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EFFECT OF MICELLAR SOLUBILIZATION ON THE GASTROINTESTINAL ABSORPTION OF INDOMETHACIN IN THE RAT

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SUMMARY

The absorption of indomethacin used as a test substance from the micellar polysorbate 80 solution was investigated and compared with that in the true solution, solutions in 60% PEG 400 and suspension. All liquid formulations were tested at different pH values comprising the range at which the indomethacin molecule was unionized (pH 2), partly ionized (pH 4) and completely ionized (pH 8). The concentration of surfactant added was 5% and 10%.

The availability parameters (AUC_0^∞, C_{max}, T_{max}) were calculated for all formulations investigated. The values of the area under the serum concentration–time curve show that the presence of surfactant does not retard absorption of drug. The enhancement of maximum serum concentration (C_{max}) is particularly distinct in case of ionized forms of indomethacin. Two-fold increase of surfactant concentration has practically no effect on the availability parameters.

INTRODUCTION

Non-ionic surfactants are frequently included in a dosage formulation in different amounts dependent upon the role they play in this form.

Their properties are mostly utilized for improving the solubility of insoluble or poorly soluble drugs as a result of micellar solubilization. The influence of these additives on absorption processes of drugs can not be neglected.

The drug absorption process from micellar solutions is usually explained on the basis of a pseudo-phase-separation model in which two phases are considered: the dispersed micellar phase and the continuous aqueous phase surrounding the micelles. The drug is partitioned between these two phases with a constant partition ratio.

It is generally accepted that a drug solubilized in micelles is not available for absorption and passive diffusion through biomembranes is governed by thermodynamic activity of the drug in aqueous phase. Therefore, micellar solubilization can have a negative effect on drug absorption.

The studies of many authors seem to support these considerations. Their experiments performed with the recirculating method in situ or by the 'everted sac' technique showed that non-ionic surfactants like polysorbates retarded the absorption of different drugs from the rat colon (Riegelman and Crowell, 1958a and b; Levy and Reuning, 1964), stomach (Kakemi et al., 1965) or small intestine (Yamada and Yamamoto, 1965; Yamada et al., 1966; Matsumoto, 1966; Saski, 1968).

It has also been demonstrated that non-ionic surfactants retard the absorption of drug after intramuscular injection (Kobayashi et al., 1974). In all cases studied the relationship between the absorption rate and the concentration of surfactant was observed.

On the other hand, several authors report the enhancement of drug absorption when surface-active agents were present in the formulation. For example, specific vitamins are much better absorbed from solubilized solutions than from oily solutions (Elworthy et al., 1968; Thoma et al., 1978). These conflicting opinions might be due to the different properties and concentrations of surface-active agents used or different experimental methods as well as different characteristics of the drugs tested.

Indomethacin used in this study is highly lipophilic, and practically insoluble in water. Encouraging results obtained in attempts to increase its solubility and stability in aqueous solutions of polyethylene glycols or different non-ionic surfactants (Krasowska, 1976; Krasowska et al., 1972; Krasowska, 1979) led the author to also investigate the influence of these additives on the absorption of drug in vivo.

The present study was undertaken to investigate the influence of micellar solubilization of indomethacin at different concentrations of surfactant and different pH values of solutions tested.

MATERIALS AND METHODS

Materials

Metindol (indomethacin) was kindly supplied by Pharmaceutical Works, Polfa (Poland); polyethylene glycol 400 (Gurr, England), polysorbate 80 (Schuchardt, München). All other chemicals were of analytical reagent grade. Double-distilled water was used throughout. Liquid formulations, containing 0.1% of indomethacin were tested as follows: (1) the drug micellularly solubilized by 5% and 10% of polysorbate 80 in buffer solution adjusted to pH 2.0, 4.0 or 8.0; (2) the solutions of drug in 60% polyoxyethylene glycol 400 buffer mixture adjusted to pH 2.0 or 8.0; (3) the true solution of drug in phosphate buffer pH 8.0; and (4) the drug in aqueous suspension prepared with 1% tragacanth adjusted to pH 2.0.

