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Hyaluronidase ameliorates rejection-induced edema

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Abstract Hyaluronan, a glucosaminoglycan with unique water-binding capacity, is accumulated in the interstitial edematous tissue in rejecting organs. We here investigated whether the increased tissue content of water and hyaluronan seen during allograft rejection can be prevented by treatment with the hyaluronan-degrading enzyme hyaluronidase. Heterotopic heart transplantations between PVG and Wistar/Kyoto rats were performed. Recipient rats were treated with hyaluronidase prophylactically or therapeutically, either alone or in combination with cyclosporine. Daily intravenous injections of hyaluronidase induced a significant reduction of the cardiac content of both hyaluronan and water, as evaluated on day six after transplantation. Morphological examination revealed grafts with better preserved morphology and fewer infiltrating mononuclear cells, compared to untreated controls. Hyaluronidase therapy, alone or combined with cyclosporine, resulted in prolonged graft survival times. Hyaluronidase infusion for two hours also reduced already established edema five days after transplantation. This study confirms the hypothesis that hyaluronan accumulation plays a critical role in edema formation, and that hyaluronidase therapy can be used to reduce edema after organ transplantation.

Key words Oedema, transplantation · Rejection · Inflammation · Hyaluronan · Hyaluronidase

Introduction

Hyaluronan (hyaluronic acid), a glucosaminoglycan macromolecule composed of the repeating structure of disaccharide N-acetyl-D-glucosamine-D-glucuronic acid, is an important constituent of the extracellular matrix, with a stabilizing function within the loose connective tissue [2]. Hyaluronan synthesis by mesenchymal cells undergoes dynamic regulation during embryogenesis, tumour invasion and inflammation. In these processes, hyaluronan exerts its function by providing a provisional matrix for the support of cellular migration and adherence [1, 4, 14, 31, 32, 35]. Certain functions of hyaluronan, e.g. recruitment of leukocytes to inflammatory sites, are mediated by the hyaluronan-interaction with the cell adhesion molecule CD44 and the receptor for hyaluronan-mediated motility (RHAMM) [for review see 26].

In previous clinical and experimental studies we have demonstrated an interstitial accumulation of hyaluronan in various inflammatory conditions; e.g. alveolitis, adult respiratory distress syndrome, myocarditis and myocardial infarction [for review see 4]. The increased hyaluronan content of the inflamed tissue is probably due to an enhanced local synthesis of hyaluronan induced by proinflammatory cytokines and growth factors released by inflammatory cells [3, 8, 9, 28, 38]. Furthermore, the degree of interstitial hyaluronan accumula-

tion was found to be strictly related to the magnitude of the interstitial edema [21]. Since hyaluronan attracts water by osmotic forces, and also resists water flow it is of importance for water homeostasis [2, 16]. It was therefore suggested that hyaluronan played a critical role in the development of inflammatory edema.

Organ allograft rejection is a powerful inflammation with interstitial edema, inflammatory cells, and a massive release of cytokines. The interstitial accumulation of hyaluronan in transplanted organs during rejection has been thoroughly documented [5, 6, 12, 34, 36] and found to correlate with the development of edema in the graft. Rejection-induced interstitial edema may be of such a magnitude that it threatens the function and viability of the graft. Theoretically, inhibition of hyaluronan synthesis or enzymatic degradation of accumulated hyaluronan should reduce the interstitial edema. In the present study, our aim was to test the possible therapeutic effects of treatment with the hyaluronan degrading enzyme hyaluronidase in a heterotopic heart transplantation model in the rat.

Materials and methods

Animals

Male PVG ($RT1^c$) and Wistar/Kyoto ($RT1^{lv}$) rats weighing 175–250 g were used for the experiments. The animals were obtained from Møllegaard (Skensved, Denmark) and were allowed to settle for at least one week before operation with free access to food and water. Heart transplantations were performed under anaesthesia with a mixture of chloral hydrate (180 mg/kg body weight), pentobarbital (40 mg/kg body weight) and magnesium sulphate (90 mg/kg body weight), administered intraperitoneally. For the infusion experiments, the rats were anaesthetised by intraperitoneal injections of Inactin (Byk, Gulden, Konstanz, Germany), 120 mg/kg body weight. The experiments were approved by the regional ethical committee, and the handling of the animals conformed with the guidelines for the care and use of laboratory animals [20].

