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CONTRIBUTION OF THE KALLIKREIN/KININ SYSTEM TO THE MEDIATION OF ConA-INDUCED INFLAMMATORY ASCITES

KÁROLY BAINTNER*

Dept. of Physiology, Faculty of Animal Science, University of Kaposvár, Kaposvár, Hungary

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Intraperitoneal administration of concanavalin A (ConA, 25 mg/kg b.w.), a cell-binding plant lectin was used for inducing inflammatory ascites, and potential inhibitors were tested in 1 h and 2.5 h experiments, i.e. still before the major influx of leucocytes. At the end of the experiment the peritoneal fluid was collected and measured.

The ConA-induced ascites was significantly ($p<0.01$) and dose-dependently inhibited by icatibant (HOE-140), a synthetic polypeptide antagonist of bradykinin receptors. Aprotinin, a kallikrein inhibitor protein also had significant ($p<0.01$), but less marked inhibitory effect. L-NAME, an inhibitor of NO synthesis, and atropine methylnitrate, an anticholinergic compound, were ineffective.

It is concluded, that the kallikrein/kinin system contributes to the mediation of the ConA-induced ascites by increasing subperitoneal vascular permeability, independent of the eventual vasodilation produced by NO. It is known, that membrane glycoproteins are aggregated by the tetravalent ConA and the resulting distortion of membrane structure may explain the activation of the labile prekallikrein.

Complete inhibition of the ConA-induced ascites could not be achieved by aprotinin or icatibant, which indicates the involvement of additional mediators.

Keywords: ascites, concanavalin, kallikrein, bradykinin, aprotinin, icatibant, L-NAME, fetuin

Introduction

The peritoneal fluid originates mainly from the subperitoneal vessels of the intestines and provides lubrication for the movement of abdominal organs. The excess fluid is returned to the lymphatics through the lymphatic stomata [1], lo-

*E-mail: rakiab22@gmail.com

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cated mainly at the diaphragm. Introduced substances may be absorbed also across the mesothelial lining.

The large molecules, due to their slow peritoneal absorption, are more suitable for the study of ascites induction, than the small ones. In an earlier study [2] a screening of inducer macromolecules was performed. Three groups of compounds were effective, namely the cell-surface binding lectines, the polykations (polyLys, polyArg and polyethyleneimine) and a heterogeneous group of polysaccharides, e.g. the well-known inflammatory agents zymosan and carrageenan.

In the present work the ConA, a tetravalent, mannose/glucose specific, cell binding plant lectin [3] was used for the routine induction of inflammatory ascites to test various compounds for inhibitory activity. The results indicate the contribution of the kallikrein/kinin system to the mediation of the ConA induced ascites.

Materials and Methods

The chemicals were Sigma products, except the Kunitz-type soyabean trypsin inhibitor (Reanal). The agents were dissolved in physiological saline, the ConA always freshly. MK886 was first dissolved with a drop of ethanol, then filled up with saline to the required volume.

Animals: Female, 24 g NMRI mice of SPF origin were kept in cages and had free access to commercial chow and water. – The experiments complied with the Hungarian Animal Welfare Act XXVIII/1998 and Edict 243/1998 (amended in 2012) and were approved by the Veterinary Office of Somogy County 1126/001/SOM/2005 and the local ethical committee.

Protocol: The animals were used in groups of five or six, except mentioned. Both i.p. and s.c. injections were administered in 0.1 ml volume. Inflammatory ascites was induced by injecting ConA (25 mg/kg b.w.) intraperitoneally with or without inhibitor. One or 2.5 h later the animals were decapitated under ether anaesthesia, the abdomen was opened along the *linea alba*, and the ascitic fluid was carefully collected with a pipette, always performed by the same person. The volume was measured by pipetting the fluid into another vessel and expressed as % of body weight. Blood-stained samples were discarded.

Aprotinin (in the 2.5 h experiment), fetuin and icatibant were administered mixed with the ConA inducer. Other inhibitors were injected s.c., 20 min before the induction. In the 2.5 h experiment half of the L-NAME dose was added before the induction by ConA and the other half 1 h later.

Statistics: Significance of differences was calculated with ANOVA of the SPSS program.

