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Medicament Release From Ointment Bases: I. Indomethacin. In Vitro and In Vivo Release Studies.

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ABSTRACT

The in vitro release of indomethacin from 1%, 3%, and 5% indomethacin ointments and its in vivo absorption through the skin of rabbits was investigated. The in vitro release of indomethacin followed zero-order kinetics and was better from an absorption base ointment. No significant differences ($F=3.047$ and $P=0.079$ for the absorption base) and ($F=2.15$ and $P=0.14$ for the hydrophilic base) in the release rate of indomethacin in 1%, 3%, and 5% indomethacin ointments were observed. Indomethacin was most effectively absorbed from absorption ointment bases. A correlation between the in vitro release and the in vivo absorption was found; also, a correlation between the in vivo release pattern of the bases used and the in vivo data reported in the literature was observed.

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INTRODUCTION

Indomethacin was first synthesized in 1963 by Shen and his co-workers. It has anti-inflammatory, antipyretic and analgesic effects (Hort et al., 1965; Hodgkinson et al., 1973; Norcross et al., 1965; O'Brien, 1968; Smyth, 1965; Thompson et al., 1966; Wright et al., 1973; Zachariae, 1966). Unfortunately, like other anti-inflammatory agents it carries the risk of gastrointestinal irritation and a number of other side effects, e.g., nausea, vomiting, headaches, etc.

Relatively little information appears in the literature with regard to the *in vitro* release rate of indomethacin from ointments and the *in vivo* bioavailability. The purpose of this investigation was to examine the influence of different indomethacin-containing ointment bases on its *in vitro* release and *in vivo* availability in rabbits, and thus to correlate, if possible, the *in vitro* and *in vivo* data.

EXPERIMENTAL

Chemicals

The following reagents were used as received from the manufacturers. Indomethacin and 1-p-fluorobenzoyl-5-methyl-indole acetic acid (MSD, West Point, PA), emulsion base (hydrophilic ointment base, Clay-Park Labs, Bronx, N.Y.), absorption base (Acquaphor, Pharmaderm, Melville, N.Y.), cellophane membrane (M.W. cut-off point 1,000, Spectrum Medical Industries, L.A., Calif), and acetonitrile, acetic acid and methanol of chromatographic grade (Waters Associates, Millford, MA).

Preparations of Ointments

Indomethacin, previously reduced to a fine powder in a mortar, was incorporated at 1, 3, and 5% concentrations into two different bases by levigation. A 1% indomethacin suspension for oral use was also prepared by dissolving the appropriate amount of indomethacin in 0.1N HCl.

Release from Ointments

Five one ounce glass ointment jars were filled with ointment, and the excess was removed with the edge of a spatula to produce an even surface. The open end was covered by a natural cellulose membrane with a molecular weight cut off point of 1000 and sealed by a silk thread, so that the entire exposed surface of the ointment was in contact with the membrane. The ointment jars were then immersed in a 200 ml beaker containing 100 ml of pH = 8 phosphate buffer (0.2N). The beakers were then placed in a constant temperature water bath at 37° ± 1° to allow the diffusion to proceed. At each sampling interval an aliquot of the diffusion medium was drawn off and assayed spectrophotometrically. The volume of the solution was kept constant by replacing the volume of the samples with equal volumes of the diffusion medium. Blank ointment samples were run simultaneously to check for any interference.

In Vivo Study

New Zealnad white male rabbits weighing 2-4 kg were utilized. Three rabbits were designated for each ointment strength and for the oral suspension. The rabbit's skin was shaved and an area of 3" x 3" was uniformly covered with each ointment. Blood samples were obtained by cardiac puncture at time intervals of 15, 30, 45, 60, 90, 120, 150, and 180 minutes. The blood was allowed to clot at room temperature for 15 minutes and then centrifuged at 2000 rpm for 30 minutes. The serum was separated and kept in a freezer until the analysis was carried out. A quantity of 1% indomethacin suspension equal to the quantity of the ointment applied to each rabbit was administered orally ino the stomach of the rabbitts with the use of a syringe.