Heterotopic heart transplantation

Heterotopic heart transplantations were performed using PVG-rats as donors and Wistar/Kyoto-rats as recipients. The aorta of the donor heart was anastomosed to the right common carotid artery of the recipient, and the pulmonar artery to the right jugular vein, using a non-suture technique [22]. In brief, the carotid artery and the jugular vein were dissected free, cross-clamped caudally and cut cranially. Short plastic tubes were placed around the vessels, and the vessels were then turned inside out over the tubes and fixed with ligatures. The donor heart was anastomosed by pulling the vessels of the graft over the tubes and fastening them with ligatures.

After transplantation, a single dose of cefuroxim (Zinacef®, Glaxo, Greenford, UK), 20 mg/rat, was administered intramuscularly. Graft function was monitored by daily palpation, and the hearts were regarded as rejected when no pulsations could be detected in the transplant any longer.

Intravenous hyaluronidase-treatment

Immediately after reperfusion of the graft, the recipient rat was given 20,000 U/kg body weight of sheep testes hyaluronidase, Type V (Sigma Chemical Co., St. Louis, MO, USA), administered intravenously. Hyaluronidase was dissolved in phosphate-buffered saline (PBS) with 2% albumin (Pharmacia AB, Stockholm, Sweden). Intravenous doses of 20,000 U/kg body weight were then given once daily on days 1, 2, 3, 4, and twice daily on day 5; the last dose of 20,000 U/kg body weight was given on day 6, 2 h before harvesting. In addition, in this experimental group ($n = 11$) the donor heart was flushed with hyaluronidase at the time of procurement; first the heart was flushed with 0.5 ml FW-solution [11] via the aorta, and thereafter with another 0.5 ml of FW-solution containing 2,500 U hyaluronidase. The rats in the control group ($n = 11$) were left untreated until harvested on day 6 after transplantation. In this group, the donor hearts were flushed with 1.0 ml of FW-solution without hyaluronidase.

Intravenous hyaluronidase-treatment and graft survival

The effect of hyaluronidase-treatment on graft survival was investigated in rats receiving daily intravenous doses of hyaluronidase, 20,000 U/kg, as long as the grafts were functioning ($n = 11$). Untreated heart graft recipients served as controls ($n = 10$). All animals were followed until the grafts were rejected.

As a separate experiment, the effect of hyaluronidase-treatment in combination with cyclosporine was investigated. These animals were given daily intravenous doses of either hyaluronidase, 20,000 U/kg, ($n = 11$) or vehicle ($n = 10$) from day 0–8. Both groups received cyclosporine (Sandoz AG, Basel, Switzerland), 10 mg/kg, starting at day 6, as long as the grafts were functioning. Cyclosporine was mixed with Intralipid® (Pharmacia AB, Stockholm, Sweden) and administered orally via a gastric feeding tube (CH 05). The experiment was terminated at graft rejection or, if the grafts were still functioning, 30 days after transplantation. The grafts of some animals in this experiment ($n = 7$) underwent ultrasound examinations on day 7 ($n = 3$ hyaluronidase, $n = 4$ vehicle).

Ultrasound examination

Ultrasound examinations were performed using a linear transducer (15L8) with variable center frequencies from 8–13 MHz (Acuson, Sequoia 512, Mountain View, Calif., USA). The same protocol was used in all cases. For the ultrasound exam, the animal was anaesthetised and transducer gel was applied to the shaven skin covering the graft. The optimal frequency (normally 13 MHz) that provided a clear image of the heart was chosen. The valve plane was identified, the heart was measured in three perpendicular diameters, and the volume calculated.

The type of perfusion in the myocardium, developed with colour Doppler energy (CDE), was subjectively evaluated and scored as normal, semi-coarse or coarse. All ultrasound examinations were performed by the same person (AE) without knowledge of what treatment the animals had received.

Short-term hyaluronidase-infusion

Five days after transplantation, previously untreated rats were anaesthetised and a catheter was inserted into the left femoral vein. The catheter was connected to a mechanical pump through which hyaluronidase ($n = 4$) or PBS ($n = 6$) was infused at a flow rate of

1.5 ml/h for 2 h. Before starting the infusion, an intravenous bolus dose of hyaluronidase (20,000 U/kg) or PBS (0.3 ml) was given. When the infusion was finished, the rats were killed and the cardiac grafts harvested.

Harvest of cardiac grafts

At harvest, one third of the transplanted heart was fixed in buffered 4% formalin, pH 7.3, with 1% cetylpyridiniumchloride, one third was snap frozen in cold isopentan, and the remaining third was taken for determinations of the water- and hyaluronan-contents. For control purposes, hearts were obtained from four untreated, non-transplanted rats.

