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The inhibitory effect of liposome-encapsulated indomethacin on inflammation and platelet aggregation

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Abstract—A comparison has been made between liposome-encapsulated and free indomethacin for their anti-inflammatory activities in the carrageenan paw oedema test in rats, and their inhibitory effect on platelet aggregation induced by adenosine 5-diphosphate (ADP) in-vitro. Free indomethacin, 3 mg kg^{-1}, strongly inhibited carrageenan-induced oedema and a similar inhibitory activity was shown by 0·3 mg kg^{-1} of encapsulated drug. For the inhibition of platelet aggregation, the threshold concentration of free drug was 0·559 mM. At this concentration, at least 5 min incubation was needed to achieve 12·5% and 45 min for 50% inhibition. The inhibition was much stronger with encapsulated drug, and pre-incubation of 28 μM encapsulated drug for 10 min with platelet-rich plasma before addition of ADP completely inhibited platelet aggregation.

The side effects of indomethacin detract from its merit as an anti-inflammatory drug and, in an attempt to reduce the dose, an evaluation of the drug encapsulated in positively charged multilamellar liposomes has been made relative to free drug for its anti-inflammatory activity in rats and inhibitory effect on platelet aggregation in-vitro.

Materials and methods

Materials. Egg yolk phosphatidylcholine (Type XI-E), stearylamine, (±)-α-tocopherol and indomethacin were from Sigma Chemicals Co., St Louis, Mo, USA, cholesterol from Boehringer Manheim GmbH, FRG, Triton x-100 from BDH Poole, UK, Sephadex G-50 fine from Pharmacia, Uppsala, Sweden. The phospholipid test system used was an enzymatic colorimetric test from Boehringer Manheim GmbH, FRG. Adenosine 5-diphosphate (ADP) was from Agregatest, Diagnostica stago, France. All other chemicals were of analytical reagent grade or better. Phosphate-buffered saline (pH 7·2) contained 1·420 g disodium hydrogen phosphate 2-hydrate, 0·270 g potassium dihydrogen phosphate and 7·5 g sodium chloride in 1 L distilled water.

Preparation of liposomes. Positively charged multilamellar liposomes containing indomethacin were prepared essentially as described by Gürsoy & Akbuğa (1986) and were composed of phosphatidylcholine cholesterol and stearylamine in the molar ratio (10:5:1) and tocopherol ($2·3 \times 10^{-4}$ M). The lipids and indomethacin were dissolved in chloroform and evaporated to dryness under vacuum. Phosphate-buffered saline was added and the mixture vigorously agitated until all the lipid had dispersed. Liposomes were gel-filtrated on a Sephadex G-50 column to remove free indomethacin and to improve the size distribution by removing the smallest liposomes. The mean diameter of liposomes as measured by electron microscope (jem-100, Jeol) with the negative staining technique of Bangham & Horne (1964), was 1·02 μM (±0·260 s.d.). The indomethacin content was assayed spectrophotometrically at 320 nm after the addition of Triton x-100 to release the drug. The phosphatidylcholine concentration was determined by the phospholipid test system.

Animal experiments. Five groups of adult male Wistar albino rats, 125–165 g, were fasted for 18 h but had free access to water. Then, immediately before the i.p. administration of each compound, the basal volume of the right hind paw was measured by means of a volume differentialmeter (Ugo Basil). The rats were then injected with: (i) phosphate-buffered saline (1 mL kg^{-1}), (ii) empty liposomes (4·8 mg phosphatidylcholine mL kg^{-1}), (iii) and (iv) a solution of free indomethacin (0·3 or 3·0 mg indomethacin kg^{-1}), (v) liposome-encapsulated indomethacin (0·3 mg indomethacin kg^{-1}). 30 min later the right hind paw was injected intraplantarly with 0·05 mL of 1% carrageenan in saline. Each hour thereafter, for 4 h, the volume of the carrageenan-challenged paw was measured and the difference, as a % of base line volume, determined.

Preparation of platelet-rich plasma (PRP). Venous blood samples, from volunteers who had not taken any medication known to affect platelet aggregation for at least one week, were taken into 3·8% trisodium citrate solution and centrifuged at 1500 rev min^{-1} for 12 min to give PRP with platelet counts between 150 000–350 000 mm^{-3}.

Platelet aggregation test. Platelet aggregation was assessed by the turbidimetric method of Born (1962), using an aggregometer (Bio/Data Corporation Model PAP-2A USA). ADP was used as an aggregation agent, and its concentration was so chosen to yield an aggregation curve for each sample of PRP, its final concentration in PRP ranging between $1·1 \times 10^{-6}$ and $2·3 \times 10^{-6}$ M. The inhibition (percent) of ADP-induced platelet aggregation was calculated according to Kobayashi & Didisheim (1973). The maximum increase in light transmission that occurred after addition of ADP to the control PRP sample at 37 °C was assigned the value of 100%. The inhibition (percent) was determined by comparing that value with those from PRP samples similarly treated with free indomethacin at 37 °C for 5, 10, 15, 45 min before, or encapsulated indomethacin for 10 min and just before, addition of ADP.

Statistics. The data were analysed for significance of difference by Student's *t*-test.