Analytical Method

There are a few methods available for the analysis of indomethacin, but all of them are time consuming and difficult.

Therefore, an accurate and rapid method of analysis was developed utilizing HPLC (Kazmi et al., 1981).

A HPLC (Waters Associates, Milford, MA) equipped with a UV detector (Bondapak C-18) and a microparticulate reverse phase HPLC column 4 mm x 20 cm was used. The mobile phase consisted of an acetonitrile-0.1M acetic acid (55:45 V/V mixture. The operating temperature was ambient, and the flow rate was 1.0 ml/min with an operating pressure of 1500 psig. The column effluent was monitored continyously at 254 nm with a sensitivity setting of 0.1 mcg/ml; the chart speed of the recorder was maintained at 0.2 inch/min.

Internal Standard

About 10 mg of 1-(pfluorobenzoyl)-5-methyl indole acetic acid (MSD, West Point, PA) was accurately weighed out, transferred to a 10 ml volumetric flask, and dissolved with methanol. A 0.1 ml volume of this solution was pipetted to a second 20 ml volumetric flask and diluted with methanol.

Serum Assay

A 0.5 sample of serum spiked with 0.1 ml of the internal standard was pipetted into a glass stoppered centrifuge tube and brought with methanol 20 2.5 ml. The mixture was shaken for 120 seconds and centrifuged for 30 minutes at 2000 rpm. The organic phase was removed and filtered through an organic filter. A 10 ml portion was injected into the column of the HPLC through a stop-flow injection port.

Calibration curves were prepared, using known concentrations of indomethacin with plasma, by plotting the concentration of indomethacin (mcg/ml) against the respective peak ratios. The standard curve developed was based on the average of three determinations, and the regression line slope was calculated to be 1.3 with a standard correlation matrix of 0.9986 indicating excellent linearity (Kazmi et al., 1981).

RESULTS AND DISCUSSION

The release characteristics of different concentrations of indomethacin from the two ointment bases over a one hour period is shown in Figures 1 and 2. The increase in the indomethacin concentration of the dissolution medium at different time intermals was used to assess the rate of the drug release from the ointments. The linearity of the plots appears to indicate that the release of indomethacin follows apparent zero-order kinetics. A one-way ANOVA with repeated measures analysis (Table 1) between 1%, 3%, and 5% indomethacin ointments in the absorption base indicated that there was no significant difference ($F=3.047$ and $P=.079$) in the release rate of indomethacin. The same holds true for the different concentrations of indomethacin in the hydrophilic base ($F=2.15$, $P=0.14$). A significant difference, however, was found from sample to sample within the same concentrations.

In Vivo Studies

The plasma indomethacin concentration - time relationships obtained from three rabbits after application of different concentration ointments are shown in Table 2, and Figure 3 and 4. As can be seen from the data, the peak levels obtained were 1.19, 1.69 and 2.37 mcg/ml for the 1, 3, and 5% concentrations, respectively, in the hydrophilic ointment and 1.12, 1.72, and 2.68 mcg/ml for the 1, 3, and 5% concentrations, respectively, in the absorption base, and 1.33 mcg/ml for the 1% indomethacin suspension. Furthermore, the average peak of indomethacin concentration obtained after percutaneous application was greater from the hydrophilic base for all indomethacin ointment concentrations except the 1%. These findings are in agreement with previously reported data (Naito et al., 1981).

However, Naito reported a lag time of about two hours for the percutaneous absorption of indomethacin. We were able to