Quantitative assay of hyaluronan and determination of water content

Immediately after harvesting, specimens were put on filter paper and weighed three minutes later (wet weight, w.w.). The specimens were lyophilised and weighed again (dry weight, d.w.). After grinding, the hyaluronan was extracted from the tissues for 16 h with 0.5 M NaCl. Following centrifugation for 15 min at 2,000 g, the hyaluronan-content of the supernatants was analysed using a commercially available radiometric assay (Pharmacia Diagnostics, Uppsala, Sweden). The technique is based on the binding of hyaluronan to specific hyaluronic acid binding proteins (HABP) [30]. In brief, a 100 µl sample is incubated for 60 min at 4–7 °C with 200 µl ^{125}I-labelled HABP. 100 µl hyaluronan-Sepharose is added, and the incubation continued for 45 more minutes at the same temperature. Before centrifugation at 2,000 g for 10 min, 2 ml washing solution is added. After decantation, the radioactivity in the pellet is measured in a gamma counter. A standard curve is constructed from samples with known amounts of hyaluronan. Double analyses were performed on each sample, with a variability of less then 10%. The relative water content, expressed as percent water of the total weight of the tissue, was calculated as $100 \times$ (w.w. – d.w.)/w.w.

Histochemistry

In order to determine the distribution of hyaluronan and the existence of CD44 positive cells, histochemical stainings were performed. For the detection of hyaluronan, an avidin-enzyme biotin-protein system was used mainly, as described earlier [5]. In brief, paraffin-embedded, 4 µm thick sections were incubated with bovine serum albumin (10 mg/ml, Fraction V, Sigma Chemical Co, St. Louis, MO, USA) to block non-specific binding sites and, thereafter, in 3% H_2O_2 in PBS to inhibit endogenous peroxidase. After incubation for 2 h with HABP, the sections were incubated with ABC Vectastain Reagent (Vector Laboratories, Burlingame, CA, USA) for 1 h. Finally, H_2O_2 as substrate and 3-amino-9-ethyl-carbazole (AEC) as electron donor were added, whereafter the specimens were counterstained with Mayer's haematoxylin. Control sections were incubated for 2 h with Streptomyces hyaluronidase. For the visualisation of CD44 positive cells, the monoclonal antibody OX50 (Serotec, Oxford, UK), was used [23]. The stainings were performed on 6 µm thick, acetone-fixed cryostat sections of frozen biopsies using a peroxidase-antiperoxidase (PAP) technique. In brief, the sections were incubated in 0.3% H_2O_2 in PBS to inhibit endogenous peroxidase and, thereafter, with normal goat serum (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) to prevent non-specific background staining, before incubation with the primary antibody. A secondary antibody, goat anti-mouse IgG (Jackson ImmunoResearch Laboratories), was used and added in excess. Then, the sections were incubated with horseradish peroxidase-mouse antiperoxidase (Dakopatts, Glostrup, Denmark). Finally, H_2O_2 as substrate, and 3-amino-9-ethyl-carbazole (AEC) as electron donor were added to react with the horseradish peroxidase. Counterstaining was performed with Mayer's haematoxylin. Negative controls were obtained by omitting the primary antibody. All slides were evaluated blindly.

Morphology

Cardiac grafts fixed in formalin were embedded in paraffin, cut into 4 µm thick sections and stained with eosin and Mayer's haematoxylin. All slides were evaluated blindly and ranked manually according to general morphology; edema, infiltrating cells and myocyte necrosis.

Statistical analysis

Data are given as mean values and standard errors of the mean (SEM). Comparison between groups were evaluated by Student's unpaired t-test for hyaluronan- and water-contents, and by Mann-Whitney U-test for graft survival times, ultrasound examinations, and for the ranking of slides. Correlation between hyaluronan and water was evaluated by linear regression. A P level of less than 0.05 was regarded as statistically significant.

Results

Effects of intravenous hyaluronidase-treatment on hyaluronan, edema and graft morphology

The relative water content of hearts from untreated, non-transplanted rats was 76.3 ± 0.3%, and the hyaluronan content 206 ± 18 µg/g d.w. In cardiac grafts from untreated rats, the water content had increased to 81.8 ± 0.3%, when harvested 6 days after transplantation, and the hyaluronan content to 789 ± 47 µg/g d.w. Daily hyaluronidase therapy significantly reduced this accumulation of water and hyaluronan. Thus, the relative water content in the hyaluronidase treated group was 80.6 ± 0.3% ($P < 0.01$ compared with the untreated group) and the hyaluronan content 494 ± 43 µg/g d.w. ($P < 0.001$ compared with the untreated group). There was a significant correlation between the relative water content and the hyaluronan content in both groups ($r = 0.77$, $P < 0.01$ for the untreated group and $r = 0.93$, $P < 0.001$ for the hyaluronidase treated group; Fig. 1).

