Prolongation of Effect of Asparaginase by Implantation in Polyacrylamide in Rats

PETER EDMAN and INGVAR SJÖHOLM ˣ

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Abstract □ L-Asparaginase was immobilized in spherical polyacrylamide microparticles, which were inserted in polyacrylamide gel for implantation in rats. Intraperitoneal implantation of this gel depressed systemic L-asparagine for the same duration as was achieved with microparticles, *i.e.*, 8–10 days. The duration of the effect was prolonged up to about 25 days when the gel with the enzyme was implanted subcutaneously on the back of the rat. In both cases, a collagenous capsule formed around the gel, eventually preventing contact with the circulation. The effect of a second implantation was of the same magnitude as the first one.

Keyphrases □ L-Asparagine—polyacrylamide microparticles, prolongation of effect by implantation of immobilized enzyme, rats □ Polyacrylamide—microparticles containing L-asparaginase, prolongation of effect by implantation, rats □ Microparticles—L-asparaginase-containing polyacrylamide microparticles, prolongation of effect by implantation of immobilized enzyme, rats

L-Asparaginase has been used to control and depress the growth of L-asparagine-dependent tumors (1–3), and its administration in immobilized form offers some advantages (4). L-Asparaginase was immobilized in polyacrylamide microspheres (mean diameter of about 0.3 μm) in such a way that the enzymatic properties were retained. When these microspheres (1000 IU of L-asparaginase/kg) were administered in a single dose intraperitoneally, the effect on the serum L-asparagine level was prolonged from 4 to 14 days when compared with the effect of soluble enzyme. Moreover, the acrylic microspheres were cleared readily from the abdominal cavity and circulation by the reticuloendothelial system (5).

The enzyme is still active in the microparticles after the endocytosis but cannot control the systemic L-asparagine level. The duration of the effect thus is directly dependent on the time the particles stay in contact with the systemic circulation. In the present paper, data show that L-asparaginase given in polyacrylamide implants can depress L-asparagine for up to about 25 days.

EXPERIMENTAL

Materials—L-Asparaginase[1] (EC 3.5.1.1) was isolated from *Escherichia coli* (ATCC 9637 and ATCC 11303). Polyacrylamide microparticles with L-asparaginase were prepared as described previously (4, 6).

Polyacrylamide Gel Preparation for Implantation—Microparticles of polyacrylamide containing L-asparaginase (20 and 100 IU) were suspended in 0.7 ml of monomeric acrylamide (T − C = 16–25[2]) in a cylinder with a height of 0.7 cm and a radius of 0.6 cm. A catalyst system of *N,N,N′,N′*-tetramethylethylenediamine (50 μl) and 50 μl of ammonium peroxydisulfate (500 mg/ml of water) then was added to the monomer suspension. The polymerization started immediately and was completed in 10 min. The gel tablet was washed with physiological saline for 7 days to free it from any remaining catalysts.

Determination of L-asparagine was done according to Cooney *et al.* (8).

L-Asparaginase was determined according to Yellin and Wriston (9) as described previously (4).

Animals—Sprague–Dawley male rats were used.

RESULTS AND DISCUSSION

L-Asparaginase Implanted Intraperitoneally—Figure 1 shows the changes in the serum L-asparagine concentration in rats given single doses (20 IU) of L-asparaginase in soluble and immobilized forms. The enzyme was immobilized in polyacrylamide microspheres (T − C = 8–25), which subsequently were inserted in polyacrylamide gel (T − C = 16–25). The gel was prepared in the form of a cylinder with a height of 0.7 cm and a radius of 0.6 cm. The gel tablet was implanted in the abdominal cavity of a 110-g male rat.

The serum L-asparagine level was measured intermittently in the rats as well as in an untreated control taken from the same litter. The soluble enzyme had a short effect on the L-asparagine level, which was essentially normalized after 2 days (Fig. 1). When the enzyme was given in the polyacrylamide gel, L-asparagine immediately dropped to a level corresponding to ~25–30% of the initial value. After 8 days, the L-asparagine level was normalized.

The effect seen after intraperitoneal implantation of the gel was comparable to the effect obtained after intraperitoneal injection of L-asparaginase in microparticles (4). However, the dose in the implant corresponded to 182 IU/kg, while there were 1000 IU/kg in the microparticles.

On the 31st day, the abdominal cavity was opened. The gel was surrounded by a nonvascularized tough ligamentous capsule. No capillaries had penetrated the polyacrylamide implant, and the gel could be removed easily from the capsule. A significant amount of L-asparaginase activity still remained in the implant.

L-Asparaginase Implanted Subcutaneously—For subcutaneous administration, polyacrylamide gels (T − C = 16–25) were prepared containing 100 IU of L-asparaginase in microparticles (T − C = 8–25). The cylinders formed were about the same size as those used for intraperitoneal implantation. The gel implants (dose of ~700 IU/kg) were placed subcutaneously in the scapular line in two male rats weighing 147 g. The L-asparagine level was followed and compared to that in an untreated rat (Fig. 2).

A significant depression of the L-asparagine level was seen for at least 25 days, and the level was normalized on the 31st day. On the 61st day when the rats weighed 350 g, the implants were removed and a new gel tablet containing 100 IU of L-asparaginase (~300 IU/kg) was implanted in the other side of the back in the scapular line. The effect of this second implantation lasted for about 19 days (Fig. 2). In these cases also, the systemic effect of L-asparaginase was interrupted by encapsulation.

