main absorption enhancing component of a medium chain glyceride mixture (Van Hoogdalem et al., 1988). Furthermore, it is known that not only medium chain monoglycerides are absorbed rectally but also linoleic acid rich monoglycerides (Christophe et al., 1987). These monoglyceride derivatives from *cis*-unsaturated fatty acids which are the main components of Dimodan LS (Table 1b), can disorder the hydrophobic region of the membrane's interior and interact with the polar groups of phospholipids (Muranishi, 1990) and in this way increase the absorption of drugs. A transcellular permeability enhancing effect was correlated with the solubilization of cholesterol, a membrane stabilizer, by monoglycerides (Van Hoogdalem et al., 1988). The observation that the t_{max} for the Dimodan LS supplemented formulations was not increased vs the pure triglyceride is an indication that the monoglyceride exerts an absorption enhancing effect during the early period after suppository administration.

A totally different behaviour was seen for the Dimodan P supplemented formulation where a significant increase in t_{max} and a lower bioavailability were obtained in comparison with the Dimodan LS supplemented formulation. The in vitro results suggested that less indomethacin should be available for absorption from the Dimodan P formulation compared to the Dimodan LS supplemented formulation (De Muynck and Remon, 1992). The lower bioavailability for the Dimodan P supplemented suppositories as compared to the Dimodan LS supplemented formulation correlates well with this finding. The lower release was correlated with the high melting temperature of the monoglyceride (De Muynck and Remon, 1992). Higher melting monoglycerides caused less enhancement in the permeability of cells for procainamide absorption (Muranushi et al., 1981). The reason why a higher C_{max} and $AUC_{0-8\,h}$ were obtained for the Dimodan P supplemented formulation in comparison with the unsupplemented formulation is unclear, since in addition to a lower in vitro availability of the monoglyceride supplemented formulation (De Muynck and Remon, 1992), the long chain saturated fatty acids which are the main components of Dimodan P monoglycerides (Table 1b) have been shown not to alter the epithelial cell bilayer interior (Muranushi et al., 1980). Since the bioavailability of indomethacin increased less after the addition of Dimodan P to Mesuro PS as compared to Dimodan LS addition and since the bioavailability seems not to be influenced by the triglyceride composition (Witepsol H 15 or Mesuro PS), suppositories composed of 5% Dimodan P in Witepsol H 15 were not included in the study.

The important increase in C_{max} and $AUC_{0-8\,h}$ after addition of 9% fatty acids and 1% methyl esters to both triglycerides cannot be attributed

to the presence of the methyl esters, since these esters of fatty acids have been shown not to be active as absorption enhancers (Muranishi, 1985). In contrast, *cis*-unsaturated long chain fatty acids increase membrane permeability (Muranushi et al., 1980). The composition of the fatty acids used in this mixture is the same as that of the Mesuro PS glyceride fatty acids (Table 1); in this mixture, oleic acid, a *cis*-unsaturated long chain fatty acid, is present in a large amount (67.5% w/w). Furthermore, the influence of oleic acid on the protein-lipid interactions has been shown to result in both transcellular and paracellular permeability enhancement (Muranushi et al., 1981). Long chain fatty acids are soluble in the triglyceride bases but at a pH higher than 7.4, the pH of the rectal fluid, they become partially ionized and their affinity for monolayers of the epithelial cell wall becomes comparable to that of membrane phospholipids (Scow and Blanchette-Mackie, 1985). The absorption enhancing effect of long chain fatty acids on the rectal absorption of indomethacin in rats has also been reported using oleic acid in a hydrogel (Kamiya et al., 1983).

The results with the FA/FAME and the Dimodan LS supplemented formulations in dogs are promising in relation to the safer use of absorption enhancers. In previous work, we have shown that compositions containing these additives could reduce rectal irritation after subchronical application in rabbits also when compared to the excipients used in Indocid® and Dolcidium® (De Muynck et al., 1991). These components have apparently a rapid and reversible action on the mucosal membrane without inducing major histological damage.

In rabbits the use of pure triglyceride bases resulted in low C_{max} values, being enhanced by the coadministration of Dimodan LS. Compared to the Indocid® formulation, the C_{max} values were increased for the Dimodan LS supplemented formulations. With the Witepsol H 15 formulation this increase was not significantly different from that in the case of Indocid®. In accordance with the results obtained in dogs, a trend towards greater availability with the Dimodan LS formulations was observed.

On comparing the C_{max} values for both animal models, some striking differences were observed. The blend of fatty acid/fatty acid methyl esters and the addition of Dimodan P did not result in a higher maximal plasma concentration of indomethacin in rabbits compared to the pure triglyceride formulations. The observation that the C_{max} after Indocid® administration is not significantly different from that after Dolcidium® administration in rabbits is in accordance with the bioequivalence observed in human volunteers (Jonkman et al., 1984) and with the similar in vitro release rates for both formulations (De Muynck and Remon, 1992) but is in contrast to what was found in dogs. These experiments suggest that the effect of absorption enhancers on the availability of indomethacin was dependent on the animal model.

In conclusion, we can state that adding Dimodan LS monoglycerides to triglycerides enhanced the rectal absorption of indomethacin in both animal models. The increase in bioavailability due to the fatty acids and fatty acid methyl ester blend depended on the animal model and was only seen in dogs. The marketed Indocid® and Dolcidium® formulations showed a different availability when administered to dogs. This study also demonstrated the difficulty of predicting bioavailability data from in vitro results and from in vivo results obtained in animals.

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