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Enhancement of anti-inflammatory effects of biphenylylacetic acid by its incorporation into lipid microspheres

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Using lipid microspheres (LM), average diameter 0·2 μm, and containing methyl and ethyl esters of biphenylylacetic acid (BPAA) in the carrageenan paw oedema tests in rats, it was found that their anti-inflammatory activities were enhanced 3 to 8 times over that of free BPAA. By electron microscopy, LM were seen to be taken up into the endothelial cells of the blood vessels and macrophages at the inflamed sites. In a study using dogs, after an intravenous injection of BPAA-methyl ester incorporated into LM (lipo-BPAA-Me), lipo-BPAA-Me rapidly disappeared from the blood and the BPAA serum level was gradually elevated. These results, together with previous findings, suggest that part of lipo-BPAA-Me was immediately transferred to the inflamed site and taken up by prostaglandin (PG)-producing cells as macrophages. It is considered that the anti-inflammatory effects of BPAA are enhanced by incorporating it into LM.

As Mizushima et al (1982, 1983a, b) reported previously, lipid microspheres (LM) with a particle size of 0·2 μm can be used as a drug delivery carrier of anti-inflammatory drugs. LM, having a similar cellular and tissue distribution to liposomes, are widely used for parenteral nutrition in man without any side effects (Wretlind 1978). We have prepared LM containing biphenylylacetic acid (BPAA) esters (lipo-BPAA) and examined the preparations for its anti-inflammatory effects. An electron microscopy study was also undertaken to observe the accumulation of LM in the inflamed tissues and distribution into PG-producing cells which are targets of non-steroidal anti-inflammatory drugs. In addition, pharmacokinetics of BPAA-Me and BPAA were studied in dogs to examine the distribution and metabolism of lipo-BPAA after its intravenous injection.

MATERIALS AND METHODS

Animals

Male, Wistar rats of 160–220 g (Japan Clea Co.), male, ICR mice of 25–30 g (Japan Charles River Co.), and male beagles of 8·5–10 kg were used.

Drugs

4-Biphenylylacetic acid methyl ester (BPAA-Me), 4-biphenylylacetic acid ethyl ester (BPAA-Et) and sodium 4-biphenylylacetic acid (BPAA-Na) were synthesized at the Chemical and Formulation Laboratory, Lederle (Japan), Ltd. These products were identified by infrared spectrophotometry and nuclear magnetic resonance. λ-Carrageenan was purchased from PASCO International Co., Ltd, Tokyo.

Incorporation of the drugs into LM

BPAA-Me and BPAA-Et were dissolved in soya bean oil at 200 mg ml^{-1} as BPAa, soya bean lecithin was added, and the preparation was made in a manner similar to that described previously (Mizushima et al 1982) to make a final concentration of 20 mg ml^{-1} as BPAA (lipo-BPAA-Me and lipo-BPAA-Et). LM alone were used as a control.

Carrageenan paw oedema

Carrageenan 1% in 0·05 ml 0·9% NaCl (saline) was injected into the left hind paw of rats. Two hours later the rats were given i.v. injections of lipo-BPAA-Me, lipo-BPAA-Et, BPAA-Na, LM alone, or saline alone at a volume of 0·1 ml 100 g^{-1} body weight. Lipo-BPAAs were diluted with 10% LM to appropriate concentrations. The oedema volume, measured with a plethysmometer, was expressed by deducting the pretreatment volume from that measured after the carrageenan injection.

Preparations of specimens for electron microscopy

Two hours after the carrageenan injection, 0·1 ml of LM alone was administered intravenously to rats

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which were killed 10 min later. Tissues were removed from both carrageenan-injected and non-injected paws, and were immediately fixed with 1% solution of osmium tetroxide. The specimens for electron microscopy were prepared in the usual way.

