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THE EFFECT OF INDOMETHACIN MICROCAPSULES ON INTESTINAL ULCERATION IN THE RAT.

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ABSTRACT

The absorption of indomethacin in the rat was studied following a single oral dose of indomethacin in the form of the powdered drug or microcapsules.

Serum levels of unmetabolized drug were measured and gastrointestinal ulceration was assessed 72 hours after dosing by measuring the tensile strength of the intestine after its removal from the animal and by counting the number of ulcers present on the intestinal wall.

In vitro dissolution of the powdered drug and microcapsules was carried out in water, in Polyethylene Glycol solution and in 40mM sodium cholate solution for a comparison with the in vivo results.

Both in vitro and in vivo results for the microcapsules were similar to those of the powdered drug and therefore encapsulation of indomethacin offered no advantage over conventional dosage forms.

INTRODUCTION

Many drugs have been microencapsulated to reduce gastric and other gastrointestinal tract irritation including ferrous sulphate (1) and potassium chloride (2). Indomethacin has been shown to produce intestinal ulcers in rats after a single toxic dose (3,4) and the incidence of ulceration after indomethacin administration appears to be related to the initial systemic drug concentration (5).

Modification of the absorption of indomethacin by controlled release from indomethacin microcapsules into the gastrointestinal tract might reduce the incidence of ulceration.

In this study, an ulcerative dose of indomethacin in the form of the powdered drug and microencapsulated drug were administered as a single oral dose to rats to evaluate their effect on the incidence of ulceration.

MATERIALS AND METHODS

Materials.

Indomethacin and indomethacin microcapsules (14.79% w/w indomethacin on dry basis) (Nicholas Labs., Slough.U.K.), Cholic acid, sodium salt (Sigma Chemical Co.,Poole.U.K.). Polyethylene Glycol, m.wt.300, pure (Koch-Light Labs., Berks.U.K.) were used as received.

METHODS

Polymorphic Characterization.

The polymorphic form of the powdered drug and that present in the microcapsules was determined by differential scanning calorimetry (D.S.C.) and infrared (I.R.) spectroscopy as described by Tuladhar et al (6).

Surface area determinations of the powdered drug and microcapsules were carried out using the Fisher Sub Sieve Sizer as described previously (7).

Solubility Studies.

The equilibrium solubility of indomethacin (form γ) was determined in water, 0-10% w/v PEG300 solutions (pH 4.15), phosphate buffer (pH7.35) and 40mM sodium cholate solution (pH7.35) using a rotating wheel assembly immersed in a water bath ($37^{o}C \pm 0.5^{o}C$) as described previously (7).

Dissolution Studies.

Dissolution of the powdered drug and microcapsules was carried out using a 1L flat bottomed beaker in which the dissolution media was stirred by means of a teflon paddle as described previously (7). The dissolution media were water, PEG300 solution and 40mMol sodium cholate solution.

Reverse phase HPLC methods.

The system consisted of a reciprocating pump (Metering Pumps, London) fitted with a 10µl injection valve, a Cecil Instruments UV variable detector (Model CE212) connected to a Servogor 120 pen recorder (John Minister Instruments). A Partisil 10 ODS-3 column (Whatman,UK) and a precolumn packed with Pellicular Media (Co:PELL,ODS,Whatman,UK) were used. Aqueous drug samples were assayed for drug contents using the reverse phase HPLC method 1 (7), and drug samples containing sodium cholate were assayed using method 2 (7) because the retention time of the sodium cholate was the same as that of indomethacin in method 1.

The extracted serum samples were assayed for drug contents using a third HPLC method (5).

IN VIVO METHODS

Experimental Procedure.

Groups of 4 non-fasted Wistar rats (Bantin and Kingman,Hull) weighing 200-250g were administered a single oral dose of

indomethacin (15mg/kg) as powder or microcapsules freshly suspended in 1ml of water as described by Hilton and Summers (5).

0.1ml blood samples were withdrawn from the tail vein at intervals for 3 hours after dosing.The blood samples were then extracted as described previously (5) and assayed for unmetabolized drug using the HPLC method 3. The resulting drug serum levels were analysed by the Student t-test.

The rats were sacrificed 72 hours after dosing by placing them in a CO_2 chamber. Their intestines were removed and emptied of any intestinal contents before evaluation of ulceration.

Evaluation of Ulceration.

Ulceration was evaluated by 2 methods. The first method determined ulceration by a tensile strength inflation technique (8) since ulcerogenesis leads to a weakening of the intestinal wall.

