Prevention and suppression by azathioprine of venom-induced protein-losing gastropathy in dogs

(cobra venom/Naja naja/immunosuppression/Heidenhain pouches)

HORACE W. DAVENPORT

Department of Physiology, The University of Michigan, Ann Arbor, Mich. 48109

Contributed by Horace W. Davenport, January 7, 1976

ABSTRACT Irrigation of the dog's oxyntic glandular mucosa contained in a chronically prepared, vagally denervated, separated pouch of the dog's stomach with a solution (0.5 mg ml⁻¹) of lyophilized venom of the hooded cobra (Naja naja) increases the permeability of the mucosa. If irrigation with venom solution is repeated at weekly intervals, the mucosa responds with increasing plasma-shedding which reaches a peak of 1–2 ml min⁻¹ from roughly 60 cm² of mucosa in 4–6 weeks. Plasma shedding in response to irrigation with venom gradually declines, leaving a permanent residual response of different magnitude in different dogs. Giving naive dogs the immunosuppressant azathioprine by mouth in a dose of 5 mg kg⁻¹ day⁻¹, beginning 1 week before the first irrigation with venom solution and continuing for 4 weeks, postpones the plasma-shedding response until the sixth or seventh week of venom irrigation. The plasma-shedding response is wholly or partially suppressed by further administration of azathioprine by mouth in a dose of 3.3 mg kg⁻¹ day⁻¹. These data support the hypothesis that the plasma-shedding response to repeated venom irrigation involves the immune system of the stomach.

Irrigation of the dog's oxyntic glandular mucosa contained in a chronically prepared, vagally denervated, separated (Heidenhain) pouch of the stomach with a solution (0.5 mg ml⁻¹, pH 7.4) of lyophilized venom of the hooded cobra (Naja naja) causes an immediate but transitory increase in the permeability of the gastric mucosa to sodium and hydrogen ions (1). During the first irrigation of a pouch with the venom solution the volume of fluid shed by the pouch is no greater than the volume shed during control irrigations, and there is no plasma shedding. If irrigation with venom solution is repeated at weekly intervals, a large volume of fluid containing plasma proteins begins to appear during the period of irrigation, and the output of fluid and plasma reaches a peak of as much as 60 ml of fluid containing more than 30 ml of plasma in 30 min. Because the area of the mucosa in the pouch is about 60 cm², this output is as much as 0.5 ml of plasma coming from 1 cm² of mucosa in 30 min, a rate of plasma shedding which matches that encountered in severe human protein-losing gastropathy (2). Frequent irrigation with venom solution is not necessary to establish the plasma-shedding state. If a pouch is irrigated only once with the venom solution, a second irrigation 4 weeks later may evoke a large response. The response to repeated irrigation gradually dies down, leaving a residual response of different magnitude in different dogs, but a definite positive response may be obtained several years after the last previous irrigation of the pouch with venom. The response is partially suppressed by daily intramuscular injections of prednisolone tert-butyl acetate in a dose of 1 mg kg⁻¹ day⁻¹ (3). I have suggested that this model protein-losing gastropathy is the result of an immune reaction of the gastric mucosa (4). The permeability of the gastric mucosa is increased by some component of the venom solution, perhaps the phospholipase which attacks lecithin in the membrane of the mucosal cells. Increased permeability allows some antigenic component of the venom to reach immunologically competent mucosal cells. Upon further exposure to venom, an immune reaction occurs in the mucosa, and histamine is released. By some unknown mechanism, the tight junctions of the surface epithelial cells become leaky to plasma protein (5), and the mucosa sheds a large volume of plasma together with interstitial fluid. To test this hypothesis, I have treated dogs with azathioprine, an immunosuppressive drug (6), before and during responses to venom irrigation. The results presented in this paper show that azathioprine treatment postpones and suppresses the plasma-shedding which is a consequence of venom irrigation.

