Liposomes as Carrier for Antibiotics: A Comparative Study on the Immune Response against Liposome-Encapsulated Penicillin and Other Penicillin Preparations

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Abstract

Liposomes do not stimulate a primary or secondary immune response against entrapped penicillin in mice.

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Several recent communications deal with the administration of liposome-entrapped antibiotics such as streptomycin [1], piperacillin [2] and penicillin-G [3]. The protective effect of the liposome-encapsulated antibiotics was equal to or exceeded that of an equivalent amount of similar antibiotics administered in free solution. It has also been shown that liposome-entrapped penicillins may inhibit the growth of some penicillin-resistant bacteria in vitro [4].

Administration of a liposome-encapsulated antibiotic may be advantageous compared to that of the free antibiotic for several reasons:

Enhancement of protective effect [2, 4];

Targeting of the drug to its site of action. For example, encapsulation of ampicillin in liposomes results in an 80-fold increase in therapeutic efficacy of the drug in the treatment of *Listeria* mono-cytogenes infection in normal mice [5];

Allergic reactions such as anaphylactic reactions, e.g. due to penicillin administration, may be avoided when the antibiotic is injected in a liposome-encapsulated form;

Reduction of toxic effects, e.g. of the antibiotic vi-olamycin-BI [6].

Although encapsulation of penicillin in liposomes may be advantageous compared to that free penicillin, the side effects of penicillin, i.e. reactions due to immunological responses still remain. In earlier studies [7, 8] we showed that administration and particularly long-term administration of penicillin may elicit substantial immune responses in man against the drug, due to adjuvantia (oil and alummonostearate)

in preparations. We demonstrated earlier that liposomes have strong immunoadjuvant activity with respect to associated antigens [9, 10].

Furthermore when liposomes are ingested and digested by macrophages, penicilloylation of their proteins is favoured by a high local concentration of penicillin. Therefore we investigated the immune response to liposome-entrapped penicillin and compared it with that to free penicillin and protein-coupled penicillin.

8 C3D2 mice (Bomholtgard, Denmark) were injected intravenously with penicillin-G [1 mg/ml phosphate-buffered saline (PBS), pH 7.4; Sigma Chem. Comp., Mo., USA], 8 C3D2 mice with penicillin-G in liposomes (2 mg/ml, according to [11]) and 8 C3D2 mice with KLH-Pen conjugate (1 mg/ml PBS). On day 5 after injection the animals were bled and their spleen was removed and snap-frozen for the detection of the primary immune response.

24 C3D2 mice were injected intravenously on day 0 with penicillin-G (8 mice), penicillin-G in liposomes (8 mice) or KLH-Pen conjugate (8 mice). Booster injections were given on day 7 and day 14. The animals were bled and their spleen removed and snap-frozen in liquid nitrogen on day 21 for the detection of the secondary immune response.

KLH was penicilloylated according to the method of Nishida et al. [12]. The penicilloyl load was 10 groups/100,000 dalton KLH [13]. The HSA-Pen7-HRP conjugate was prepared as described previously [12].

The localization of anti-penicillin antibody-producing cells in the spleen was determined in 8- μ m cryostat sections by incubation with HSA-Pen7-HRP followed by HRP cytochemistry [14].

Circulating anti-penicillin antibodies were determined in serum using ELISA. As antigen we used a conjugate of human transferrin and penicillin-G [15], while normal mouse serum was used as negative control serum. As positive control a monoclonal anti-penicil-loyl antibody Pen, was used [5].

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Fig. 1. Anti-penicillin antibody-containing cells in the outer part of a periarteriolar lymphocyte sheath (P) in the white pulp of a mouse spleen at 5 days after an intravenous injection with KLH-Pen conjugate in PBS. Some cells are grouped around a terminal ar-teriolar branch (T) extending in the red pulp (R). Anti-penicillin antibody-containing cells were not seen after injection of free penicillin or penicillin-entrapped in liposomes.

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Fig. 2. Results of the ELISA. Curve A: The shaded area contains the results of normal mouse serum, sera of mice injected with penicillin-G (primary and secondary immune response) and sera of mice injected with liposome-entrapped penicillin-G (primary and secondary response).

Curve B: Sera of mice injected with KLH-Pen, primary response. Curve C: Sera of mice injected with KLH-Pen, secondary response.

No primary immune response was detected in the spleen of mice injected intravenously with penicillin-G or penicillin-G entrapped in liposomes. In contrast, when KLH-Pen was used as antigen we demonstrated anti-penicillin antibody-containing cells in cryostat sections of the spleen. The bulk of these antibody-producing cells were localized in the outer parts of the periarteriolar lymphocyte sheaths and around the terminal arteriolar branches (fig. 1). Sporadically specific antibody-containing cells were also detected in the red pulp, in the inner parts of the PALS and in the follicles of the spleen. No secondary response in mice after the third injection with penicillin-G or penicillin-G entrapped in liposomes could be demonstrated. However, the spleen of mice boosted with KLH-Pen contained anti-penicillin-producing cells. The localization pattern of these cells was similar to that described for the primary immune response but more cells were found.

As can be seen in figure 2, curve A, no differences were found between normal mouse serum (negative control serum) and sera of mice taken after one injection with penicillin-G or penicillin-G encapsulated in liposomes. Also mice repeatedly injected with penicillin-G or penicillin-G incorporated in liposomes demonstrated no anti-penicillin antibody response (fig. 2, curve A). In contrast, when mice were injected with KLH-Pen, a primary response could be demonstrated (fig. 2, curve B). After repeated injections with KLH-Pen, a secondary immune response of anti-penicillin antibodies was demonstrated (fig. 2, curve C). The concentration of anti-penicilloyl antibodies in boosted mice injected with KLH-Pen was 0.2 mg/ml in respect to Peng Abs [14].

Liposomes may be considered a strong adjuvant, both for haptens [18, 19] and for antigens [20]. It is shown in the present study that, in mice, liposomes do not stimulate a primary or secondary immune response against entrapped penicillin. Neither was an immune response detected against penicillin administered in free solution. It has been shown that penicillin preparations without contamination of highly penicilloylated proteins, that may arise during their preparation, do not induce an immune response in mice [20]. As stated already, immunopotentiating activity of liposomes has been shown for different antigens [9] and for haptens coupled to phospholipids [15]. Obviously non-specific interaction between peni-

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cillin or possibly penicilloylated cellular proteins does not occur. The same holds true for the penicilloylation of cellular proteins. In contrast, when we used penicilloylated protein, i.e. KLH-Pen conjugate that can be compared with penicilloylated-protein impurities (so-called extrinsic factor) in preparation of the drug, we found an immune response. In these animals we found circulating anti-penicillin antibodies as well as anti-penicillin-producing cells in the spleen. The latter results were in agreement with those of earlier investigations of our group [21, 22]. In conclusion, this study shows that the immunogenicity of penicillin in mice is not enhanced by encapsulation in liposomes.

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