Histochemical studies of the normal heart tissue demonstrated positive staining for hyaluronan in the adventitia of arteries and veins and in the connective tissue of the perimysium, and only a minute staining in the narrow endomysium (Fig. 2a). During rejection, the accumulation of hyaluronan was localised to the widened endomysium, which showed a homogenous staining for hy-

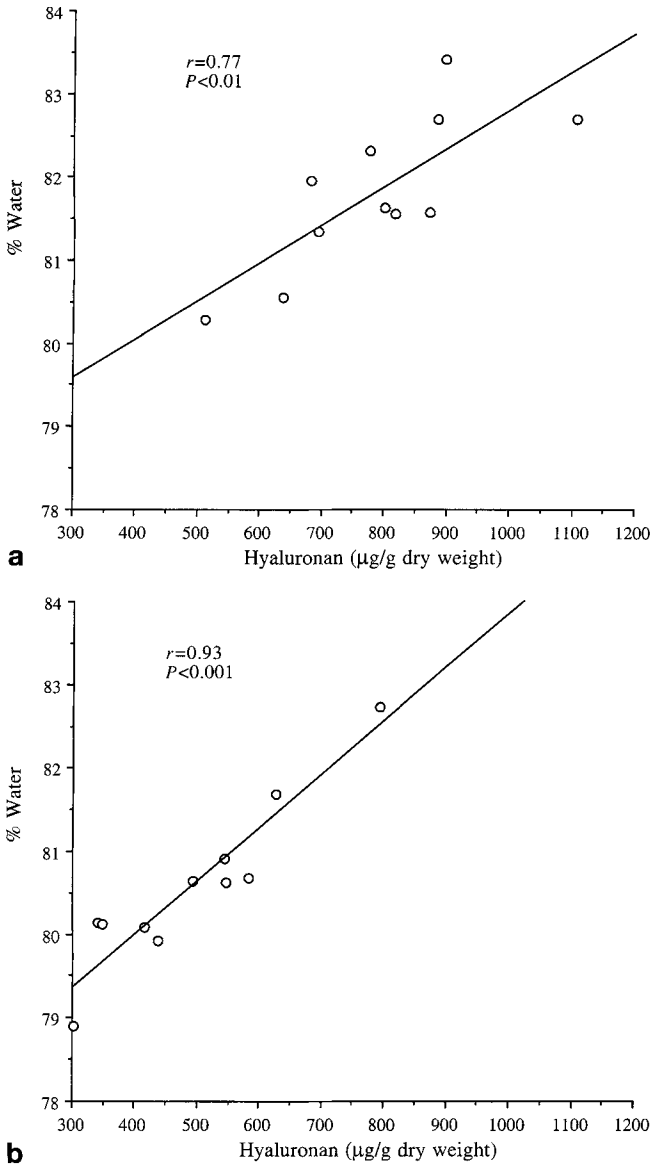

Fig. 1 Correlation between hyaluronan and water contents in cardiac allografts 6 days after transplantation. Recipient rats were either untreated **(a)**, or treated with daily intravenous injections of hyaluronidase **(b)**

aluronan (Fig. 2b). Patchy positive staining for hyaluronan was seen in the cell-infiltrated areas (Fig. 2c–d). Hyaluronidase treatment ameliorated or totally inhibited such hyaluronan accumulation (Fig. 2e–f), and blind morphologic evaluation of the cardiac grafts revealed a better morphology in the enzymatically treated group ($P < 0.01$) with fewer infiltrating cells, milder edema and a better preserved myocardium. In normal heart tissue, just a few scattered mononuclear cells stained positive for the CD44-marker OX50. In rejecting grafts, most of the infiltrating cells were CD44-positive. In addition, there was a faint staining for CD44 in the myocardial interstitium, a staining that most probably reflects shedding of the receptor. There was no obvious difference in CD44-staining between treated and non-treated animals.

Effects of hyaluronidase-treatment on graft survival

In untreated rats, the cardiac grafts survived for 8.3 ± 0.3 days. Daily intravenous treatment with hyaluronidase significantly prolonged graft survival time to 9.7 ± 0.5 days ($P < 0.01$). Histological evaluation of these rejected grafts also indicated a less pronounced edema in grafts from hyaluronidase treated animals, compared to grafts from untreated rats. No obvious negative effects of the enzymatical treatment were seen, since the animals remained healthy throughout the study.

