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Rapid Communication

Macrophage activity may be modified in vivo by galenical excipients

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Summary

Excipients present in drug formulations may have an impact on the immune system. We present evidence of modifications of macrophage activation observed after administration to mice of galenical excipients commonly used in drug manufacturing, including sugars (glucose and mannitol), surfactants (polysorbate 80, cremophor EL, egg, lecithin, pluronic F68), polysaccharides (dextran 20 and 40), and pyrogenated silica (cabosil). They were dissolved in distilled water at 0.1% (5% for mannitol and glucose), and sterilized at 120°C for 20 min. Macrophage phagocytic activity was measured in terms of chemoluminescence following engulfment of opsonized zymosan. Marked macrophage recruitment to the peritoneal cavity occurred after dextran 40 and mannitol injection. Phagocytic activity was increased after the injection of dextran 40, dextran 20, pluronic F68, mannitol, glucose, cremophor EL and cabosil (in increasing order of efficiency), but not polysorbate 80.

Excipients are used to improve the preparation, administration, stability and storage of drugs. They should not interact with the active agents, other galenical materials or with physiological functions. To study the impact such substances might have on the immune system, we tested additives which are delivered in large quantities (glucose, mannitol, and dextran) and those which can be more specifically considered as excipients (egg lecithin, cremophor EL, polysorbate 80, pluronic F68 and cabosil). We studied how these substances affected macrophage activation, as these cells play an important role in the initiation of immune responses, in particular in the immediate and sustained control of infectious and parasitic diseases, and in the expression of hypersensitivity. Mice were injected intraperitonealy and macrophage phagocytic activity was measured 4 days later.

Chemicals: Glucose, mannitol, egg lecithin (phosphatidylcholine) and polysorbate 80 (sorbitan mono-9-octadecenoate poly(oxy-1,2-ethanediyl) derivative were obtained from Cooper, France. Cremophor EL (polyoxyl 35 castor oil) and pluronic F68 (poloxamer 188) were purchased from BASF France. Cabosil (pyrogenated silica) was obtained from Cabot, Neuilly sur Seine, France. Dextran 20 and 40 were supplied by Fluka, Switzerland. These substances were dis-

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solved or dispersed in distilled water at 0.1 or 5% (glucose and mannitol) and sterilized at 120°C for 20 min.

Animals: 6-week-old female Swiss mice were purchased from the Centre de Production Animale (45160 Olivet France).

Mice received intraperitoneal (i.p.) injections of 2 ml of the preparations, 2 ml of water for injection or 2 ml of thioglycolate (classically used to attract cells to the peritoneum). 4 days later, peritoneal cells were harvested by washes with 6 ml of Hanks' saline without phenol red (pH 7.2). Viable cells were counted in the trypan blue exclusion test. The cell population contained more than 90% of macrophages and was adjusted to 10^6 cells/ml.

When engaged in phagocytosis, macrophages liberate free oxygen radicals (OH^-, OH, H_2O_2, O_2^-) which emit photons when they return to their stable electronic state: the intensity of photon emission is increased by the presence of photon amplificators. The intensity of the photon emission determines the degree of cellular activation.

1 ml of cell suspension was placed in a plastic Clinicon cuvette maintained at 37°C in the dark, then 20 μl/mol of luminol (5-amino-2,3 dihydro 1,4-phthalazine dione) (Sigma Chemicals) and 50 μl of fresh mouse serum-opsonized zymosan (15 mg/mol) were added. Emitted light was measured every 10 min for 10 s with a photomultiplier coupled to a picoamperometer (LKB 1250 luminometer). Each point represents three measurements. Chemoluminescence, expressed in mV, was plotted against time and the areas under the curve were calculated (Bravo-Cuellar et al., 1987). Results are expressed in reference to a control group (untreated or injected with thioglycolate), as follows:

$$\frac{\text{value of the treated group} - \text{value of the control group}}{\text{value of the control group}} \times 100$$

Distilled water did not modify peritoneal cell mobilisation (+7%) but increased phagocytic activity (+68%). Since the excipients were prepared in distilled water, this was taken as the control value (Table 1).

Mannitol, dextran 40 and cabosil were very effective in attracting cells to the peritoneal cavity (+160, +304 and +102%, respectively).

Glucose, dextran 20, cremophor EL, pluronic F68 and cabosil strongly activated peritoneal macrophage phagocytosis (+320, +489, +536, +164 and +3341%, respectively).

The preliminary results we report here were obtained under particular conditions, i.e., mice were injected only once with 2 ml of the preparation at only one concentration, and macrophage recruitment and activation were measured only once (day 4). The increased macrophage numbers in the peritoneum after injection of the various

TABLE 1

In vivo recruitment and activation of peritoneal macrophages by galenical excipients and preparations

	Cell numbers in the peritoneum on day 4 expressed as % of untreated control values	Activation of peritoneal macrophages area under the curve expressed as % of untreated control values
Distilled water	+ 7	+ 68
Thioglycolate	+142	+ 52
Glucose, 5%	+ 21	+320
Mannitol, 5%	+160	+167
Dextran 20, 0.1%	+ 52	+489
Dextran 40, 0.1%	+304	+122
Egg lecithin, 0.1%	+ 20	+ 44
Cremophor EL, 0.1%	+ 26	+536
Polysorbate 80, 0.1%	+ 47	+ 1
Pluronic F 68, 0.1%	+ 36	+164
Cabosil, 0.1%	+102	3341

preparations probably resulted from cell recruitment from peripheral blood or proliferation.