Animals

Male rats of the Wistar strain weighing between 180 and 230 g, randomly chosen, were used.

The animals were fasted for 24 h prior to the experiment but were allowed free access to water. Dosage of 5 mg kg^{-1} of indomethacin was given orally by a stomach tube. At 0.5, 1, 1.5, 2, 4, 6, 8, 16 and 24 h after administration animals were anaesthetized by intraperitoneal injection of 0.1 g kg^{-1} of methohexital sodium.

The blood samples were withdrawn from the heart after opening the chest, centrifu-

TABLE 1

MEAN SERUM CONCENTRATION ($\mu g\ ml^{-1}$) OF INDOMETHACIN ± S.E.M. AFTER ORAL ADMINISTRATION OF DOSE 5 $mg\ kg^{-1}$

	Dosage form of indomethacin	pH	Time after administration (h)								
			0.5	1	1 5	2	4	6	8	16	24
I	Suspension in 1% tragacanth aqueous solution	2	7.39 ± 1 16	10.01 ± 1.50	21.56 ± 5.19	19.48 ± 2.87	17.48 ± 3.14	10.76 ± 0.852	10.44 ± 4.97	8.27 ± 1.05	1.42 ± 0.455
II	Solution in 60% PEG–buffer mixture	2	12.68 ± 2.41	17.88 ± 2.02	23.03 ± 0.50	16.93 ± 1.16	19.00 ± 1.87	13.88 ± 1.41	16.37 ± 1.37	6.6 ± 1.56	2.27 ± 0.20
III	Micellar buffered solution with 5% of polysorbate	2	20.12 ± 2.37	21.30 ± 3.18	24.01 ± 2.16	20.92 ± 2.01	19.16 ± 1.34	17.16 ± 3.61	11.71 ± 0.57	4.73 ± 0.63	1.35 ± 0.20
IV	Micellar buffered solution with 10% of polysorbate	2	14.47 ± 1.84	22.39 ± 2.06	24.24 ± 2.72	19.62 ± 1.72	17.96 ± 1.39	17.33 ± 2.10	10.36 ± 1.33	7.62 ± 1.31	2.05 ± 0.35
V	Micellar buffered solution with 10% of polysorbate	4	28.92 ± 2.31	30.79 ± 6.35	34.84 ± 4.17	27.93 ± 0.53	19.64 ± 1.37	17.08 ± 1.84	13.17 ± 0.79	3.90 ± 0.489	0 85 ± 0.17
VI	True solution in phosphate buffer	8	22.20 ± 3.85	28.04 ± 2.00	29.66 ± 2.07	25.59 ± 2.47	14.71 ± 0.57	10.31 ± 0.91	12.2 ± 0.74	4.58 ± 0.79	0.76 ± 0.23
VII	Solution in 60% PEG–buffer mixture	8	17.72 ± 1.56	26.92 ± 2.13	26.79 ± 2.72	21.12 ± 1.35	21.72 ± 1.75	14.44 ± 2.21	9.06 ± 1.28	4.04 ± 1.04	1.26 ± 0.18
VIII	Micellar buffered solution with 5% of polysorbate	8	27.8 ± 3.81	28.02 ± 2.93	35.02 ± 0.75	21.69 ± 2.27	23.14 ± 4.19	17 46 ± 1.63	9.61 ± 1.23	3.09 ± 1.22	0.92 ± 0.2
IX	Micellar buffered solution with 10% of polysorbate	8	28.6 ± 4 63	30.18 ± 4.73	36.43 ± 1.40	18.74 ± 1.67	14.57 ± 1.09	13.88 ± 0.89	13.82 ± 1.62	5.88 ± 1.42	1.73 ± 0.35

