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Inflammatory response to polyacryl starch microparticles, role of arachidonic acid metabolites

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Summary

The effect of polyacryl starch microparticles on arachidonic acid metabolism in macrophage cultures and in mice was studied. When macrophages were incubated with the microparticles, significant amounts of arachidonic acid metabolites were released. Two of the major metabolites were identified and found to be prostaglandin E_2 and leukotriene C_4. Intravenous administration in mice of slowly degradable polyacryl starch microparticles, having a mean diameter of 1 μm, resulted in hepatomegaly and occasionally, inflammatory granulomas in the liver. Rapidly degradable microparticles did not give the same response. When the mice were treated with inhibitors of arachidonic acid metabolism, i.e. indomethacin or timegadine, the microparticle-induced hepatomegaly was partly inhibited. The microparticle-mediated effects were compared with those obtained with a well-characterized macrophage-activating/inflammatory agent, 1,3-β-glucan particles.

Introduction

When injected intravenously, microparticulate drug carriers are usually rapidly phagocytosed by the macrophages of the reticuloendothelial system (Edman et al., 1986). However, few studies have been made on the interaction between the carriers and their target cells. Available data suggest that macrophages can ingest large amounts of the carriers and that the i.v. uptake may result in stimulation of intracellular metabolism and secretion of different inflammatory mediators, such as reactive oxygen intermediates (ROI), hydrolytic enzymes and arachidonic acid metabolites (Artursson et al. 1987a and b; Smith and Hunneyball, 1986).

Similarly, little is known of the effects of drug carriers on the target organ (mainly the liver). Large doses of undegradable drug carriers are likely to saturate the macrophages and thereby induce a (reversible) RE-blockade (Biozzi et al., 1953). A dose-dependent hepatosplenomegaly, combined with an inflammatory response with infiltration of granulocytes and mononuclear cells may follow (Edman et al., 1983; Laakso et al., 1988).

A microparticulate drug carrier that has shown some promise in experimental therapeutics is the polyacryl starch microparticle. These particles have been used as enzyme carriers in an experimental lysosomal storage disease (Artursson et al., 1984b)

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and as carriers of antiparasitic drugs in the treatment of experimental leishmaniasis in mice (Baillie et al., 1987). The polyacryl starch microparticles are extensively phagocytosed by macrophages after i.v. injection. As a consequence of the particle uptake, the macrophages are stimulated to release hydrogen peroxide and interleukin-1 (IL-1) (Artursson et al., 1987a, b). Even though the polyacryl starch microparticles are comprised of 95% polysaccharide and can be dissolved upon incubation with α-amylase, a recent investigation shows that these particles can induce dose-dependent alterations in liver tissue (Laakso et al., 1988). In this study some dose-dependent effects of polyacryl starch microparticles on the liver were investigated by the use of inhibitors of arachidonic acid metabolism. The results suggest that polyacryl starch microparticles can stimulate macrophages to release prostaglandins and leukotrienes and that these compounds may contribute to the effects of the microparticles on liver tissue.

Materials and Methods

Preparation of microparticles

Microparticles were prepared from acryloyl-substituted starch (maltodextrin, Stadex AB, Malmö, Sweden; m.w. 5000) as described previously (Artursson et al., 1984a). Briefly, polyacryl starch microparticles were prepared from a solution of acryloyl starch monomer (10% w/v) in sodium phosphate buffer (0.1 M, pH 7.4) containing EDTA (1×10^{-3} M). Ammonium peroxidisulphate (0.43 M, pH 7.4, 200 μl) was added to the deoxygenated monomer solution (5 ml) and the water phase was homogenized in an organic phase consisting of toluene/chloroform (4 : 1, v/v, 300 ml) containing poloxamer 188 as a detergent. Upon addition of a suitable amount of *N*,*N*,*N*′,*N*′-tetramethylenediamine (TEMED), the monomer in the water droplets polymerised to microparticles. The microparticles were freed from the organic phase by extensive washes with distilled water and physiological saline. The microparticles were autoclaved and stored in sealed injection vials until further use. Only microparticles that were free from endotoxins, as determined with the *Limulus* amoebocyte assay, were used.