METHODS

The preparation of the pouches, the care and treatment of the dogs, the method of irrigating their pouches, and the measurement of plasma shedding have been fully described (1, 3). Briefly, female dogs in good health were provided with vagally denervated, separated pouches of the oxyntic glandular mucosa which drained to the outside through a cannula. A dog was deprived of food but not water 18 hr before the experiment. The dog's pouch was rinsed out with 15 mM sodium phosphate buffer, pH 7.4, made isotonic with NaCl. The dog was given an intravenous injection of T-1824 (Evans blue) in a dose of approximately 25 mg, and its pouch was then irrigated for 30 min with 30 ml of the phosphate buffer. The volume of fluid put out by the irrigated mucosa in this period was usually 1–3 ml, and there was no plasma shed. Then the pouch was irrigated for another 30 min with a solution (0.5 mg ml⁻¹) of lyophilized venom of Naja naja in the same phosphate buffer. A blood sample was taken at the midpoint of the irrigation period. The fluid removed from the pouch was optically clear after centrifugation, and its plasma content was measured by comparing its absorbance at 615 nm with that of a suitably diluted portion of the plasma. Electrophoretic analysis of fluid obtained during irrigation with venom solution demonstrated that the blue color of the fluid actually represented plasma shed. Venom was obtained from Ross Allen Reptile Institute Silver Springs, Inc., Silver Springs, Fla., and no differences have been observed in the responses to many different batches received over more than 6 years.

RESULTS

Control observations

The pouches of seven dogs were irrigated with a solution of venom of the hooded cobra once a week or at longer in-
were again given after dogs were in azathioprine dose control not are volume their Fig. 2. Suppression of response at peak during stomachs, denervated ly
numbers solution of for put. Values were irrigation Six outputs venom plasma-shedding one and the probability that the plasma outputs are the same as those in the corresponding week in Fig. 1 is less than 0.001, and the upper asterisks indicate that the probability that the total volume outputs are the same as those in the corresponding week of Fig. 1 is less than 0.001.

Because zero response to venom irrigation did not occur in all three dogs in the same week, Fig. 2 does not show complete suppression. The data obtained in one of the dogs are shown in Fig. 3. They demonstrate both prolonged prevention of the response during and after the initial course of azathioprine treatment and complete suppression for 1 week during the second course of treatment. I began a third course of azathioprine administration, hoping to find another cycle of suppression and recovery, but in the 17th week the dog pulled her cannula out, developed peritonitis, and died.

All dogs responding to venom irrigation with plasma-shedding vomit. The vomiting experience of the dog whose data are given in Fig. 3 is indicated by the V's at the tops of the bars. Venom irrigation also stimulates motility of the pouch, but I have no quantitative data on this point.

DISCUSSION

These data demonstrate that administration of azathioprine to dogs prevents the development of plasma-shedding by the oxyntic glandular mucosa in response to repeated irrigation

FIG. 2. Ordinate: as in Fig. 1. Azathioprine was given by mouth in the dose and for the period indicated. The pouches of four dogs were irrigated for weeks 1 through 12 with a 0.5 mg ml⁻¹ solution of lyophilized cobra venom, and the pouches of three of those were similarly irrigated for the remaining weeks. The lower asterisks indicate that the probability that the plasma outputs are the same as those in the corresponding week in Fig. 1 is less than 0.001, and the upper asterisks indicate that the probability that the total volume outputs are the same as those in the corresponding week of Fig. 1 is less than 0.001.

FIG. 3. Ordinate: as in Fig. 1. The pouch of one dog was irrigated with a 0.5 mg ml⁻¹ solution of lyophilized cobra venom for 30 min once a week from week 1 through week 16. Azathioprine was given by mouth in the dose and for the periods indicated.
of the mucosa with a solution of the venom of *Naja naja*. They also show that the response, once developed, can decline to zero during a second course of azathioprine treatment. The response to venom spontaneously declines, and it is possible that the decline seen during the second course of treatment was merely coincidental. However, I have never encountered a decline to zero in the absence of azathioprine or prednisolone treatment. The fact that the response increased substantially after termination of azathioprine treatment supports the conclusion that the decline during treatment was actually caused by the drug. Because a major effect of azathioprine is to suppress immune responses, these data validate the hypothesis that venom-induced protein-losing gastropathy involves an immune reaction by the oxyntic glandular mucosa.

Two major problems remain: the identification of the cells in the gastric mucosa which respond to venom, and the mechanism by which the mucosa is made leaky to plasma proteins.

I am grateful to Dr. Gordon L. Kauffman, Jr., for help. This work was supported by Grant AM-08716 from the U.S. Public Health Service.