The second method involved viewing and counting the number of lesions present in the small intestine in any condition (5).

RESULTS AND DISCUSSION

Polymorphic Form.

The polymorphic form present in the both the powdered drug and the microcapsules was the stable form, form γ. D.S.C. and IR spectra were not affected by the presence of gelatin and acacia in the microcapsules.

Equilibrium Studies.

The increased indomethacin solubility in aqueous P.E.G. solutions (Fig 1) suggests a soluble complex was formed between the amido group of indomethacin and the -OH groups of the P.E.G. molecule via hydrogen bonding.

The equilibrium solubility of indomethacin was greater in 40mM sodium cholate solution at pH7.35 compared to its solubility in phosphate buffer at pH7.35 (Table 1). This result confirms

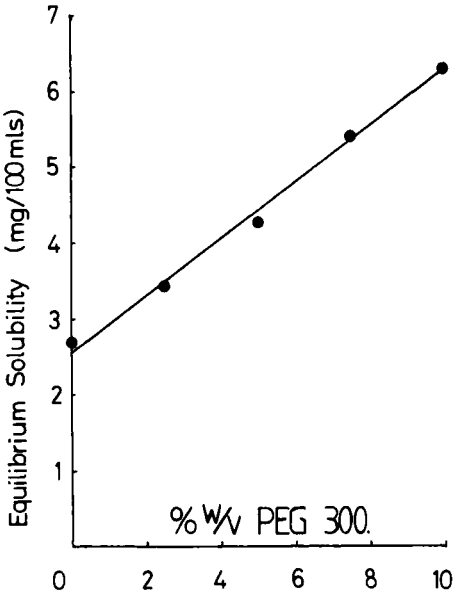

FIGURE 1.

Equilibrium Solubility of Indomethacin in aqueous P.E.G. 300 solutions at 37°C + 0.5°C, pH 4.15.

TABLE 1.

Equilibrium Solubilities of Indomethacin in 40mM Sodium Cholate Solution (pH7.35), 0.1M Phosphate Buffer (pH7.35) and Water (pH5.6).

Solvent	Equilibrium Solubility of Form δ (mg%)
40mM Sodium Cholate Solution (pH 7.35)	175.93
0.1M Phosphate Buffer (pH 7.35)	136.55
Water (pH 5.6)	1.85

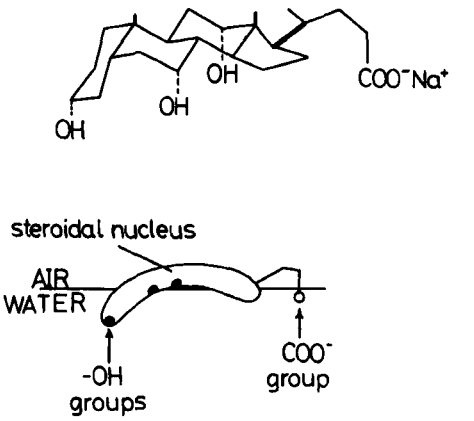

FIGURE 2.

The Bile Salt Structure.
(The A ring is cis to the B ring).

that of Miyazaki et al (9) who suggested the increase in indomethacin solubility in bile salt solution may be due to micellar solubilization.

In water, bile salts can arrange themselves in a saucer shaped micelle (Fig.2).

At pH7.35, the bile salt is negatively charged due to protonation of the carboxylate group. Indomethacin also has a carboxylate anion and the ionic form carries a relatively large hydrophobic portion in the molecule. Thus there is an electronic similarity between bile salts and indomethacin;both have polar groups (carboxylate groups) at one end of their molecules. Sodium Cholate, a trihydroxy bile salt forms primary and secondary micelles and incorporation of indomethacin into the palisade layers of the micelles results in mixed micelle formation and increased indomethacin solubility (10,11).