By varying the amount of TEMED, microparticles with different sensitivity to α-amylase (Boehringer, Mannheim, F.R.G.) were obtained (Laakso et al., 1988). Thus, "rapidly" biodegradable microparticles, i.e. particles that are completely dissolved upon incubation with α-amylase (0.5 mg of ^{14}C-labelled microparticles in 10.0 IU/ml of α-amylase, 15 min; 37° C), were produced by addition of 500 μl of TEMED to the monomer solution, while "slowly" degradable microparticles, i.e. particles that were only partly affected during short term incubation with α-amylase, were produced by adding 100 μl of TEMED to the monomer solution.

Radioactive microparticles were produced by including [^{14}C]acryloyl starch in the monomer solution. About 90% of the microparticles had a diameter of 0.5–2.2 μm as determined by scanning electron microscopy (Artursson et al. 1987a) and the radioactive microparticles had a specific activity of 1.26×10^6 cpm/mg dry weight. The microparticles were characterized by the D-T-C nomenclature according to Edman et al. (1980). The microparticles had a D-T-C value of 10–0.5–0.

Glucan particles

1,3-β-Glucan particles (Lichenan; prepared from *Cetraria islandica*; Sigma, St. Louis, MO), were suspended in physiological saline, washed 3 times, autoclaved and stored in sealed injection vials at a concentration of 10 mg/ml until further use. The mean diameter of the particles was determined with a particle counter (Coulter Counter TA II, Coulter Electronics) and was in the region of 1–2 μm. No particles were larger than 4 μm.

Macrophages

Resident peritoneal macrophages were collected from the peritoneal cavity of 6–12 week old male NMRI-mice (A-lab, Stockholm, Sweden), and were seeded on cell culture dishes (Nunc, Roskilde, Denmark; 3.5 and 1.6 cm diameter) at a density of 4×10^6 cells/ml. The macrophages were washed and characterised as described previously (Artursson et al. 1987a), giving cell cultures containing more than 98% of macrophages. The cells were

incubated in RPMI 1640 supplemented with 10% fetal calf serum, benzylpenicillin (100 U/ml) and streptomycin (10 μg/ml) at 5% CO_2 in air and 95% humidity for 24 h before addition of the microparticles. The cell culture media and buffers contained < 0.1 ng of endotoxin/ml, as determined with a *Limulus* amoebolysate microassay (Melvaer and Fystro, 1982).

Stimulation of macrophages

After 24 in culture, the macrophages were rinsed, counted and incubated with polyacryl starch microparticles or glucan particles (final particle concentration 0.5 mg/ml) for up to 3 h.

In some experiments, arachidonic acid metabolism was inhibited by the addition of indomethacin (1×10^{-6} M; MSD, U.S.A.) or timegadine (5×10^{-5} M; Leo Pharmaceuticals, Malmö, Sweden) to the medium.

[^{3}H]Arachidonic acid assay

Two h after seeding, [^{3}H]arachidonic acid (aa), (New England Nuclear, F.R.G.) was added to the macrophage cultures at a concentration of 0.5 μCi/ml. After 20 h incubation, the monolayers were rinsed 4 times and the uptake of aa by the macrophages was determined to be between 58 and 82%. The incorporation was higher when a new batch of aa was used and then declined with time, indicating decomposition of the radioactive tracer. The cells were allowed to rest for up to 2 h after the rinsing, before addition of the particles (Scott et al., 1980). The culture medium was analysed for the release of aa before and 0.25, 0.5, 1.0, 2.0 and 3.0 h after addition of 0.5 mg/ml of the various particles.

Radioimmunoassays

Macrophages were incubated with the particles as described above and the culture supernatants were collected and analysed for the content of PGE_2 and LTC_4, using commercial available radioimmunoassay kits (New England Nuclear, F.R.G.).

Animal experiments

Male NMRI-mice, weighing 18–22 g, were given a single i.v. dose of microparticles or glucan particles in one of the tail veins. To minimize the interindividual variations, the mice were matched according to weight so that the maximal weight difference between the mice in a single experiment was 2 g.