Peritoneal macrophages were collected from ICR mice treated with an intraperitoneal injection of 3% thioglycolate (Difco Laboratories, USA) 4 days before sample collection. The exudate was centrifuged at 1500 rev min^{-1} for 5 min and washed 3 times with saline. Then, the packed cells were fixed with 1% solution of osmium tetroxide and specimens were prepared for electron microscopy using a JEOL (Nippon Denshi) electron microscope.

Measurement of serum BPAA and BPAA-Me levels

A dose of 5 mg kg^{-1} as BPAA of lipo-BPAA-Me or BPAA-Na was injected into a catheter inserted into the vein of the left forefoot of dogs. Blood samples were collected every hour from a catheter in the vein of the right forefoot. Immediately after the sample collection, 2·0 ml of methanol was added to 0·1 ml of whole blood in order to terminate the esterase activity and to remove the proteins. The mixture was then centrifuged at 2500 rev min^{-1} for 10 min and the supernatant tested. BPAA and BPAA-Me were quantified by high pressure liquid chromatography on a Cosmosil C_{18} (Nakarai Co.) column with a Water Associates Model 441 absorbance detector. flow rate was 1 ml min^{-1}, wavelength for detection was 254 nm, and MeOH–H_2O–MeCOOH (80 : 20 : 1) was used as a flow solvent.

Solubility in soya bean oil

Each drug was added to refined soya bean oil (Ajinomoto Co., Ltd) at concentrations of 5, 10, 15, 20, 50, 100 and 200 mg ml^{-1}. The suspensions were vigorously shaken at room temperature (20 °C) for 30 s every 5 min for a total of 30 min.

Statistics

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

RESULTS

Anti-inflammatory effects of lipo-BPAA.

Fig. 1 shows changes over time of the oedema volume following administrations of BPAA-Na, lipo-BPAA-Me, and lipo-BPAA-Et. All the preparations inhibited significantly the carrageenan oedema in a dose-dependent manner. LM alone showed no significant inhibition. The dose-response curves of lipo-BPAA-Me, lipo-BPAA-Et, and BPAA-Na, plotted from the data 4 h after the drug injection shown in Fig. 1, are exhibited in Fig. 2. When the doses that attained 20% inhibition are compared, the anti-inflammatory activities of lipo-BPAA-Me and lipo-BPAA-Et were approximately 8 and 3 times greater than that of BPAA-Na, respectively.

Distribution of LM to the inflamed site and PG-producing cells

Electron micrographs of the carrageenan-induced inflamed tissues showed that the endothelial cells of blood vessels contained LM and that LM penetrated

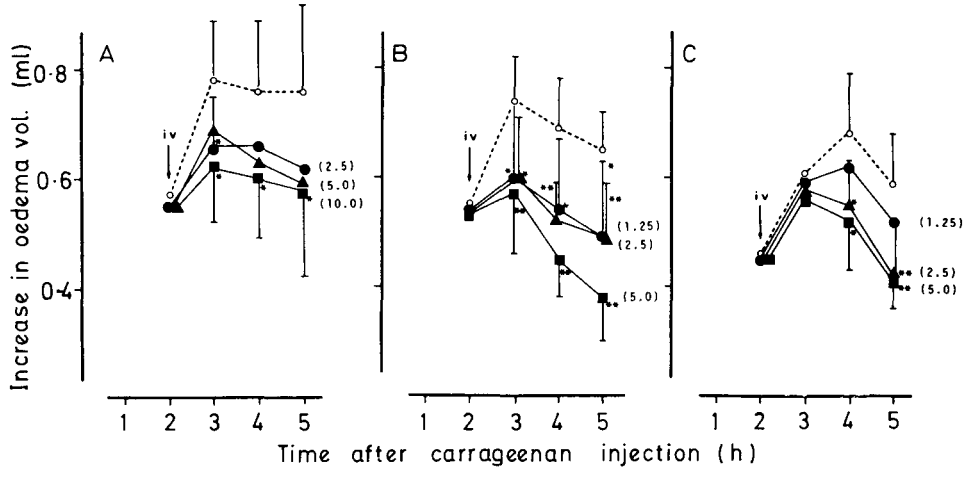

FIG. 1. Inhibitory effects of BPAA-Na (A), lipo-BPAA-Me (B), and lipo-BPAA-Et (C) on carrageenan oedema in rats. Drugs were injected intravenously 2 h after carrageenan. O—O: control (saline alone). Significantly different from the control groups at $P < 0·05$ (*) and $P < 0·01$ (**). Numbers in parentheses show drug dose as BPAA in mg kg^{-1}.