When hyaluronidase therapy was combined with cyclosporine, 64% of the animals were alive with beating grafts 30 days after transplantation (Fig. 3). However, one graft was rejected during the study period (day 12), whereas 3 animals had to be sacrificed with functioning grafts on days 18, 18 and 20, due to the development of ileus, probably related to the intraperitoneal anaesthesia. In the control group, receiving vehicle and cyclosporine, 20% of the grafts functioned for more than 30 days ($P < 0.01$; Fig. 3).

Evaluations of the ultrasound examinations performed on day 7 after transplantation revealed that grafts from hyaluronidase treated animals had a volume of 0.87 ± 0.13 cm^3, and grafts from vehicle treated animals a volume of 1.55 ± 0.22 cm^3 ($P < 0.05$). The perfusion of the myocardium in the hyaluronidase group was on average better than that of the vehicle group (semi-coarse vs coarse).

Effects of short-term hyaluronidase-infusion

Grafts from rats with transplanted hearts that received a 2-h infusion of PBS on day 5 had a relative water content of 81.2 ± 0.5%, and a hyaluronan content of 504 ± 82 μg/g d.w. (Fig. 4). In contrast, grafts from rats that received hyaluronidase infusion contained 78.8 ± 0.4% water ($P < 0.01$) and 174 ± 37 μg/g d.w. of hyaluronan ($P < 0.05$).

Discussion

Treatment of rats with transplanted hearts with the hyaluronan-degrading enzyme hyaluronidase was found to ameliorate the development of interstitial edema after allogeneic transplantation. The reduced water con-

