

Treatment of experimental immune complex glomerulonephritis by sodium alginate

Abbas Mirshafiey^{a,*}, Zohreh Borzooy^a, Reza Safari Abhari^a, Alireza Razavi^a,
Mohammad Tavangar^a, Bernd H.A. Rehm^b

^aDepartment of Immunology, School of Public Health, Tehran University of Medical Sciences, P. O. Box 6446, 14155 Tehran, Iran

^bInstitut für Mikrobiologie, Westfälische Wilhelms-Universität Münster, Münster, Germany

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Abstract

We have studied the therapeutic efficacy of the sodium alginate in experimental immune complex glomerulonephritis. Bovine serum albumin (BSA) nephritis was induced in rats by a subcutaneous immunization and daily intravenous administration of BSA. Sodium alginate at two different doses (25 and 50 mg/kg) was administered intraperitoneally at regular 72-h intervals for 6 weeks. Onset of treatment was day 42. Urinary protein was measured weekly and serum anti-BSA antibody was assessed by ELISA method at different intervals. Animals were euthanized at the 12th experimental week and blood samples and kidney specimens were obtained. BUN, serum creatinine and serum cholesterol and triglyceride were measured at the time of sacrifice. Kidney specimens were processed for light and immunofluorescent microscopic examination. The tolerability and inhibitory effect of LVA on matrix metalloproteinase 2 (MMP-2) were tested using WEHI-164 cell line and zymography method. Results of this experiment showed that treatment with sodium alginate could significantly reduce the urinary protein excretion and serum creatinine in treated rats vs. nontreated controls. Anti-BSA antibody titers were lower in treated rats than in controls at the 12th week post-immunization. There was no significant difference in the level of BUN and serum lipids between two groups. Whereas, glomerular hypercellularity, PMN infiltration and glomerular deposition of BSA were less intense in treated rats vs. controls. Moreover, in vitro examinations revealed that treatment with LVA, as a very safe agent could diminish MMP-2 activity. These results suggest that treatment with sodium alginate as a new immunosuppressive agent can reduce proteinuria, inhibit MMP-2 activity and suppress the antibody production as well as the development of glomerular lesions in a rat model of immune complex glomerulonephritis. © 2005 Elsevier Inc. All rights reserved.

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1. Introduction

Alginates are natural copolymers comprised of β D-mannuronate (M-block) and α L-guluronate (G-block) linked by 1→4 glycosidic linkage. They are synthesized by bacteria belonging to the genera *Pseudomonas* and *Azotobacter* and brown sea-weeds, and the M-blocks of the bacteria, but not sea-weed polymers, are to a variable extent

acetylated at positions 0–2 and/or 0–3 (Skjak-Braek et al., 1986; Bucke, 1987; Rehm, 1998).

The variability in monomer block structures and acetylation which are associated with the source of alginate, strongly affect the physicochemical and rheological properties of the polymer, and the biological basis for the variability is therefore of both scientific and applied importance (Rehm and Valla, 1997).

The alginate gels are well known as biocompatible, degradable, and nontoxic. Thus, they are widely used as carriers for drug delivery, haemostatic wound dressing and immunoisolation systems for transplantation using uncoated alginate microsphere and devices anastomosed to the

* Corresponding author. Fax: +98 21 6462267.

E-mail address: a_mirshafiey@yahoo.com (A. Mirshafiey).

vascular system as arteriovenous shunts such as alginate-impregnated polyester vascular graft (Odell et al., 1994; Lanza et al., 1995; Lee et al., 1997; Kuipers et al., 2002; Streitenberger et al., 2002; Efentakis and Buckton, 2002). On the other hand, inhibitory effects of various types of alginic acid on hyaluronidase and mast cell degranulation were examined, so that alginic acid with an M/G ratio of 1.0 exhibited the strongest inhibition of both activities (Asada et al., 1997). Moreover, the protective and reparative effects of sodium alginate on radiation stomatitis and suppression of radioactive absorption by this compound in animals and human subjects were investigated (Hasegawa et al., 1989; Oshitani et al., 1990; Gong et al., 1991).