Miyazaki et al (12) suggested hydrophobic interaction was the mechanism of aggregation of bile salt monomers into micelles and that the hydrophobic side of the bile salt micelles (C_{18} and C_{19} methyl groups) interacted with the hydrophobic portion of

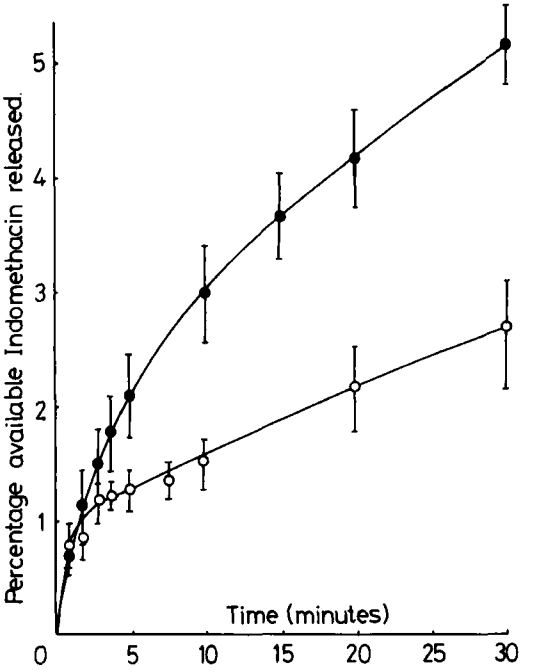

FIGURE 3.

Dissolution of Powdered Indomethacin and Indomethacin Microcapsules in 600mls distilled water at 37°C + 0.5°C (pH 5.6). Key: ○ powdered drug; ● microcapsules.

indomethacin molecules, thus enhancing the solubilization process.

Dissolution Studies.

The indomethacin microcapsules were well wetted in water and produced an approximately constant dissolution rate (Fig.3). Upon exposure to PEG solution, the same dissolution rate was produced, indicating that PEG did not affect the release mechanism of the drug from the microcapsules (Fig.4).

The microcapsules consist of an inner solid core of indomethacin surrounded by a coating of acacia and gelatin which swelled upon addition to water. The release mechanism of drug

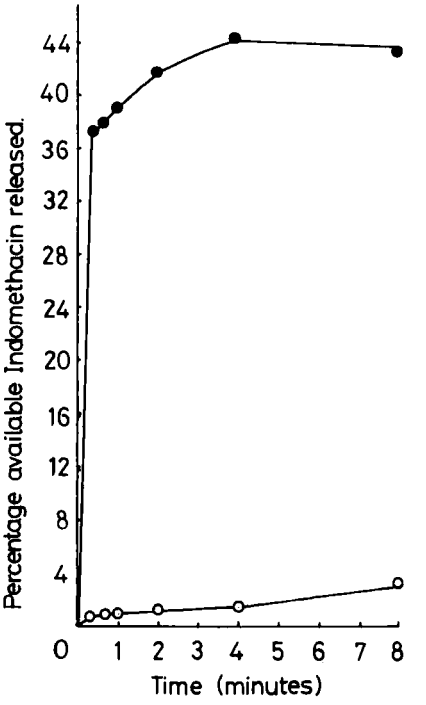

FIGURE 4.

Dissolution of Powdered Indomethacin and Indomethacin Microcapsules in 600mls PEG300 at 37°C + 0.5°C (pH 5.5).
Key: ● powdered drug; ○ microcapsules.

during dissolution may have been achieved by a "pore" diffusion mechanism proposed by Walker (13) (Fig.5).

In the initial stage, water permeated the coating (R_1). Next, an aqueous solution of the solid was formed within the structure (R_2) and this solution permeated through microchannels or pores into the continuous water phase (R_3). The rate of release was controlled by pore size and the molecular volume of the solute. In practice, the rate R_3 is the rate controlling step, eg studies by Levy et al (14) and Khalil and El-Gamal (15). Hence the release rate is a function of the permeability of the

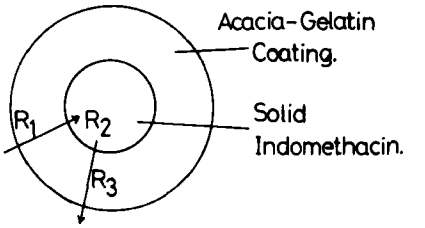

FIGURE 5.

Indomethacin Microcapsules.

R_1 = rate of penetration of solvent into microcapsule.

R_2 = rate of core dissolution.

R_3 = rate of removal of dissolved core material.

coating to water, the solubility of the microencapsulated solid and the permeability of the coating to the saturated solution.

Being a large molecule, PEG could not penetrate the pores in the coating and therefore could not complex with the drug as it dissolved. If it had been able to penetrate the pores, the drug dissolution rate may have increased due to complexation.