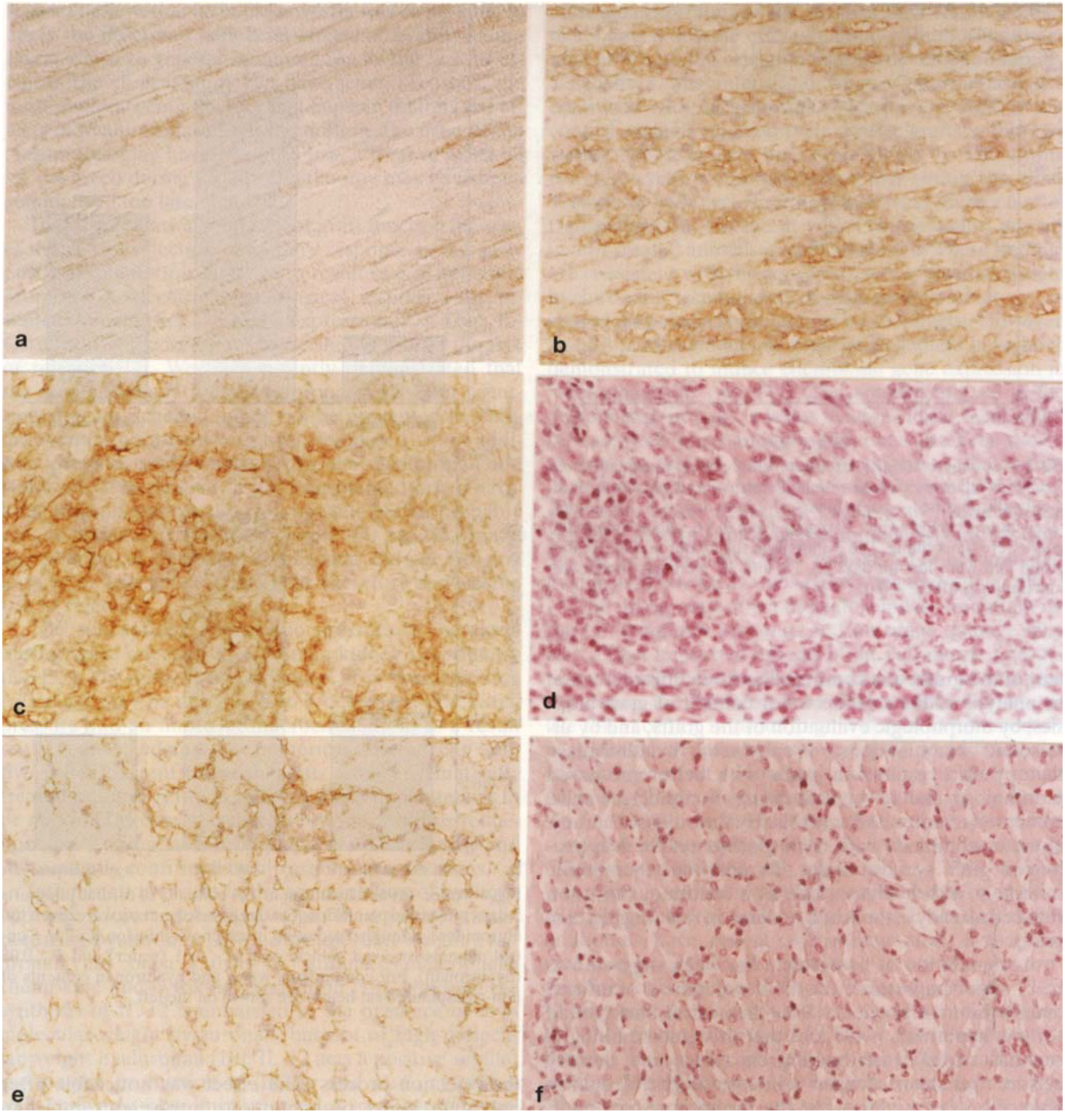

Fig. 2 Tissue sections from normal rat heart **(a)**, and cardiac grafts 6 days after allogeneic transplantation **(b–f)**. Staining for hyaluronan reveals a minute staining in the endomysium in the normal heart **(a)**, and an intense staining of the cell-rich widened interstitium in the rejecting heart **(b)**. The accumulation of hyaluronan is more pronounced in areas with more profound inflammatory reaction **(c)** as seen in haematoxylin staining **(d)**. In animals treated with hyaluronidase for 6 days there is a considerable reduction in tissue hyaluronan accumulation **(e)** paralleled with a decreased widening of the interstitium and less infiltration by inflammatory cells **(f)**

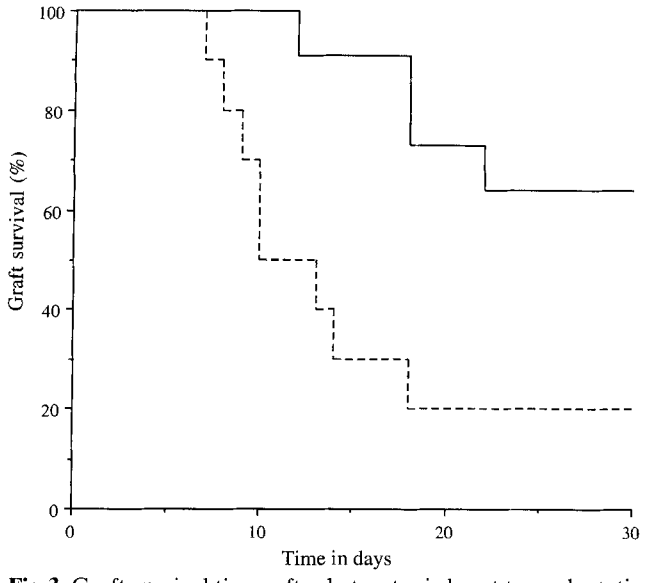

Fig. 3 Graft survival times after heterotopic heart transplantation. Recipient rats were treated with daily intravenous injections of either hyaluronidase (—) or vehicle (- - -) from days 0–8. All animals received oral cyclosporine, 10 mg/kg, from day 6, and as long as the grafts were functioning. The 3 animals in the hyaluronidase treated group that were lost on days 18, 18, and 20, were sacrificed with functioning grafts. $P < 0.01$