TABLE 2

ABSORPTION CHARACTERISTICS OF INDOMETHACIN ADMINISTERED ORALLY AT A DOSE OF 5 mg kg^{-1} IN VARIOUS LIQUID FORMULATIONS

Dosage form of ID		pH	C_{max} ($\mu g\ ml^{-1}$)	T_{max} (h)	AUC_0^∞ ($\mu g\ h^{-1}\ ml^{-1}$)
I	Suspension in 1% tragacanth aqueous solution	2	21.56 ± 12.71 [6] *	1.5	230.80
II	Solution in 60% PEG 400 – buffer mixture	2	23.03 ± 1.12 [5]	1.5	274.54
III	Micellar buffered solution with 5% of polysorbate 80	2	24.01 ± 4.33 [4]	1.5	222.0
IV	Micellar buffered solution with 10% of polysorbate 80	2	24.24 ± 9.02 [11]	1.5	259.01
V	Micellar buffered solution with 10% of polysorbate 80	4	34.84 ± 10.22 [6]	1.5	224.46
VI	True solution in phosphate buffer	8	29.66 ± 4.62 [5]	1.5	225.95
VII	Solution in 60% PEG 400 – buffer mixture	8	26.79 ± 8.18 [9]	1.5	225.85
VIII	Micellar buffered solution with 5% of polysorbate 80	8	35.02 ± 2.10 [6]	1.5	240.0
IX	Micellar buffered solution with 10% of polysorbate 80	8	36.43 ± 4.43 [10]	1.5	250.88

* Figures in parentheses refer to the number of animals.

ged and serum obtained was kept in a refrigerator until analysis.

Assay of indomethacin in serum was performed spectrofluorimetrically with Aminco Bowman spectrofluorimeter according to the method of Holt and Howkins (1965) modified by Hvidberg et al. (1972).

Serum concentrations were calculated as the values representing the mean ± S.E.M. of at least 4-animals (Table 1). The bioavailability parameters: maximum serum concentration (C_{max}), time taken to reach the maximum serum concentration (T_{max}) and area under the serum concentration–time curve (AUC_0^∞), estimated according to the trapezoidal rule, are shown in Table 2. C_{max} values obtained after the administration of different liquid formulations of indomethacin were statistically evaluated with Student's *t*-test, *F*-test and C-test of Cochran and Cox. A probability value (*P*) of 0.05 or less was considered significant.

RESULTS AND DISCUSSION

In the light of the results obtained in the present work it could be generally stated that solubilization of indomethacin in the aqueous-buffered (pH 2–8) solution of non-ionic surface-active agents did not reduce the gastrointestinal absorption of the drug in rats – relative to the systems containing no surfactant.

The presence of polysorbate 80 in the formulation did not markedly affect the area