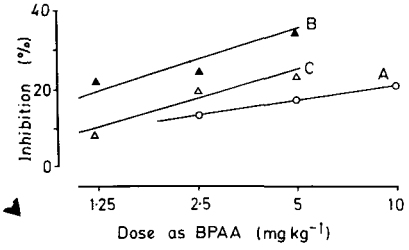

FIG. 2. Dose response curve of the inhibitory effects of BPAA-Na (A), lipo-BPAA-Me (B) and lipo-BPAA-Et (C) at 4 h after carrageenan. Drugs were injected intravenously 2 h after carrageenan and the volume of rat paw oedema was measured 4 h later.

from basement membrane to the outer layer of the vessels and accumulated in the inflamed tissues (Fig. 3). No LM were observed in the normal tissues. Peritoneal fluid was obtained 10 min after an intraperitoneal injection of 0·1 ml of LM to the mice

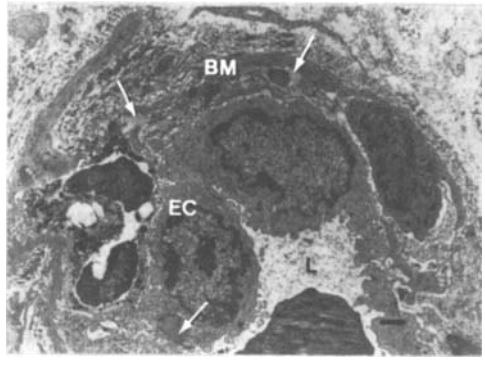

FIG. 3. Electron micrograph of inflamed tissue induced by carrageenan 10 min after LM injection. LM are taken up by endothelial cells of blood vessels. Penetration and accumulation of LM in outer layer of blood vessels are also recognized. (see arrows). Some LM fuse to each other and make larger particles. EC, endothelial cells; BM, basement membrane; L, lumen; bar = 1 μm.

treated previously with thioglycolate. It was shown that many LM were taken up by peritoneal macrophages (Fig. 4).

Pharmacokinetics of lipo-BPAA-Me and BPAA in dogs

When an intravenous injection of 5 mg kg^{-1} of lipo-BPAA-Me was given, most BPAA-Me disappeared from the blood in 3 min, whereas the concentration of BPAA in the blood increased gradually (Fig. 5A). When BPAA-Na was injected intravenously, BPAA reached the peak in 2·5 min,

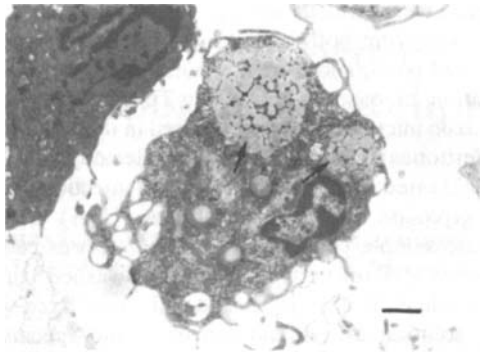

FIG. 4. Uptake of LM by peritoneal macrophages 0·1 ml of LM was injected intraperitoneally into mice treated with thioglycolate 4 days before and peritoneal macrophages were gathered 10 min after the LM injection. Many LM (arrows) were taken up by peritoneal macrophages; bar = 1 μm.

and then decreased gradually (squares in Fig. 5B). The circles in Fig. 5B show the sum of concentrations of BPAA and BPAA-Me after the intravenous injection of lipo-BPAA-Me. A comparison of the areas under the curve (AUC) of total BPAA between lipo-BPAA-Me and BPAA-Na gave a smaller AUC for lipo-BPAA-Me (Fig. 5B). In this experiment, the concentration of BPAA-Na and BPAA-Me was expressed as free BPAA.