In this investigation, sodium salt low viscosity alginate (LVA) purified from *Macrocystis pyrifera* (Kelp) was used. The present study was carried out to evaluate the therapeutic efficacy of the i.p administration of different doses of LVA in a rat model of bovine serum albumin (BSA) nephritis, the experimental model relevant to the human proliferative glomerulonephritis (Fujita et al., 1991).

2. Materials and methods

2.1. Materials

Sodium salt low viscosity alginate (Sigma-Aldrich Co., USA) with viscosity of 2% solution (25 °C) was used at 5 g/l concentration in demineralized water. BSA (Fluka, The Netherlands) was dissolved in sterile 0.15 M NaCl solution at 5 and 30 mg/ml concentrations; complete Freund's adjuvant (CFA) was obtained from Difco Laboratories, Detroit, MI.

2.2. Animals

A total of 66 female Sprague–Dawley rats weighing 180 ± 20 g were obtained from the Razi Institute (Karaj, Iran) and were housed in our animal facility (temperature, 20–22 °C; humidity, 55–56%; 12-h light/12-h dark cycle; unlimited access to food and water) for at least 1 week before the experiment. Rats were divided randomly into four groups. N: normal group ($n=10$); P: patient group ($n=24$); T1 and T2: treatment groups ($n=2 \times 16$). Experimental procedures were performed in accordance with the recommendations and policies of the Iran Pasteur Institute for the protection of animals used for experimental and other scientific purposes.

2.3. Experimental protocol and treatment

To induce immune complex glomerulonephritis, the method of Yamamoto et al. (1978) was used. Briefly, 8 weeks after subcutaneous immunization with 1 mg of BSA in complete Freund's adjuvant, the animals received an intravenous dose of 2 mg BSA daily for 4 weeks. On day

42, intraperitoneal injections of sodium salt low viscosity alginate (LVA) solution at two different doses (25 and 50 mg/kg) to the treatment groups (T1 and T2) were given. Fourteen sodium alginate intraperitoneal injections were administered at regular 72-h intervals. The experiments were terminated on day 85.

2.4. Measurement of anti BSA antibody

Blood samples were collected by orbital plexus bleeding in the 3rd, 6th, 9th, and 12th experimental weeks. Polyvinylchloride ELISA plates (Nunc-Immuno Module, Denmark) were coated with BSA diluted in 0.1 M sodium carbonate buffer pH=9.6 by incubation for 1 h at 37 °C, followed by incubation for 16 h at 4 °C (1 µg/well). The wells were washed three times with 0.05% Tween 20 in PBS (PBS-T) and incubated for 1 h at 37 °C with the rat sera diluted $1:25 \times 10^3$. Unbound material was removed by three washes with PBS-T. Rabbit anti-rat polyvalent Ig-peroxidase conjugate diluted 1:250 in PBS-T was incubated at room temperature for 1 h, followed by three washes with PBS-T. The peroxidase was reacted with *O*-phenylenediamine (Sigma) and H₂O₂ (Merck, Germany) for 60 min, and 2 NH₂SO₄ was added for stopping the reaction. The reaction product was quantified by measuring extinction at 492 nm (Hogendoorn et al., 1990).

2.5. Assessment of kidney function

Measurement of proteinuria was carried out during 6 stages, from weeks 3 to 12 post immunization; urine was collected before the intraperitoneal injections of LVA. Urine protein was measured using precipitation by trichloroacetic acid. Serum creatinine was calculated by the alkaline picrate method (Bousnes and Taussky, 1945). Blood urea nitrogen (BUN) was assessed by the oxime method (Evans, 1968).

2.6. Evaluation of serum lipid levels

Serum triglycerides and serum cholesterol were determined by routine laboratory tests on the day of sacrifice.

2.7. Histological examinations

Kidney specimens were processed by light and immunofluorescence microscopic examination. For light microscopy, renal tissues were fixed by immersion in 10% buffered formalin, embedded in paraffin, and 4-µm sections were stained with hematoxylin–eosin and periodic acid-Schiff. The severity and extent of glomerular lesions were blindly evaluated in four parameters: hypercellularity; glomerular infiltration of PMN; fibrinoid necrosis and interstitial infiltration. In addition, the existence of tubular casts was considered. These parameters were evaluated by a semi-quantitative method of renal histology using a grading scale of 0–3 (0, negative; 1, mild; 2, moderate; 3, severe).

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