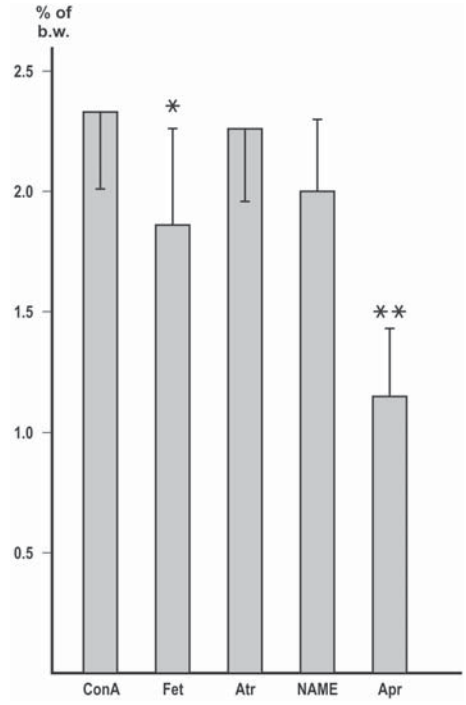

Figure 1. Inhibition of ConA-induced ascites, 2.5 h experiment.
Collected ascitic fluid in % of body weight. Mean±SD. Asteriscs indicate significant differences as compared to the ConA control: *p<0.05; **p<0.01. See the text for the doses.
ConA = control without inhibitor (n = 5); Fet = fetuin (n = 5); Atr = atropine methylnitrate (n = 6); NAME = N-nitro-L-arginine-methyl-esther (n = 6); Apr = aprotinin (n = 4)

Results

I.p. injection of 0.1 ml of physiological saline was used as a negative control. Due to the absorption of the saline, this control produced very low peritoneal fluid volumes, which could not be exactly measured, and were not considered.

I.p. administered ConA (25 mg/kg b.w.) was used as positive control. Its repetition did not produce significant differences. The same treatment was used routinely in the experiments with inhibitors. In the icatibant study another ConA charge with slightly lower activity was used.

In the 2.5 h experiment (Figure 1) fetuin (75 mg/kg b.w.), atropine methylnitrate (0.4 mg/kg), L-NAME (N-nitro-L-arginine-methyl-esther. HCl, 30 mg/kg) and aprotinin (a trypsin and kallikrein inhibitor protein, 100 mg/kg) were tested

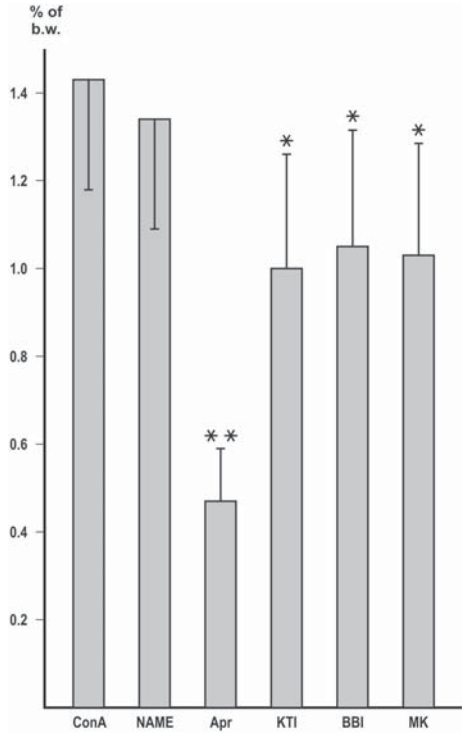

Figure 2. Inhibition of ConA-induced ascites, 1 h experiment. See Fig. 1. for explanation.
ConA = control without inhibitor (n = 5); NAME = N-nitro-L-arginine-methyl-esther (n = 8);
Apr = aprotinin (n = 6); KTI = Kunitz trypsin inhibitor (n = 5);
BBI = Bowman-Birk trypsin-chymotrypsin inhibitor (n = 4); MK= MK886 (n = 8)

as inhibitors. Fetuin ($p<0.05$) and aprotinin ($p<0.01$) significantly reduced the volume of the of ascitic fluid. Other treatments did not reach the level of significance.

In the 1 h experiment (Fig. 2) two treatments were repeated with similar results as before: aprotinin (100 mg/kg) was markedly inhibitory ($p<0.01$), and L-NAME (20 mg/kg) was ineffective. Three additional compounds exerted slight inhibitory effect ($p<0.05$), namely, the Kunitz (100 mg/kg) and Bowman-Birk (100 mg/kg) trypsin inhibitors of the soyabean, and a leukotriene synthesis inhibitor (MK886, 3 mg/kg).

In a third experiment (Fig. 3) icatibant acetate (HOE-140), a bradykinin receptor antagonist polypeptide was tested, and highly significant ($p<0.01$) inhibition of fluid accumulation was observed at both 5 and 25 mg/kg b.w. dose level.