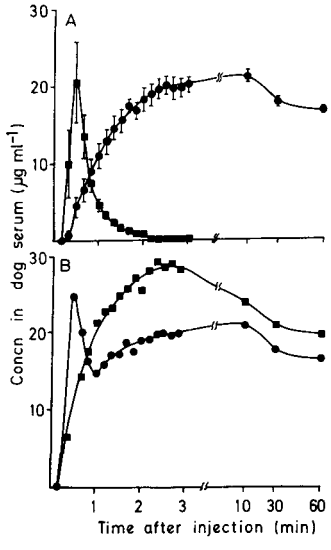

FIG. 5. A, Concentration of BPAA (●) and BPAA-Me (■) in canine serum after i.v. injection of lipo-BPAA-Me (5 mg kg^{-1} as BPAA). All values are mean ± s.e. of 4–7 animals. B, Sum of concentration of BPAA after i.v. injection of lipo-BPAA-Me (●) or BPAA-Na (■). (■) Blood concentrations of BPAA after the injection of BPAA-Na. (●) sum of concentrations of BPAA and BPAA-Me (as BPAA).

Solubility

The solubility in soya bean oil was measured for BPAA-Me, BPAA-Et, indomethacin ethyl ester, and indomethacin ethoxycarbonylmethyl ester. The solubility was between 10 and 15 mg ml^{-1} for indomethacin ethyl ester, between 15 and 20 mg ml^{-1} for indomethacin ethoxycarbonylmethyl ester, and more than 200 mg ml^{-1} for BPAA-Me and BPAA-Et.

DISCUSSION

Our previous study (Mizushima et al 1982) with radiolabelled dexamethasone palmitate incorporated in lipid microspheres indicated that LM accumulate in the inflamed lesion induced by carrageenan. In that study, the approximate distribution ratio of dexamethasone palmitate which had been incorporated into lipid microspheres was based on the drug concentration in non-inflamed paw being 1·0, 2·5 for inflamed paw, 1·3 for muscle, 4·8 for spleen, and 9·5 for liver. When incorporated into LM, indomethacin ethoxycarbonylmethyl ester was 6 times greater in anti-inflammatory activity than free indomethacin (Mizushima et al 1983a). However, the solubility of indomethacin esters in soya bean oil is low, so a stable LM incorporating sufficient indomethacin to produce satisfactory clinical effects was not possible. We therefore, incorporated BPAA esters into LM (lipo-BPAA), because these esters are more lipophilic than indomethacin esters and we found lipo-BPAA to be 3 to 8 times greater in anti-inflammatory activity than free BPAA. The lipo-BPAA preparation containing 20 mg ml^{-1} of BPAA-Me or BPAA-Et was proved stable and is considered to be potent enough for clinical application.

In the experiment in dogs, BPAA-Me disappeared from the blood within a few minutes after the injection of lipo-BPAA-Me and serum BPAA increased gradually. BPAA-Me was not markedly released for a few minutes from the LM incubated in canine serum (unpublished observation). The AUC for BPAA was less following the injection of lipo-BPAA-Me than that of free BPAA-Na (Fig. 5b). These results suggest that most of the lipo-BPAA-Me was immediately transferred to the tissues in the form of LM, and produced free BPAA in the tissues.

The electron microscopy study also supports our previous findings on tissue distribution of LM when studied with radiolabelled LM. The study also revealed that LM were delivered to the inflamed tissues and into macrophages and endothelial cells which are PG-producing cells (Weissmann et al 1979; Weksler et al 1977).

In conclusion, part of the BPAA esters incorporated into LM were distributed immediately to the targeted inflamed sites after the intravenous injection and were taken up by PG-producing cells, thus the anti-inflammatory activity of lipo-BPAA was greater than that of free BPAA.

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