In the kinetic experiments, the mice were killed 1, 3, 5 or 7 days after the injection and the total body weight and liver and spleen wet weights were determined. Thin pieces of tissue specimens were rapidly cut out from the liver and immersed in ice-cold 5% formaldehyde in phosphate buffer (0.15 M, pH 7.2). The specimens were embedded in paraffin, cut at 2–3 μm, stained with haematoxylin and eosin and examined in the light microscope (Edman et al., 1983).

In the dose–response study, the mice were injected i.v. with 0.1, 0.5, 1.0 or 2.0 mg of microparticles in 200 μl of saline. All mice were sacrificed 5 days after the particle administration and were studied as in the kinetic experiments.

In separate experiments, blood samples were taken from the right (day 1) or the left (day 5) orbital plexus from untreated and particle-treated mice, and analysed for alanine-aminotransferase and total leucocyte counts.

In some experiments, the mice were given two daily i.v. doses of indomethacin (Confortid, 0.05 mg × 2) or two oral administrations of timegadine in carboxymethylcellulose (CMC 2%; 1 mg/100 μl; according to the manufacturers specifications). The drug treatment was started one day before the injection of the particles. Indomethacin was administered i.v. since administration of an oral dose in CMC resulted in weight loss and even death of some of the mice.

Alanine-aminotransferase assay

Alanine-aminotransferase (ALAT) was assayed with a commercial kit (Boehringer Mannheim, F.R.G.) according to the manufacturer's specifications. Seronorm (Nyegaard, Denmark), a standard serum with defined ALAT content, was used as reference sample throughout the experiments.

Leucocyte counts

Total leucocyte counts were determined in the light microscope using a Bürker chamber, after

appropriate dilution of the blood samples in Wright's stain (Way et al., 1985).

Statistics

The *t*-test for the comparison of two independent means was used to analyse the results. A difference was considered significant if $P < 0.05$. All figures are expressed as mean values ± S.D.

Results

Characteristics of the microparticles

When ^{14}C-labelled slowly degradable microparticles were autoclaved at 120 °C for 20 min, they became more susceptible to degradation by α-amylase than non-treated microparticles (Table 1). However, although their uptake by mouse peritoneal macrophages in vitro was not significantly altered, the increased amylase degradation resulted in a slightly decreased liver uptake after i.v. injection. Thus, the liver uptake of autoclaved microparticles was 55.4 ± 8.2% as compared with 67.9 ± 0.1% for non-treated microparticles. Repeated autoclavation resulted in microparticles that were completely degradable, and upon i.v. injection only 21.2 ± 4.3% of the radioactivity was found in the liver. Based on the results in Table 1, the microparticles used in this study were autoclaved once.

TABLE 1

Characterization of polyacrylstarch microparticles

Autoclavation [1]	Degradation (%) [2]	Macrophage uptake (% μg/10^6 cells) [3]	Liver uptake (%) [4]
none	22.0 ± 0.3	3.0 ± 0.4	67.9 ± 0.1
once	54.0 ± 0.8	2.7 ± 0.3	55.4 ± 8.2
twice	104.8 ± 2.2	n.d. [5]	21.2 ± 4.3

^{14}C-Labelled microparticles, D-T-C = 10–0.5–0, were prepared with 100 μl of TEMED per 5 ml water phase as described in Materials and Methods. Values are means ± S.D.

[1] Microparticles were suspended in 0.15 M NaCl (10 mg/ml) and autoclaved for 20 min at 120 °C.

[2] ^{14}C-labelled microparticles were incubated with α-amylase (10 U/ml) as described in Materials and Methods ($n = 5$).

[3] Macrophages were incubated with 0.5 mg/ml of ^{14}C-labelled microparticles for 2 h as described previously (Artursson and Sjöholm, 1986; $n = 5$).

[4] Liver uptake was expressed as % of the dose given (0.5 mg/mouse).

[5] n.d. = not determined.