Results

Anti-inflammatory effect. As shown in Fig. 1, free and encapsulated indomethacin at doses of 3·0 and 0·3 mg kg^{-1}, respectively, markedly reduced the carrageenan-induced oedema in rat paw from 2 h after injection. Free indomethacin at a dose of 0·3 mg kg^{-1} or empty liposomes (4·8 mg phosphatidylcholine kg^{-1}) also caused a significant reduction but only from 3 h after injection. However, a significant

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difference was found between free and encapsulated indomethacin at the dose of 0·3 mg kg^{-1} with respect to inhibition of carregeenan-induced oedema from 2 h after injection.

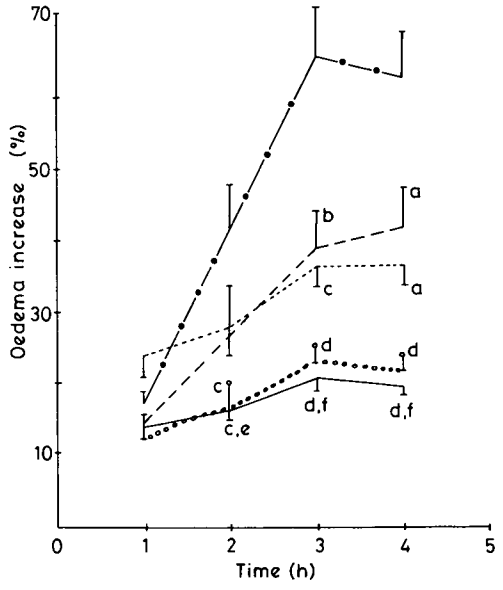

FIG. 1. Effects of free and encapsulated indomethacin on the development of oedema after intraplantar injection of carrageenan. The test compounds were given i.p. 30 min before the carrageenan injection. The basal volume of each rat paw was taken as 100% and variations from this value were given as percent difference. Vertical bars denote s.e.m. (——·——) Phosphate-buffered saline (8), (————) empty liposomes (7), (- - - - -) 0·3 mg kg^{-1} free indomethacin (10), (o o o) 3·0 mg kg^{-1} free indomethacin (10), (————) 0·3 mg kg^{-1} encapsulated indomethacin (10). The number of rats used are given in parentheses. $^{a}P < 0·05$, $^{b}P < 0·002$, $^{c}P < 0·01$, ^{d}P 0·001 versus phosphate-buffered saline group; $^{e}P < 0·02$, $^{f}P < 0·001$ versus 0·3 mg kg^{-1} free indomethacin group.

In-vitro inhibition of ADP-induced platelet aggregation. The addition of phosphate-buffered saline and different concentrations of empty liposomes to PRP had no effect on ADP-induced platelet aggregation, while the threshold anti-aggregating concentration of free indomethacin in phosphate-buffered saline was 0·559 mM. As shown in Table 1, the incubation of free drug (0·559 mM) for 5, 10, 15 and 45 min with PRP increased the percent inhibition of ADP-induced aggregation but only to 50% at 45 min.

On the other hand, the inhibition of aggregation by encapsulated indomethacin was dependent on the drug concentration and was much stronger when the liposomes were preincubated with PRP for 10 min before addition of ADP.

Table 1. The percent inhibition of platelet aggregation with 0·559 mM free indomethacin preincubated for 5, 10, 15, 45 min with PRP before addition of ADP (mean of 10 experiments ± s.d.).

	Incubation time (min)			
	5	10	15	45
% Inhibition	12·5 ±2·1	22·0 ±5·7	22·5 ±6·8	50·0 ±6·6

The difference between 5–10 min and 15–45 min incubation times was significant ($P < 0·001$).

There was a linear relation between concentration of encapsulated drug and percent inhibition of aggregation. With the 14 and 28 μM concentrations of the encapsulated drug without preincubation the inhibition of aggregation was 38 and 59 percent, respectively (n = 10). Although incubation of free drug for 45 min with PRP gave only 50% inhibition, incubation of 28 μM encapsulated drug for 10 min resulted in almost complete inhibition of platelet aggregation.

Discussion

Free indomethacin, 3 mg kg^{-1}, strongly inhibited carrageenan-induced oedema while the encapsulated drug at 0·3 mg kg^{-1} had a similar effect (Fig. 1). Also, much lower concentrations of encapsulated indomethacin than free drug were required to inhibit platelet aggregation, the inhibition by the encapsulated drug being dependent on dose. The inhibitory effect was increased by prolonging the incubation time of both free and encapsulated drug with PRP. But with encapsulated drug the inhibition was much stronger.

It is well-known that liposomes are taken into cells by fusion and endocytosis. If this is so, with platelets the indomethacin-containing liposomes will fuse directly with the platelet membrane thereby reducing the time and dose of indomethacin needed for inhibition of aggregation. This may partly explain the enhanced inhibition of aggregation with small doses of encapsulated indomethacin in such a short time.

References

Bangham, A. D., Horne, R. W. (1964) J. Mol. Biol. 8: 660–668

Born, G. V. R. (1962) Nature 194: 927–929

Gürsoy, A., Akbuğa, J. (1986) Proc. 4th Int. Conf. Pharm. Tech., Paris. Association de Pharmacie Galenique Industrielle. pp 207–211

Kobayashi, I., Didisheim, P. (1973) Thromb. Diath. Haemorrh. 30: 178–190