There was no correlation between the attractant capacity of the preparation and its capacity to stimulate phagocytic activity.

We did not expect glucose, a physiological sugar, to have such durable activity. The results obtained with dextran should be compared to those reported with other polysaccharides such as lentinan, levan, glucan, crosslinked dextran (Bogwald et al., 1984; Blanckmeister et al., 1985). Blocking of the reticuloendothelial system by dextran has been reported by (Farrow et al., 1971), who observed that plasma substitutes cause RES depression lasting for some hours in normal mice. We report here a relatively durable effect which could be described as a rebound response. D mannitol has been reported to stimulate rabbit alveolar macrophage migration in vitro via its membrane receptor (Takata et al., 1987). Cremophor EL was found to have an effect on the microviscosity of cell membranes and to be able to reverse multidrug resistance (Chervinsky et al., 1992; Woodcock et al., 1992). Such membrane activation may well be translated by increased phagocytic capacity.

Interestingly, Cremophor EL has been reported to favor immediate hypersensitive reactions and allergy to various anesthetics such as diazepam, in which it was introduced as nonionic surfactant. It may also activate complement and thus lead to the release of histamine.

We were expecting egg lecithin to have an effect, since one constituent, arachidonic acid, is a cell activator when degraded through the lipoxygenase pathway, leading to the formation of leukotrienes (LTB_4 is involved in macrophage chemotaxis) (Nathan, 1987). Pluronic F68 administration has been reported to be followed by a fall in peritoneal macrophage numbers. In man, it inhibits macrophage-monocyte migration and chemotaxis (Virmani et al., 1983). This might explain some of the side-effects of this adjuvant: when incorporated into fluorocarbon parenteral preparations, it is suspected to favour infections as an effect on granulocyte and macrophage mobilisation (Lane and Lamkin, 1986). Polysorbate 80 increased peritoneal cell recruitment without modifying phagocytic activity. Polysorbate 80 is described as an immunologic adjuvant. Fleming and McNeill (1975) observed increased responsiveness to in vitro stimulation of bone marrow colony-forming cells by colony-stimulating factor after treatment with polysorbate 80.

The last agent which markedly stimulated macrophages was cabosil. We attribute this result to its very special mode of preparation. Silica is normally available as quartz crystals or in an amorphous form which exerts cytotoxic effects on macrophages (Allison et al., 1966). Cabosil is constituted of particles with an average diameter between 70 and 500 Å in ramified chains, thus conferring remarkable physical properties to the preparation.

Although preliminary, our data indicate that some galenical preparations may have immunostimulatory properties.

References

Allison, A.C., Harington, J.S. and Birbeck, M., An examination of the cytotoxic effects of silica on macrophages. *J. Exp. Med.*, 124 (1966) 141–153.

Blanckmeister, C.A. and Sussdaf, D.M., Macrophage activation by cross-linked dextran. *J. Leukocyte Biol.*, 37 (1985) 209–219.

Bogwald, J., Johnson, E., Hoffman, J. and Selgelid, R., Lysosomal glycosidases in mouse peritoneal macrophages stimulated in vitro with soluble and insoluble glycans. *J. Leukocyte Biol.*, 35 (1984) 357–371.

Bravo-Cuellar, A., Scott Algara, D., Metzger, G. and Orbach-Arbouys, S., Enhanced activity of mouse peritoneal cells after aclacinomycin administration. *Cancer Res.*, 47 (1987) 3477–3484.

Chervinsky, D.S., Brecker, M.L. and Hoelle, M.J., Cremophor EL reverses taxol cross resistance in murine C1300 multidrug resistant neuroblastoma cells. *83th Annual Meeting of the American Association of Cancer Research*, 33, San Diego, May 20–23, 1992, p. 2551.

Farrow, S.P. and Ricketts, C.R., Blocking of RE cells by dextran. *J. Pharm Pharmacol.*, 23 (1971) 295–297.

Fleming, W.A. and McNeill, T.A., Cellular responsiveness to stimulation in vitro: increased responsiveness to colony stimulating factor of bone manow colony forming cells treated with surface active agents and cyclic 3′5′ AMP. *J. Cell. Physiol.*, 88 (1975) 323–330.

Lane, A.T. and Lamkin, G.E., Increased infection mortality and decreased neutrophil migration due to a component of an artificial blood substitute. *Blood*, 68 (1986) 351–354.

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

Schildt, B., Bouveng, R. and Sollenberg, M., Plasma substitute induced impairment of the reticuloendothelial system function. *Acta Chir. Scand.*, 141 (1975) 7–13.

Takata, I., Chida, K., Gordon, M.R., Myvrik, Q.N., Ricardo, M.J. and Kucera, L.S., L-Fucose, D-mannose, galactose and their BSA conjugates stimulate macrophage migration. *J. Leucocyte Biol.*, 41 (1987) 248–256.

Virmani, R., Warren, D., Rees, R., Fink, L.M. and English, O., Effects of perflucrochemical on phagocytic function of leukocytes. *Transfusion*, 23 (1983) 512–515.

Woodcock, D.M., Linsenmeyer, M.E., Chojnowski, G., Kriegler, A.B., Nink, V., Webster, L.K. and Sawyer, W.M., Reversal of multidrug resistance by surfactants. *Br. J. Cancer.*, 66 (1992) 62–68.