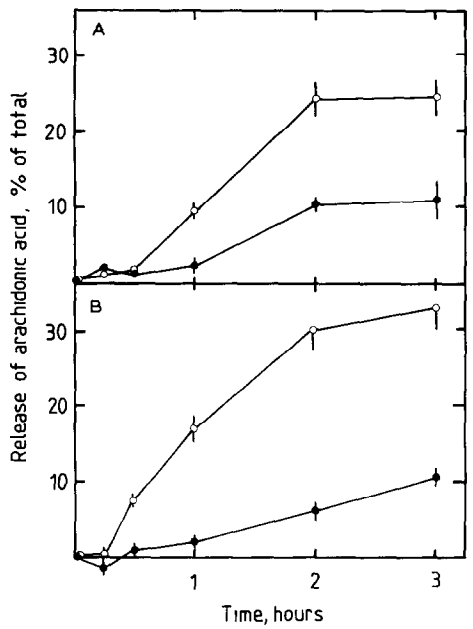

Fig. 1. Release of [^{3}H]arachidonic acid and metabolites from resident (●) and stimulated (○) macrophages ($n = 3$) A: starch, and B: glucan microparticles.

Release of arachidonic acid metabolites from microparticle-stimulated macrophages

Resident peritoneal macrophages were stimulated with microparticles (0.5 mg/ml) and the release of ^{3}H-arachidonic acid and its metabolites was followed for 3 h (Fig. 1A). 1,3-β-Glucan particles were used as positive controls since the uptake of these particles has previously been shown to stimulate macrophages leading to an increased arachidonic acid metabolism (Czop and Austen, 1985) (Fig. 1B). Both types of particles stimulated the macrophages to release significant amounts of arachidonic acid metabolites. After 3 h, the microparticle-stimulated macrophages had released 24.3 ± 2.1% of the total membrane-bound ^{3}H-label, as compared with 10.8 ± 2.4% for the unstimulated cells. The corresponding values for glucan-stimulated macrophages were 32.8 ± 2.4% and 10.5 ± 1.1%.

TABLE 2

Release of PGE_2 and LTC_4 from resident peritoneal macrophages

Stimulus	PGE_2 (ng/10^6 cells)	LTC_4 (ng/10^6 cells)
none	1.6 ± 0.4	0.1 ± 0.1
starch	11.7 ± 2.5	2.6 ± 0.2
starch/indomet *	3.2 ± 0.6	3.5 ± 0.1
starch/timegad *	1.1 ± 0.1	1.1 ± 0.0
glucan	34.6 ± 4.2	3.2 ± 0.4

Values are mean ± S.D.
Macrophages were exposed to 0.5 mg/ml of the various stimuli for 2 h ($n = 3$).
* Indomet = indomethacin, 1×10^{-6} M; timegad = timegadine, 5×10^{-5} M.

The amounts of PGE_2 and LTC_4 released by the macrophages were determined by radioimmunoassay (see Table 2). The microparticle-stimulated macrophages released 11.7 ± 2.5 ng of $PGE_2/10^6$ cells and 2.6 ± 0.2 ng/10^6 cells of LTC_4. Indomethacin (1×10^{-6} M) was found to be a potent inhibitor of the microparticle-induced release of PGE_2. As expected, indomethacin had no inhibitory effect on the lipo-oxygenase pathway, as monitored by the release of LTC_4. When timegadine, a drug that has been shown to inhibit the biosynthesis of both cyclo-oxygenase and lipo-oxygenase pathways (Myers and Siegel, 1983), was included in the tissue culture medium in a nontoxic concentration (5×10^{-5} M), a total inhibition of PGE_2 and about 60% inhibition of LTC_4 was obtained (Table 2).

Effects of the microparticles on liver

The effects on liver weight and liver morphology of a single i.v. injection to mice of 0.5 mg of autoclaved and endotoxin-negative microparticles, were followed for 7 days and are summarized in Fig. 2. Endotoxin-positive 1,3-β-glucan particles, known to induce hyperplasia and hypertrophy of RES organs (Deimann and Fahimi, 1980) were used as positive controls. Neither the rapidly nor the slowly biodegradable starch microparticles induced morphological changes or hepatomegaly at the aforementioned dose level. However, as expected, the 1,3-β-glucan particles induced a significant liver enlargement and occurrence of numerous inflammatory granulomas 3 days after injection, Fig. 2. The granulomas were haphazardly located within the lobules and varied considerably in size. They were mainly composed of cells of epitheliod type, while leucocytes and giant cells were sparse. It appeared that the granulomas took their origin within the sinusoids. Since a previous report from our group has shown that slowly degradable microparticles can induce liver changes at corresponding dose levels (Laakso et al., 1988), the experiments were repeated with 3 different batches of slowly degradable microparticles. However, no changes in liver morphology or liver weight were observed with these preparations.