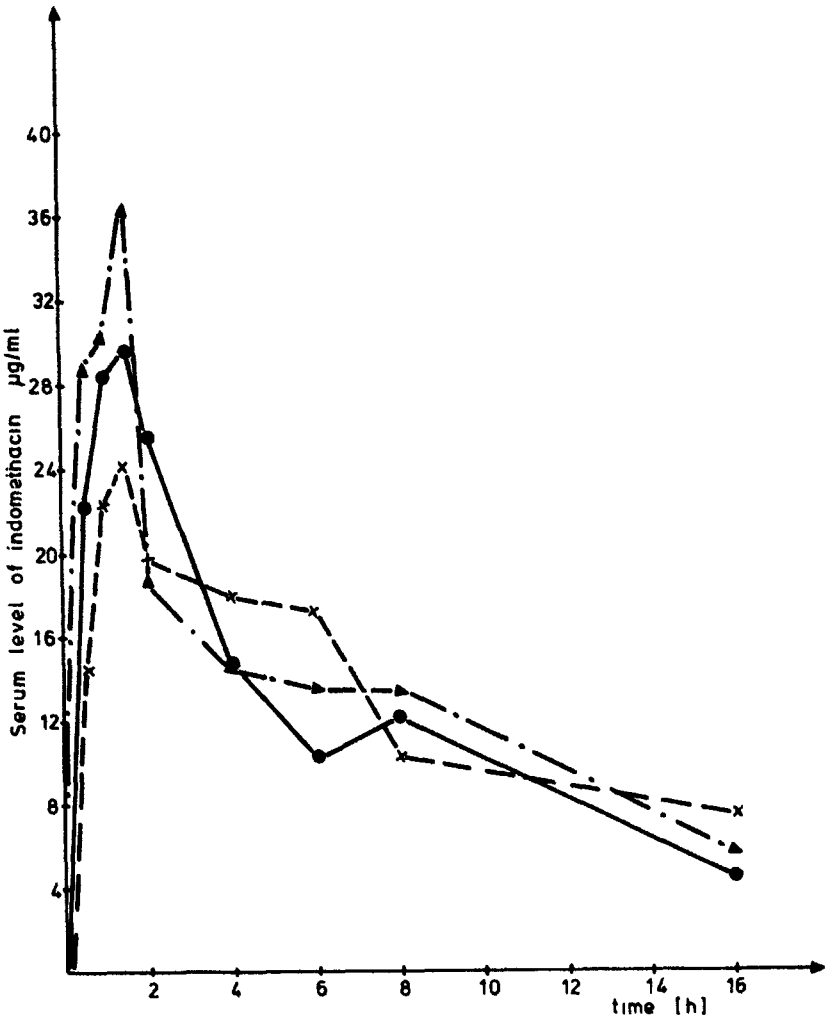

Fig. 1. Effect of surfactant added to the liquid formulation of indomethacin at different pH on mean serum level of drug after oral administration of dose, 5 mg kg^{-1}. (●———●), true solution, pH 8 without surfactant; (▲· – · – ·▲), solution with 10% of polysorbate 80, pH 8; (X– – –X), solution with 10% of polysorbate 80, pH 2.

under serum concentration–time curve (AUC_0^∞) in the most of the cases or the time taken to reach the maximum serum concentration of the drug (C_{max}) (Table 2).

The value of C_{max} after administration of indomethacin in phosphate-buffered solution (pH 8) indicates that this drug is much more readily absorbed in ionized form (pK_a 4.6) than in unionized (pH 2).

The differences in C_{max} values between the formulations I and VI or II and VI are statistically significant ($P < 0.05$). Significant differences are also seen in the case of micellar solutions at extreme values of pH (formulation III and VIII or IV and IX).

The influence of surfactant on C_{max} values in case of unionized form of indomethacin (pH 2) or partly ionized (pH 4) is negligible but quite distinct in case of completely ionized molecules (pH 8). The differences in C_{max} values between formulation VI and VIII or VI and IX are statistically significant ($P < 0.05$; Fig. 1).

The magnitude of surfactant concentration seems to have no effect on the bioavailability parameters. Two-fold increase of this concentration at pH 2 as well as at pH 8 (formulation III and IV or VIII and IX) does not reflect the differences in C_{max}.

It was stated previously (Krasowska, 1979) that indomethacin at pH 8 is quite well solubilized in polysorbate 80 solution. If the statement that a drug entrapped into micelles does not have the ability to penetrate the membrane is valid, the results obtained in this study might suggest that in the GI tract the free indomethacin molecules are easily released from the drug–micelle species, especially when an ionized form is considered. It is very likely that ionized molecules of indomethacin, which have a greater affinity to water, occupy the sites near the surface of the micelle, i.e. in the outer hydrophilic polyoxyethylene layer. The release of molecules from this region is much easier than from the vicinity of hydrophobic interior where undoubtedly unionized molecules at pH 2 are buried.

High lipophilicity of the indomethacin molecule, not altering very much with increasing ionization, should also be considered when passive diffusion through a biomembrane is discussed.

Moreover, evidence from the literature (Nissim, 1960) indicates that the effect of surfactant facilitating the drug absorption could also result from the possible interaction of polysorbate 80 with the structure of mucosal membrane. Such a high concentration of surfactant as was used in this study (5% and 10%) could cause some disruption of the mucosal surface and release proteins and phospholipids from the membrane.

This action enhances the membrane permeability to solute (Whitmore et al., 1979).

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