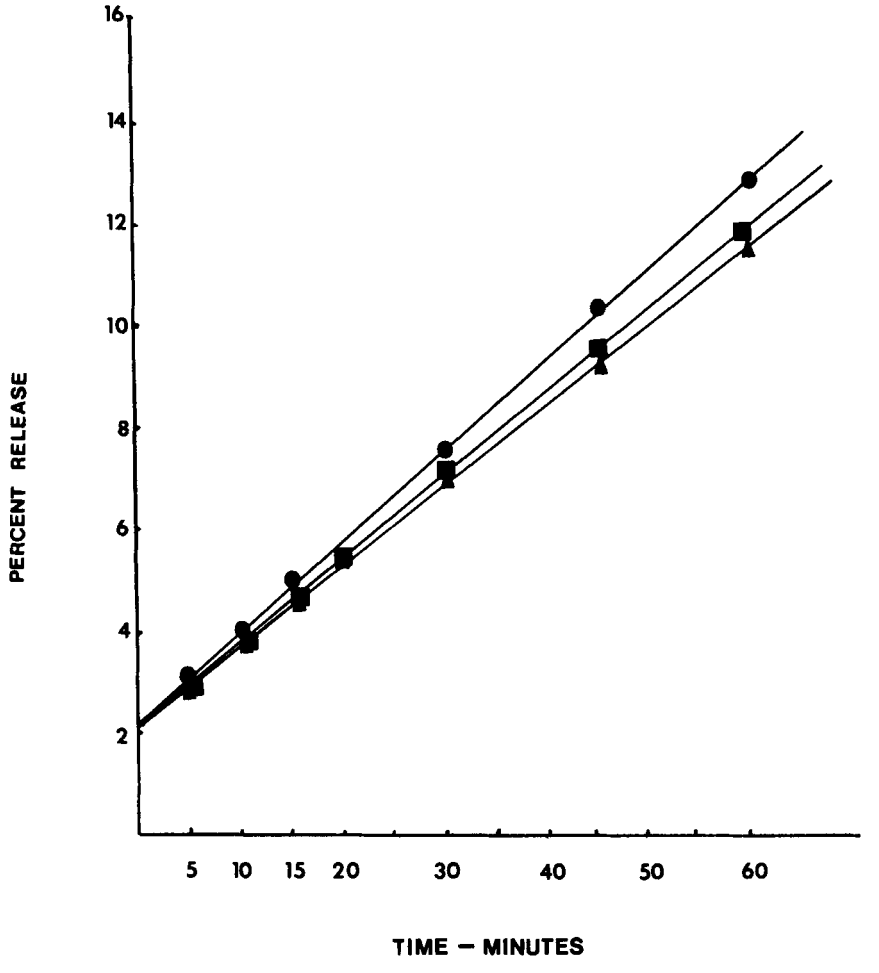

FIGURE 1.

Results of indomethacin release from hydrophilic base.
Key: ● 1% indomethacin; ▲ 3% indomethacin; ■ 5% indomethacin