The effects of higher doses of microparticles and 1,3-β-glucan are summarized in Fig. 3. A dose-dependent hepatomegaly and tendency toward granuloma formation was observed for the slowly degradable microparticles and the glucan particles, while the rapidly degradable microparticles had no such effects. The granulomas induced by the microparticles were sparse and small, while those in the glucan-exposed livers were large, abundant and sometimes associated with necroses

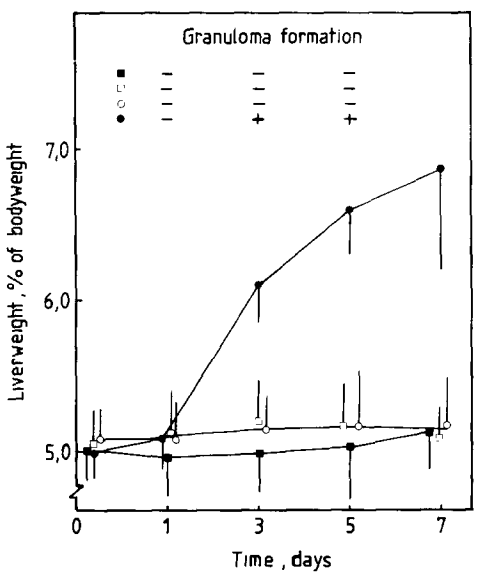

Fig. 2. Kinetics of microparticle- (□, ○) or glucan- (●) induced hepatomegaly and liver granulomas. Rapidly degradable (□); slowly degradable (○) microparticles; control livers (■); $n = 5$.

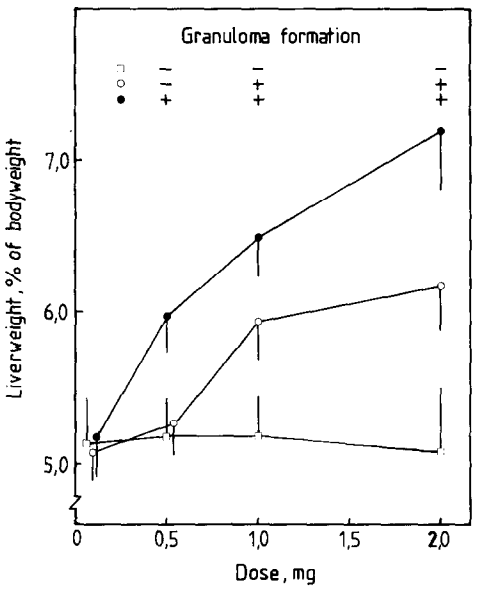

Fig. 3. Effects of various doses of rapidly degradable (□); slowly degradable (○) microparticles and glucan (●) particles on liver weight and liver granuloma formation, 5 days after i.v. injection; $n = 5$.

of variable size. Moreover, some livers in the groups treated with high doses of slowly biodegradable microparticles (i.e. 1.0 and 2.0 mg/mouse showed no histopathological changes. Since the rapidly biodegradable microparticles had no effect on the liver tissue, the experiments below were performed with slowly degradable microparticles.

TABLE 3

Total blood leucocyte counts in mice treated with polyacrylstarch or glucan microparticles

Treatment	Total leucocyte counts $\times 10^{-6}$/ml	
	day 1	day 5
None	4.9 ± 0.6	4.8 ± 0.7
Starch	5.3 ± 0.8	4.5 ± 0.8
Glucan	6.1 ± 1.4	10.3 ± 2.6

Values are means ± S.D., $n = 5$.