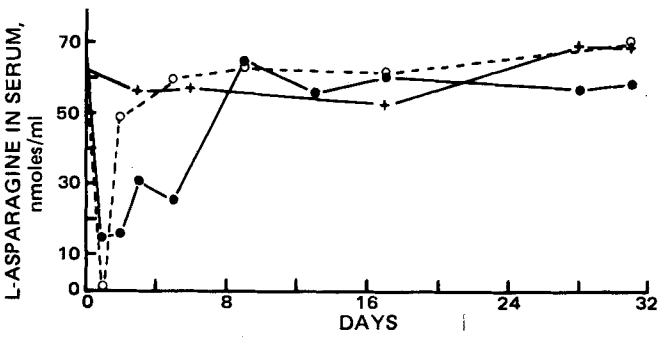

Figure 1—*L-Asparagine concentration in serum (nanomoles per milliliter) after intraperitoneal implantation of 20 IU of L-asparaginase (182 IU/kg) in rats (●) or after injection of the same amount in soluble form (○). The normal L-asparagine concentration (+) was obtained from an untreated rat taken from the same litter.*

[1] Crasnitin, Bayer (Sverige) AB, Stockholm, Sweden

[2] The nomenclature follows the suggestion made by Hjertén (7).

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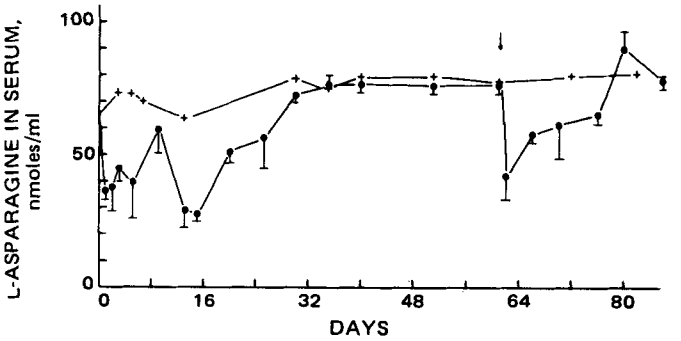

Figure 2—*L-Asparagine concentration in serum after subcutaneous implantation of 100 IU of L-asparaginase (700 IU/kg) in rats (●). The arrow shows the time when the implant was removed and a new gel tablet containing 100 IU of L-asparaginase (300 IU/kg) was inserted. Each point represents samples drawn from two rats and shows the mean and the range. The normal L-asparagine level (+) was obtained from an untreated rat from the same litter.*

The decrease of the serum L-asparagine concentration was of the same magnitude after the second implantation as that found initially, in spite of the lower dosage of L-asparaginase. This finding indicates that no significant amounts of antibodies were produced in the rats during the first 60 days of exposure to the implants. This conclusion is in accordance with earlier findings that proteins immobilized in highly cross-linked polyacrylamide are stably bound in the gel (6). Thus, it is feasible to assume that not enough of the immunogenic enzyme leaks out of the gel to activate the immune system.

By subcutaneous administration of L-asparaginase in implants of polyacrylamide, the effect can be substantially prolonged compared to the duration obtained by administration of soluble enzyme or by enzyme immobilized in microspheres. Although duration of the systemic effect *in vivo* was not limited by denaturation, the formation of a collagenous capsule around the implant effectively prevented the contact between the enzyme and the circulating L-asparagine. The encapsulation is a normal reaction to the presence of a foreign body. The same effects were detected after implantation of other acrylic polymers in rats (10, 11).

Updike *et al.* (12) also increased the duration of L-asparaginase by immobilizing the enzyme in resealed red blood cell ghosts. Undetected or very low levels of circulating L-asparagine lasted twice as long after the intravenous injection in monkeys of ghost-entrapped enzyme than after injection of the same amount of free enzyme. In this case, the duration of the enzymatic effect was limited by the survival of the red blood cell ghosts in the circulation.

Chang *et al.* (13) also showed that L-asparaginase entrapped in semipermeable microcapsules (~80 μm in diameter) can deamidate L-asparagine *in vitro*. When given intraperitoneally in mice, the entrapped enzyme (3.5 IU) was more efficient than native enzyme and cured 50% of the treated mice bearing an L-asparagine-dependent lymphoma cell line, 6C3HED (14).

The L-asparaginase implants thus were able to depress the systemic L-asparagine level significantly for prolonged periods. However, as is evident from the figures, the concentration of L-asparagine never reached zero. Therefore, it is not possible to predict whether the decreased level would influence the proliferation of L-asparagine-dependent tumors. Such tests may be performed with the 6C3HED cell line growing in mice.

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This paper is Number 3 in a series on Acrylic Microspheres *In Vivo*.

Fluorometric Determination of All-*trans* Retinol in Rat Serum

SU-CHEN WU, A. C. CAPOMACCHIA ˣ, and J. C. PRICE

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Abstract □ A rapid, specific, and sensitive fluorometric assay for retinol in rat serum is reported. Serum retinol is extracted into cyclohexane after dilution of the serum sample with aqueous acetonitrile. Sample volumes as small as 0.2 ml can be used with a limiting detectable concentration of 2.5×10^{-8} *M* for retinol.

Keyphrases □ Vitamin A—fluorometric determination in rat serum □ Retinol, all-*trans*—fluorometric determination in rat serum □ Fluorometry—determination of all-*trans* retinol in rat serum

Vitamin A (all-*trans* retinol) is well known for its importance in general growth, the growth and differentiation of epithelial tissues, visual function, and reproduction (1–4). A number of retinoids prevent or inhibit the growth of epithelial tumors (5–9). As early as 1926, it had been noticed that vitamin A deficiency leads to carcinomas in

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