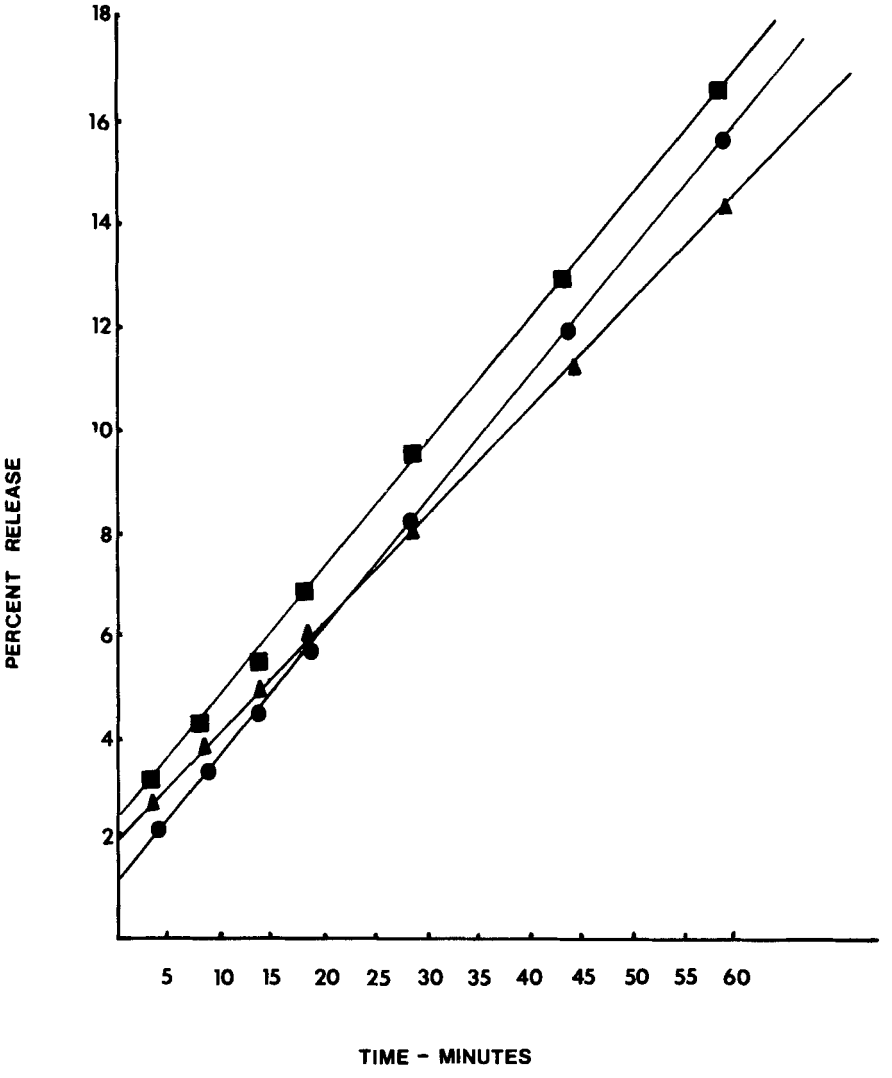

FIGURE 2

Release of indomethacin from absorption base.
Key: ● 1% indomethacin; ▲ 3% indomethacin; ■ 5% indomethacin

TABLE 1

ANALYSIS OF VARIANCE OF RELEASE OF INDOMETHACIN FROM OINTMENT BASES

Base	Concentration Indomethacin %	ANOVA F-Ratio	ANOVA Probability
Hydrophilic	1	4.19	0.936[a]
"	3	7.94	0.034[a]
"	5	9.01	0.0019[a]
Comparison between 1, 3, and 5%		2.154	0.145[b]
Absorption	1	7.444	0.0044[a]
"	3	6.437	0.0104[a]
"	5	6.082	0.0126[a]
Comparison between 1, 3, and 5%		3.047	0.0797[b]

[a]significant [b]not significant

detect indomethacin in plasma thirty minutes after indomethacin application with a mean maximum peak plasma indomethacin concentration of about 1.2 mcg/ml reached in about two hours. The absorption of indomethacin from all bases as well as from the orally administered was rapid, as reflected by the relative short time to attain the peak plasma indomethacin level (Figures 3 and 4 and Table 2). The time of occurence of the maximum peak level was longer after the hydrophilic and absorption bases (120 min.) than that after oral administration (60 min.). The area under the plasma concentration - time curve (AUC) was calculated by the trapezoidal rule. In general, the AUC for the 1% indomethacin in the absorption ointment base (w/o) was 44.30% and for the 1% indomethacin in the hydrophilic ointment base

TABLE 2

RELATED BIOAVAILABILITY PARAMETERS FROM SERUM LEVEL DATA

Parameter	Hydrophilic			Absorption base			Suspension
	1%	3%	5%	1%	3%	5%	1%
Average of peaks of individual serum concentration (time curves) (mcg/ml)	1.19	1.69	2.37	1.12	1.72	2.68	1.33
Time of peak value of individual serum concentration (time curve) (mcg/ml)	120	120	120	120	120	120	60
Average of area under individual serum concentration (time curves) (mcg/ml x min)	25.99	58.98	105.82	34.83	78.57	145.65	70.45