In a separate experiment, blood samples were taken 1 and 5 days after injection of 1.0 mg of slowly degradable microparticles and 1.0 mg of 1,3-β-glucan. The samples were analysed for alanine-aminotransferase (ALAT) activity and total blood leucocyte counts. No changes in ALAT activity could be detected in blood samples from the particle-treated mice as compared with non-treated animals. The glucan particles induced a significant increase in blood leucocytes 5 days after injection (Table 3). However, no increase was observed in the starch microparticle-treated animals.

Effects of the microparticles on spleen

In general, no effects of the microparticles on the spleen were observed. Occasionally, a slight splenomegaly could be observed in some micro-

TABLE 4

Effects of anti-inflammatory drugs on the microparticle-mediated inflammatory response

Treatment [1]	Liver weight, % of body weight	P [2]	Granuloma formation	Total leucocyte counts, $\times 10^{-6}$/ml	P [2]
None	5.1 ± 0.2		−	4.9 ± 1.1	
Starch	5.9 ± 0.2		+	5.4 ± 1.0	
Starch/indomet [1]	5.4 ± 0.2	< 0.01	+	5.8 ± 1.2	n.s.
Starch/timegad [1]	5.3 ± 0.3	< 0.01	+	4.5 ± 1.5	n.s.
Glucan	7.2 ± 0.5		+	8.0 ± 1.5	
Glucan/indomet [1]	5.9 ± 0.1	< 0.001	+	5.3 ± 0.9	< 0.01
Glucan/timegad [1]	6.1 ± 0.3	< 0.01	+	5.1 ± 0.8	< 0.01

Slowly degradable starch or glucan microparticles (1.0 mg) were injected i.v. to mice. The liver morphology and blood leucocyte counts were estimated 5 days after administration of the microparticles ($n = 5$). Values are mean ± S.D.

[1] The mice were treated with the various drugs as described in Materials and Methods.

[2] As compared with starch- and glucan-treated livers, respectively.

n.s. = not significant.

particle-treated mice. No correlation could be found between the dose given and the development of splenomegaly. The splenomegaly did not coincide with the occurrence of liver granulomas. A possible reason for the random appearance of splenomegaly may be endotoxin contamination of the injection needle as a result of the contact with the mouse tail. The spleen morphology has previously been shown to remain normal over a wide dose range of i.v. administered microparticles (Laakso et al., 1988). Therefore, spleen morphology was not further investigated in this study.

Effects of indomethacin and timegadine on the microparticle-mediated inflammation

The involvement of arachidonic acid metabolites in the microparticle-induced liver changes were studied by giving indomethacin or timegadine to the mice prior to the administration of an "inflammatory dose" of microparticles or glucan (Table 4). The mice were given the drugs throughout the experiment. The hepatomegaly in the indomethacin- and timegadine-treated mice was less extensive than in untreated mice ($P < 0.05$). However, clear differences in the extent of granuloma formation were not observed.

Similar results were obtained for the groups given 1,3-β-glucan particles. Thus, mice given 1.0 mg of glucan particles and indomethacin showed a significantly smaller increase in liver weight ($5.9 \pm 0.1\%$) than mice given the same dose of glucan without the drug ($7.20 \pm 0.55\%$). A simultaneous suppression of the number of blood leucocytes was observed in the indomethacin and timegadine-treated groups.

Discussion

It is well known that macrophages can release arachidonic acid metabolites upon phagocytosis of particulate polysaccharides, such as zymosan or 1,3-β-glucan in vitro (Czop and Austen, 1985). It is also known that, when these polysaccharides are injected i.v., they will be taken up by the macrophages of the RES and subsequently induce an inflammatory response in the liver. This effect is believed to be related to the release of arachidonic acid metabolites by liver macrophages (Kupffer cells) (Way et al., 1985). This raised the question whether or not polyacryl starch microparticles had similar effects on the macrophages as the 1,3-β-glucan particles.

The experiments with isolated macrophage cultures showed that the polyacryl starch microparticles can stimulate peritoneal macrophages to release arachidonic acid metabolites in vitro. The reason for choosing peritoneal macrophages and not Kupffer cells for the in vitro studies was that the former are easier to isolate and maintain in tissue culture without activation of the cells.