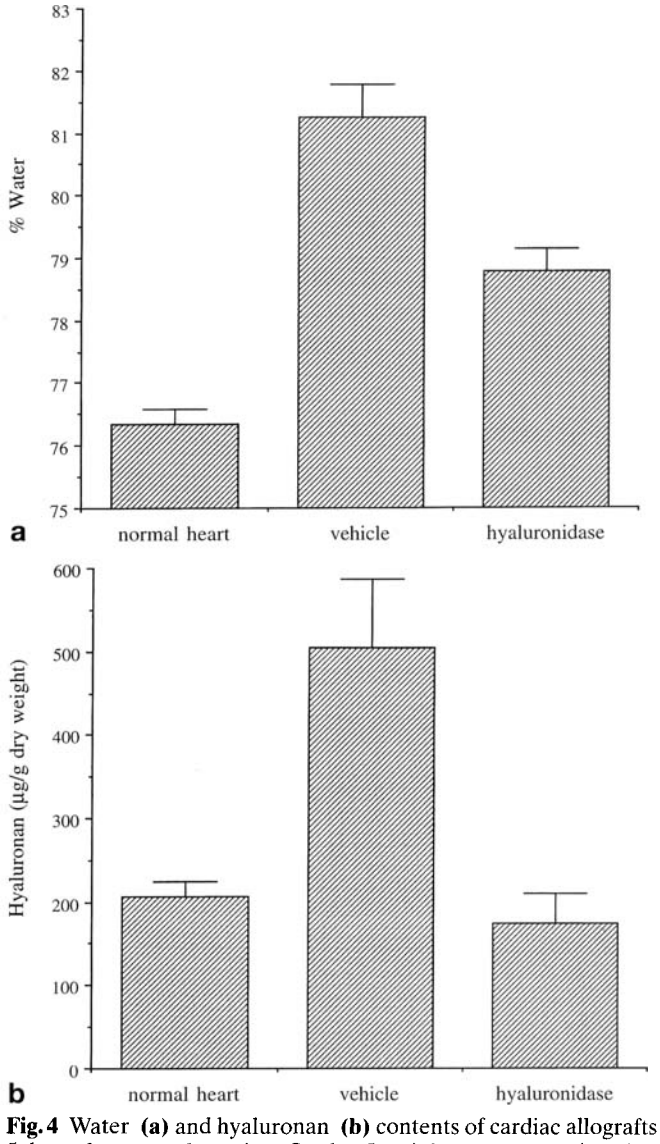

Fig. 4 Water **(a)** and hyaluronan **(b)** contents of cardiac allografts 5 days after transplantation. On day 5 recipient rats were given hyaluronidase, administered as an intravenous infusion for 2 h. Control animals received vehicle only. $P < 0.01$ (water) and $P < 0.05$ (hyaluronan). For comparison, water and hyaluronan contents of non-transplanted rat hearts are shown on the left

tent of the grafts was confirmed in three different ways; by analysis of the relative water content of graft biopsies, by morphologic evaluation of the grafts, and by ultrasound examinations. Prophylactic hyaluronidase therapy also resulted in grafts with better preserved morphology and fewer infiltrating mononuclear cells, compared to untreated controls. Hyaluronidase therapy, alone or combined with cyclosporine, resulted in prolonged graft survival times. In addition, therapeutic treatment with hyaluronidase as a continuous infusion reduced already established oedema in rejecting cardiac grafts.

Accumulation of hyaluronan has been suggested to be of major importance for the development of inflammatory interstitial edema. The theoretical background for this hypothesis is the fact that this glucosaminoglycan has strong water-binding capacities and thereby acts as a regulator of water homeostasis [2, 16]. A firm linkage between edema and the hyaluronan content in the interstitium of rejecting tissues has previously been observed [4, 5, 34]. The maintained correlation between hyaluronan and water during hyaluronidase treatment further substantiates the validity of the previous observations. The efficacy of hyaluronidase treatment was, in the present study, not only demonstrated by its ability to prevent the development of transplantation edema when given prophylactically, but also by its ability to reduce already established interstitial edema late during the rejection process. The effect was noticeable after only two hours of continuous intravenous infusion. This promptness suggests that hyaluronidase therapy would be of value in emergency situations, where an instant reduction of interstitial edema is of utmost importance, e. g. to prevent graft rupture or immediate cardiac failure due to graft edema.

Testicular hyaluronidase has previously been demonstrated as reducing experimental infarct size [15], a finding that, however, could not be reproduced satisfactorily in a large clinical study [19, 24]. Despite this negative re-

sult, we believe that it should nevertheless be possible to apply the described beneficial effects of hyaluronidase therapy also to general inflammation as the lack of effect in the clinical study can have several reasons, the most obvious being the fact that heparin inhibits the effects of hyaluronidase [37]. In addition, the dose of hyaluronidase may have been too low, a total of 4,500 U/kg was given during 48 h, or the therapy may have been commenced too late.

In transplantation, initial poor graft function increases the risk of rejection. An injury response in the early post-transplantation period, as a result of e.g. ischemia, promotes a specific immunological response, for instance by increasing the MHC expression [27]. Thus, interruption of the injury response should reduce the risk of rejection [7]. We have recently demonstrated that the renal accumulation of hyaluronan and water occurring after an ischemia injury can be prevented by daily intravenous treatment with hyaluronidase [13]. In addition, previous studies have shown that hyaluronan and water are accumulated in the transplanted organ early after transplantation also in syngeneic grafts, probably due to ischemia/reperfusion injury [4, 5]. Enzymatic degradation of excessive hyaluronan during the first days after transplantation might therefore reduce the risk of rejection.

The subcapsular hydrostatic pressure is increased during acute rejection after human kidney transplantation [33]. The reduced tissue damage after hyaluronidase treatment may be attributed to a decreased interstitial pressure and an improved microcirculation, as a result of the reduced edema. The numerical difference in relative water content between grafts from enzymatically treated rats and grafts from control rats might appear to be minor. However, the clinical relevance of this reduction was striking, as felt by palpation of the grafts and subsequently confirmed by ultrasound examinations.