The drug release rate from the microcapsules was faster in sodium cholate solution than in water (Fig.6). Indomethacin is more soluble in high pH solutions (16) and since the pH of sodium cholate solution was higher than that in water, drug solubility in the core was increased. Thus, the rate of diffusion through the microcapsule coating (R_3) increased. Sodium cholate molecules may have been too large to penetrate the pores, so the increased drug solubility was entirely due to the pH conditions because a sodium cholate: indomethacin interaction could not occur in the core.

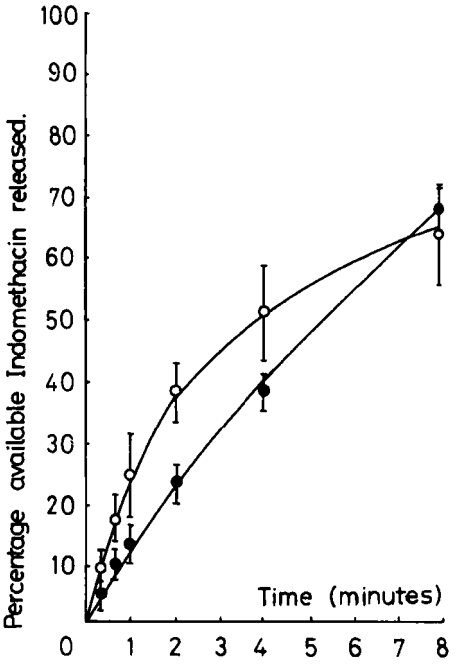

FIGURE 6.

Dissolution of Powdered Indomethacin and Indomethacin Microcapsules in 600mls 40mM. Sodium Cholate Solution, 37°C + 0.5°C (pH 7.35). Key: ○ powdered drug; ● microcapsules.

An alternative method of increased drug solubility could have occurred if the pH conditions were such as to cause the breakdown of the microcapsule coating. However, after dissolution the microcapsules did not appear to be damaged and an obvious non-linear profile did not occur as a result of erratic increases in drug solubility if the coating had broken down.

Indomethacin is a drug of low aqueous solubility and is hydrophobic. The powdered drug was therefore poorly wetted in water and the drug release rate could not be compared with that of the microcapsules (Fig.3). To improve wetting of the powder,

0.5mls of PEG300 was mixed with the drug powder prior to dissolution. However, the drug freely complexed with PEG and produced a faster release rate compared to that produced in water (Fig.4).

Dissolution in sodium cholate was therefore carried out to improve wetting of powdered drug and microcapsules using a compound which may affect their dissolution in vivo. The indomethacin powder released approximately the same percentage drug as the microcapsules within 8 minutes (Fig.6). However, the increase in drug solubility in the bile salt solution was due to improved wetting, favourable pH conditions and a possible interaction between indomethacin and the bile salt. Dissolution was carried out for 8 minutes because indomethacin:P.V.P solid dispersion systems were assessed at the same time for comparison and many of them exhibited 80-100% drug release within 8 minutes (7).

In Vivo Studies.

Table 2 shows that the initial systemic drug levels produced by the microcapsules and powdered drug were not significantly different from each other at each time interval and the gastroinestinal ulceration produced by the microcapsules was as severe as that produced by the powdered drug. However, at the same dose level, Rowe (17) found indomethacin microcapsules did not have a pathological effect on rat duodenum and jejunum. Ulceration occurred when the microcapsules were administered at higher dose levels of 20, 40 and 80 mg/kg.

The in vivo drug release rate from the microcapsules must have been similar to that of the powdered drug on the assumption that absorption of indomethacin was dissolution rate limited. Thus there appears to be a possible correlation between the in vivo dissolution and the in vitro dissolution in sodium cholate solution.

TABLE 2.

Serum Levels and Intestinal Tensile Strength values after administration of Indomethacin Powder (Form ɣ) and Microcapsules.

	Serum levels at x hours after dose (µl/ml + SEM				Tensile strength
Sample.	0.5hr	1hr	2hrs	3hrs	(mmHg + SEM)
Control					222 ± 11.0
Indomethacin (form ɣ)	30.2 ± 0.3	37.2 ± 1.6	50.3 ± 0.7	53.3 ± 3.2	38.4 ± 6.3a
Microcapsules (form ɣ)	25.1 ± 1.2	43.5 ± 3.9	54.2 ± 2.1	61.6 ± 3.9	33.5 ± 2.8a

a = many adhesions and perforation in small intestine; unable to count the number of lesions present.

In conclusion, there was no apparent advantage in encapsulating indomethacin because the microcapsules exhibited *in vitro* and *in vivo* results which were similar to those of the powdered drug which would be present in conventional dosage forms.

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