The dose-response studies showed that polyacryl starch microparticles had a relatively low toxicity as compared with the glucan particles. No morphological changes were observed in the livers after injection of the rapidly degradable microparticles. Similarly, no structural changes were observed when doses lower than 1.0 mg/mouse of the slowly biodegradable microparticles were given. With doses of 1.0 and 2.0 mg, small granulomas were found in some of the mice. Therefore, these groups were classified as "granuloma-positive". These results are not in complete agreement with those by Laakso et al. (1988) who reported that morphological changes occurred also at lower dose levels. This discrepancy may result from differences in biodegradability after autoclavation of the microspheres. It may also be of significance that all polyacryl starch particles used in the present study were sterile and endotoxin-negative.

The potential contribution of arachidonic acid metabolites to the microparticle-mediated hepatic inflammatory response with granuloma formation was indicated by the decrease in the liver weight obtained in indomethacin and timegadine-treated groups. No significant differences between the two drugs could be observed. This does not mean that the role of cyclo-oxygenase products in the inflammatory response was more significant than that of the lipo-oxygenase products. The differences in the inhibition of arachidonic acid metabolism between the two drugs in vitro was too small to allow an analysis of the relative contribution of various arachidonic acid metabolic pathways to the increased liver weights. The re-

sults are in agreement with those of Way et al. (1985) who showed that 1,3-β-glucan-induced liver inflammation in rats could be related to arachidonic acid metabolites. However, in contrast to Way et al. we could not demonstrate a significant decrease in the granuloma formation as a result of the drug treatment. Perhaps more extensive experiments with morphometric analyses may be required in order to demonstrate significant differences between the types of treatment.

Obviously, factors other than the release of arachidonic acid metabolites are likely to be involved in microparticle-induced inflammation since macrophages are known to release many products as a result of ingestion of particulate stimuli (Nathan, 1987). Moreover, a variety of non-macrophage-associated responses, such as complement activation by the carrier matrix (Artursson and Sjöholm, 1986), may also contribute to the inflammatory response.

In addition to the arachidonic acid metabolites, reactive oxygen intermediates (ROI) and interleukin-1 (IL-1) are likely to contribute to the microparticle-mediated inflammation. Both products have been shown to be released from macrophages stimulated by polyacryl starch microparticles in vitro (Artursson et al., 1987a and b). IL-1 has recently been suggested to be a more potent chemotactic factor than C5a and LTC_4 (Cybulsky et al., 1986).

In an attempt to study the effects of ROI on the microparticle-induced liver injury, superoxide dismutase (SOD), an enzyme with well-known anti-inflammatory properties (Petrone et al., 1980), was incorporated in the microparticles. Although the SOD-containing microparticles could inhibit superoxide anion-mediated reduction of Nitroblue tetrazolium upon phagocytosis in vitro, no effect of the immobilized enzyme was observed in the in vivo experiments (data not shown). This may be explained by the fact that mouse Kupffer cells have been shown to be poor producers of ROI (Lepay et al., 1985).

In conclusion, arachidonic acid metabolites seem to contribute to the dose-dependent side effects of polyacryl starch microparticles in vivo. Whether this is a general phenomenon that is also associated with the uptake of other types of microparticles or an effect restricted to carbohydrate microparticles, such as 1,4-α-glucans (starch) and 1,3-β-glucans, remains to be established.

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References

Artursson, P., Edman, E., Laakso, T. and Sjöholm, I., Characterization of polyacryl starch microparticles as carriers for proteins and drugs. J. Pharm. Sci., 73 (1984a) 1507–1513.

Artursson, P., Edman, P. and Sjöholm, I., Biodegradable microspheres. I. Duration of action of dextranase entrapped in polyacrylstarch microparticles in vivo. *J. Pharmacol. Exp. Ther.*, 231 (1984b) 705–712.

Artursson, P. and Sjöholm, I., Effect of opsonins on the macrophage uptake of polyacrylstarch microparticles. *Int. J. Pharm.*, 32 (1986) 165–170.

Artursson, P., Arro, E., Edman, P., Ericsson, J.L.-E. and Sjöholm, I., Biodegradable microspheres V. Stimulation of macrophages with microparticles made of various polysaccharides. *J. Pharm. Sci.*, 76 (1987a) 127–133.