Inflammation results in the accumulation of hyaluronan with a lower molecular weight than that of normal occurring hyaluronan [25]. Low-molecular-weight hyaluronan has been shown to stimulate macrophage chemokine and cytokine production [10]. Notably, the synthesis of IL-12 is enhanced in the presence of low-molecular-weight hyaluronan, but not of high-molecular-weight hyaluronan [10]. IL-12 acts a positive regulator for the production of IFN-γ and IL-2, cytokines well known to play important roles in allograft rejection. Thus, removal of accumulated hyaluronan should not only be advantageous because of the attained reduction of the edema of the graft, but also because of the interruption of parts of the rejection process. Indeed, 6 days after transplantation, cardiac grafts from rats on hyaluronidase therapy displayed a better preserved morphology than that of grafts removed from untreated animals. In addition, hyaluronidase treatment led to prolonged graft survival times. Single therapy with hyaluronidase prolonged the survival time for an average of 1.4 day; a notable effect in a non-immunosuppressed animal, where the rejection process develops so rapidly. This effect was intensified when hyaluronidase was combined with cyclosporine. Accordingly, 70% of the control animals rejected their cardiac grafts within two weeks, whereas just one graft (9%) in the hyaluronidase-cyclosporine group was lost within this time. Treatment with cyclosporine was started on day 6 after transplantation, a stage at which the rejection process in non-treated animals is well developed. These findings are consistent with the concept of low-molecular-weight hyaluronan, accumulated during an inflammatory response, as being a stimulator of macrophages. However, hyaluronan-fragments as small as hexamers have been demonstrated to induce chemokine gene expression in macrophages *in vitro* [17]. The enzyme utilised in the present experiments, a testes hyaluronidase, is an endo-β-N-acetyl-D-hexosaminidase that has also been shown to catalyse transglycosylation reactions, so that tetra-, hexa-, di- and octa-saccharides are formed [29]. Thus, also some of the degrading products of hyaluronidase could be able to stimulate the immune system. However, the beneficial effects of removing the accumulated hyaluronan obviously outweighed this theoretical risk.

The therapeutic effect of hyaluronidase treatment on the inflammatory response and on tissue damage may also reflect important aspects of the cell-matrix interaction that regulates leukocyte recruitment. Leukocytes expressing the hyaluronan binding receptor CD44 utilise this receptor in order to gain access to inflamed areas with hyaluronan-rich matrices [18]. In addition, the stimulatory effect of low-molecular-weight hyaluronan on macrophages have been suggested to be mediated via CD44 [10]. In the healthy myocardium, just a few scattered mononuclear cells were found to express CD44, but in the rejecting myocardium, CD44 staining was seen for infiltrating leukocytes and in the interstitial space infiltrated by leukocytes. Anti-CD44 monoclonal antibodies have potent anti-inflammatory activity *in vivo* and have been demonstrated to prevent the progression of ongoing collagen-induced arthritis [18]. These monoclonal antibodies mediate a rapid release of CD44 from the surface of CD44-positive leukocytes, thereby presumably preventing the CD44-hyaluronan mediated recruitment of leukocytes. In analogy, the hyaluronidase-mediated anti-inflammatory activity with reduced numbers of infiltrating cells might be partly mediated by preventing the CD44-hyaluronan interaction by enzymatic degradation of hyaluronan.

A potential risk with hyaluronidase therapy would be a general effect on, and degradation of, hyaluronan throughout the body. Previous experiments in a model of unilateral renal ischemia revealed that after four days of hyaluronidase treatment the contralateral, healthy kidney had normal levels of hyaluronan and water.

Thus, the single effect of the therapy was seen in the ischemically damaged kidney, where the accumulated hyaluronan had been degraded by hyaluronidase [13]. This finding indicates that the enzyme more readily gains access to inflamed tissue with an affected endothelium and/or that accumulated hyaluronan is more susceptible to degradation by hyaluronidase. From this aspect, systemic administration of hyaluronidase appears to be a safe therapy. However, the distribution of exogenous hyaluronidase in the body should be further evaluated.

In conclusion, enzymatic depolymerisation of hyaluronan in rejecting cardiac grafts resulted in a parallel reduction of water content. Prophylactic therapy also ameliorated cell infiltration and prolonged graft survival times. The potential therapeutic value of hyaluronidase treatment in transplantation may be severalfold. Thus, prophylactic treatment may diminish not only rejection-induced edema, but also ischemic edema [13]. The favourable effect, furthermore, on established edema opens a route to emergency intervention to avoid deleterious effects. The strong linkage between hyaluronan and edema in many types of inflammation, and our observed beneficial effects on allograft inflammation, also suggest that hyaluronidase therapy may become a part of anti-inflammatory treatment.

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