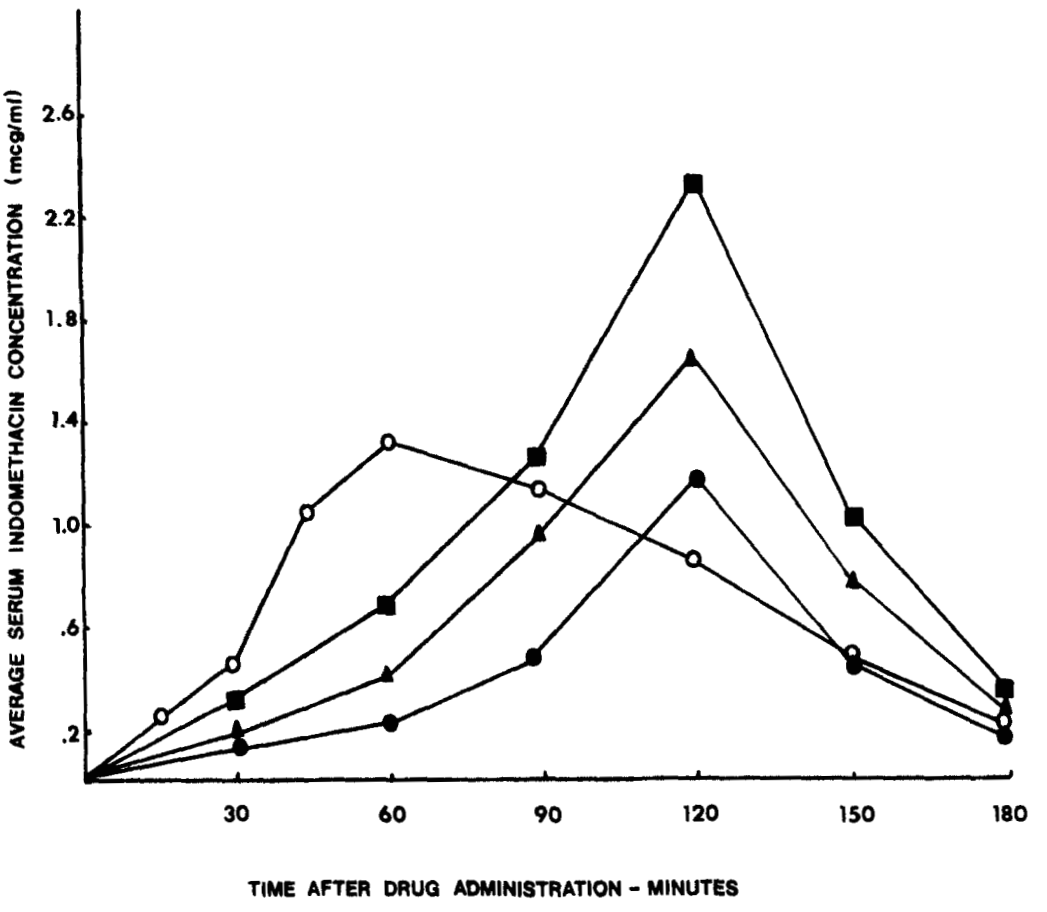

FIGURE 3

Average serum indomethacin concentration (mcg/ml) obtained for three rabbits after topical application of indomethacin ointment is hydrophilic base.
Key: O suspension; ● 1% indomethacin; ▲ 3% indomethacin; ■ 5% indomethacin

(o/w) was 33.00% in comparison to the AUC of the 1% indomethacin in suspension.

The mean plasma concentration of different concentrations of indomethacin associated with the hydrophilic base, the absorption base and the suspension were statiscially compared, based on three rabbits per each treatment and even sampling time - periods. The analysis of variance for 1-way ANOVA with repeated measures were comducted, and the results are presented in Table 3. The analysis clearly indicated that