Artursson, P., Edman, P. and Ericsson, J.L.-E., Macrophage stimulation with some structurally related polysaccharides. *Scand. J. Immunol.*, 25 (1987b) 245–254.

Baillie, A., Coombs, G., Dolan, T., Hunter, C., Laakso, T., Sjöholm, I. and Stjärnkvist, P., Biodegradable microspheres. IX. Polyacryl starch microparticles as a delivery system for the anti-leishmanial drug, sodium stibogluconate. *J. Pharm. Pharmacol.*, 39 (1987) 832–835.

Biozzi, G., Benacerrat, B. and Halpern, B.N., *Br. J. Exp Pathol.*, 34 (1953) 441–457.

Cybulsky, M.I., Colditz, I.G. and Movat, H.Z., The role of interleukin-1 in neutrophil leukocyte emigration induced by endotoxin. *Am. J. Pathol.*, 124 (1986) 367–372.

Czop, J.K. and Austen, K.F., Generation of leucotrienes by human monocytes upon stimulation of their β-glucan receptor during phagocytosis. *Proc. Natl. Acad. Sci. U.S.A.*, 82 (1985) 2751–2755.

Deimann, W. and Fahimi, H.D., Hepatic granulomas induced by glucan. An ultrastructural and peroxidase-cytochemical study. *Lab. Invest.*, 43 (1980) 172–181.

Edman, P., Ekman, B. and Sjöholm, I., Immobilization of proteins in microspheres of biodegradable polyacryldextran. *J Pharm. Sci.*, 69 (1980) 838–842.

Edman, P., Sjöholm, I. and Brunk, U., Acrylic microspheres in vivo. VII. Morphological studies on mice and cultured macrophages. *J. Pharm. Sci.*, 72 (1983) 658–665.

Edman, P., Artursson, P., Laakso, T. and Sjöholm, I., Solid microspheres as drug delivery systems. In G.M. Ihler (Ed.), *International Encyclopedia of Pharmacology and Therapeutics, Vol. 120, Methods of Drug Delivery*, Pergamon 1986, 23–37.

Laakso, T., Edman, P. and Brunk, U., Biodegradable microspheres. VII. Alterations in mouse liver morphology after i.v. administration of polyacrylstarch microparticles with different biodegradability. *J. Pharm. Sci.*, 77 (1988) 138–143.

Lepay, D.A., Nathan, C.F., Steinman, R.M., Murray, H.W. and Cohn, Z.A., Murine Kupffer cells. Mononuclear phagocytes deficient in the generation of reactive oxygen intermediates. *J. Exp. Med.*, 161 (1985) 1079–1096.

Melvaer, K.I. and Fystro, D., Modified micromethod of the *Limulus* amoebocyte lysate assay for endotoxin. *Appl. Environ. Microbiol.*, 43 (1982) 493–496.

Myers, R.F. and Siegel M.I., Differential effects of anti-inflammatory drugs on lipoxygenase and cyclo-oxygenase activities of neutrophils from a reverse passive Arthus reaction. *Biochem. Biophys. Res. Commun.*, 112 (1983) 586–594.

Nathan, C.F., Secretory products of macrophages. *J. Clin Invest*, 79 (1987) 319–326.

Petrone, W.F., English, D.K., Wong, K. and McCord J.M., Free radicals and inflammation: superoxide-dependent activation of a neutrophil chemotactic factor in plasma. *Proc. Natl. Acad. Sci. U.S.A.*, 77 (1980) 1159–1163.

Scott, W.A., Zrike, J.M., Hamill, A.L., Kempe, J. and Cohn, Z., Regulation of arachidonic acid metabolites in macrophages. *J. Exp. Med.*, 152 (1980) 324–335.

Smith, A. and Hunneyball, I.M., Evaluation of poly(lactic acid) as a biodegradable drug delivery system for parenteral administration. *Int J. Pharm.*, 30 (1986) 215–220.

Way, C.F., Dougherty, W.J. and Cook, J.A., Effects of essential fatty acid deficiency and indomethacin on histologic, ultrastructural, and phagocytic responses of hepatic macrophages to glucan. *J Leuk. Biol*, 37 (1985) 137–150.