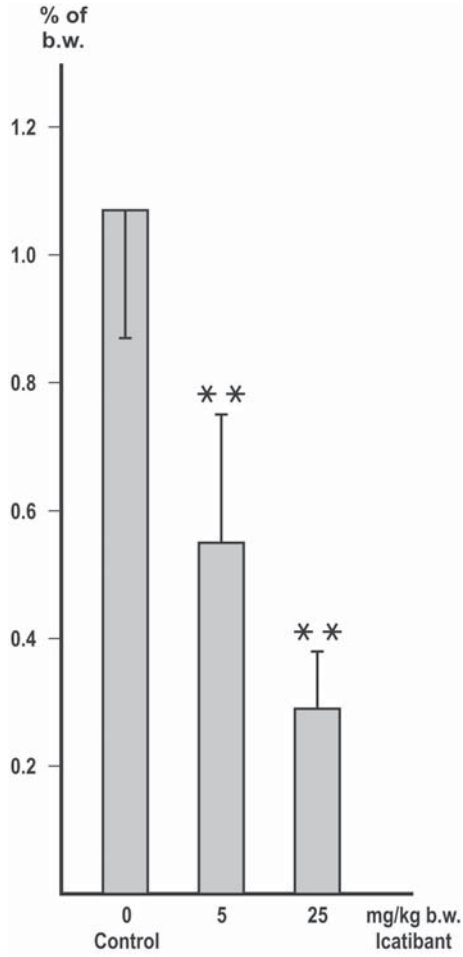

Figure 3. Inhibition of ConA-induced ascites with icatibant (HOE-140), 1 h experiment. Control = ConA without inhibitor (n = 7); 5 mg/kg (n = 5) and 25 mg/kg icatibant (n = 6)

Discussion

In an earlier study a wide range of macromolecules was screened for the ability to induce inflammatory ascites [2], still before the major influx of leukocytes [4]. In further studies ConA was chosen for routine induction of ascites to clarify the mechanism of the process. Histamine was excluded as mediator [5].

Next we applied other potential inhibitors of ascites formation to obtain indirect information about the mediators. In a preliminary screening thalidomide (NFκB inhibitor), captopril (ACE- and tissue metalloproteinase inhibitor) and ZM323881 (VEGF receptor antagonist) had no inhibitory effect (unpublished). In the present work conclusions were drawn only if the differences reached the 1% confidence level as compared to the positive control group (ConA alone).

In both the 2.5 h and 1 h experiments (Figs. 1 and 2) high aprotinin dose (100 mg/kg b.w.) was required for significant ($p<0.01$) inhibition of ConA-induced ascites. The bradykinin receptor antagonist icatibant was more effective than aprotinin and produced dose-dependent inhibition ($p<0.01$, Fig. 3). It is concluded that the kallikrein/kinin system contributed to the mediation of the ConA-induced ascites via the B2 receptor. The role of the inducible B1 receptor can be excluded, because of the short duration of the present experiments. The findings are in agreement with the ascites-inducing role of kallikrein in the ovarian hyperstimulation syndrome [6].

In the present work complete inhibition of ConA-induced ascites could not be achieved, which indicates the involvement of additional mediators.

It is known that bradykinin produces vasodilatation by the activation of eNOS. However, the inhibition of NO synthesis by L-NAME (Figs. 1 and 2) did not affect the ConA-induced ascites. Permeability of subperitoneal capillaries may be controlled by Ca^{2+} influx, another effect of bradykinin [7].

The native, tetravalent ConA makes non-covalent cross-links between the glycosyl side-chains of cell surface glycoproteins resulting in their aggregation [8]. The monovalent succinyl-ConA is ineffective [2]. The distortion of membrane structure may activate membrane prekallikrein [9] resulting in the release of kinins. Cellular mediation of the ConA-induced ascites is beyond the scope of the present paper.

In spite of being trypsin-like proteinases, kallikreins are not inhibited by the trypsin inhibitors of the soyabean. However, both Kunitz and Bowman-Birk inhibitors exerted a small, but significant ($p<0.05$) effect on the ConA-induced ascites (Fig. 2).

Parasympathic mediation was excluded, using atropine methylnitrate (Fig. 1), a molecular variant without central effect.

Fetuin is a mannose-containing, heavily glycosylated serum protein. When injected in threefold excess (by weight) of ConA, it exerted slight but significant ($p<0.05$) inhibitory effect on the accumulation of peritoneal fluid (Fig. 1). Although the mannose residue directly reacts with the functional moiety of the ConA molecule, *in vivo* the lectins are transposed to the cell membrane receptors [2], which may explain the poor inhibitory effect of fetuin.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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