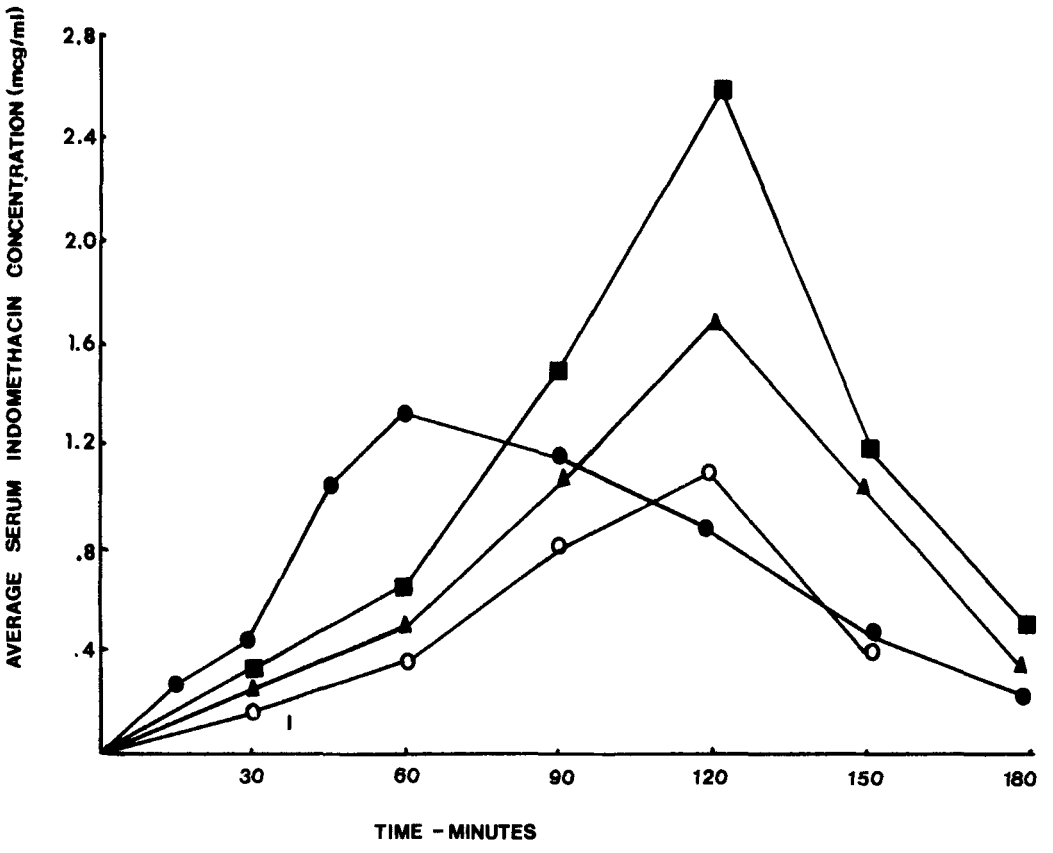

FIGURE 4

Average serum indomethacin concentration (mcg/ml) obtained for three rabbits after topical application of indomethacin ointment in absorption base.
Key: ● suspension; ○ 1% indomethacin; ▲ 3% indomethacin; ■ 5% indomethacin

there are significant differences from rabbit to rabbit both in hydrophilic and absorption bases. Furthermore, the mean plasma concentration of the treatments for the hydrophilic base are not significant while there is a significant difference between 1, 3, and 5% indomethacin in the absorption base. It is apparent from Figures 1 and 2 that indomethacin was released better from the absorption base than from the hydrophilic base. In addition, the bioavailability of indomethacin (Table 2) was better from the absorption base than from the hydrophilic base. This is in agreement with the in vivo data reported previously (Naito et al., 1981).

TABLE 3

ANALYSIS OF VARIANCE OF PLASMA CONCENTRATION
(1-WAY ANOVA WITH REPEATED MEASURE)

Indomethacin Concentration	F	Probability
Emulsion Base		
1%	4.331	0.0343[a]
3%	5.377	0.0182
5%	4.222	0.0367[a]
Comparison 1%, 3%, and 5%	1.483	0.2602[b]
Absorption Base		
1%	12.46	0.0019[a]
3%	8.209	0.0044[a]
5%	9.939	0.0021[a]
Comparison 1%, 3%, and 5%	4.039	0.0456[a]
Suspension	18.2300	0.0000

[a]significant [b]not significant

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