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Hyaluronic acid accumulation; the mechanism behind graft rejection edema

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Abstract. Hyaluronic acid (HA) is an important stabilizing consistuent of the loose connective tissue and regulates water homeostasis. Thus, excessive accumulation of HA in interstitial tissue immobilizes water and may thereby contribute to interstitial tissue edema. By the use of biotin labelled core protein and an avidin-enzyme system, we visualized HA in grafted rat kidney, rat heart, rat small bowel and also in human kidneys. By an extraction procedure the tissue amounts of HA were measured in the experimental grafts. Simple techniques for measuring water content were also employed. The extracellular amounts of HA increased between 100% and 350% in rejecting tissues as compared to syngeneic controls. The relative water content also increased and correlated well with the HA accumulation. The clinical value of these experimental observations was confirmed in human transplantation where rejecting kidney allografts demonstrated a highly significant increase in HA staining in the interstitium as compared to non-rejecting biopsy specimens. We therefore concluded that transplantation edema – a key features of graft rejection – is regulated by the accumulation of HA not only under experimental conditions but also in the clinical setting.

Key words: Hyaluronic acid – Transplantation edema – Rejection

We have shown in two recent studies that HA – an important stabilizing constituent of the loose connective tissue and regulator of water homeostasis – accumulates during graft rejection in rat renal and cardiac grafts [2, 3]. In those studies we also found a correlation between the accumulation of HA and the water content of the tissue. We drew the tentative conclusion that the HA accumulation is the major mechanism behind graft rejection edema. The aim of the present communication was to review our findings regarding rat heart and rat kidney allografts and compare those with new findings emerging from the present studies of rejecting rat small bowel and human kidney allografts.

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Materials and methods

Animals. Lewis rats and DA rats or Lewis × DA F1-hybrid rats (originally obtained from Bantin & Kingman, N Humberside, UK and Möllegaard, Skensved, Denmark) were bred in our own animal quarters. Parental strain animal transplants were performed for obtaining rejection in kidney and heart allografts, whereas F1 hybrids were transplanted into Lewis rats for small bowel transplants.

Patients. The records of 153 patients were retrospectively analyzed and biopsy reports were studied. Only biopsies where the graft status was classified as denfinitely rejecting ($n = 39$) or definitely stable ($n = 38$) were selected for the present investigation ($n = 77$). All patients received base line immunosuppression with triple drug therapy as outlined elsewhere [1]. These biopsy specimens were restained for HA according to a protocol shown elsewhere [7].

Transplant procedures. Heterotopic cardiac [4], orthotopic renal [3], and heterotopic small bowel transplants [6] were performed as described elsewhere.

Tissue preparation: extraction of tissue hyaluronan and calculation of water content. All experimental animals mentioned in this paper were sacrificed 2, 4 or 6 days after transplantation. Only the data for animals harvested 6 days posttransplantation are shown. Tissue handling and processing is describe in detail elsewhere [3]. Briefly, one part of the specimens obtained were analyzed for HA and water content and were weighed immediately on filter paper at room temperature (wet weight, ww) and later after lyophilization at −80°C for 4 days (dry weight, dw). The relative water content of the specimens was calculated according to the formula: $100 \times /(ww - dw)/(ww)/$. The HA was extracted from pulverized dried tissue with 0.5 M NaCl. The material was extracted with 2 ml of the buffer for 16 h with constant shaking at 4°C. The samples were then centrifuged for 15 min at 2,000 *g*. The supernatants were recovered and the HA concentrations were analyzed in duplicate with a radiometric assay (Pharmacia Diagnostics, Uppsala, Sweden), according to the principles previously outlined [5].

Data presentation. All data are presented as mean ± SEM. For the human tissues the intensity and the amount of HA stain in the inter-

Table 1. Amounts of hyaluronan extracted and relative water content (mean ± SEM)

Organ	Type	*n*	HA (μg/g dw)	Water (%)
Kidney	Syngeneic	5	180 ± 10	75.2 ± 1
	Allogeneic	5	350 ± 20	84.3 ± 1
Heart	Syngeneic	3	465 ± 42	79.6 ± 0.3
	Allogeneic	4	930 ± 13	82.9 ± 0.7
Small bowel	Syngeneic	3	80 ± 35	74.0 ± 1.0
	Semi allogenric	4	280 ± 25	83.0 ± 1.0

stitium was given as intensity, graded into an abritary scale from 0–3 where zero denotes no or absence of stain and 3 represents intense staining. Due to lack of sufficient material for further sectioning only 32 of the stable grafts and 35 of the acute rejecting grafts were available for the final evaluation.

Statistical differences between group means were carried out by means the Student's *t*-test for the experimental animals and with the Mann-Whitney U-test for the clinical material.

Results

The results for the animal experiments are summarized in Table 1. Table 1 depicts the HA concentrations in kidney, heart, and small bowel grafts when performed in either allogeneic or syngeneic conditions. Table 1 also depicts the water content of the tissues. The HA concentration on the 6th day after allografting increased 100–350% as compared to the syngeneic situation. The water concentration also increased significantly. There was a significant correlation between the relative water content and HA content of the allogeneic grafts ($r = 0.51$, $P < 0.01$ for kidneys; $r = 0.62$, $P < 0.05$ for hearts and $r = 0.72$, $P < 0.01$ for small bowels).

Non rejecting human kidney grafts were scored to have an interstitial HA content of 1.031 ± 0.08 and acutely rejecting kidney grafts were arbitrarily scored to have a mean of 1.9 ± 0.13.

Discussion

In this communication we showed that the HA content increased in three different experimental settings; that is, non-immunosuppressed rejection of cardiac, renal and small bowel allografts. We also found a good correlation between HA and the increased water content in the tissues. We were also able to confirm these findings of increased HA content in a clinical setting. These findings have been extensively discussed elsewhere [2, 3]. The finding of a good clinical correlation to the experimental findings strengthens the validity of the interpretation put forward that HA binds water and thereby immobilizes water in the interstitial tissue. Two clinical implications are clear: (1) HA staining, may in certain instances, add to the diagnostic potency of routine histology, and (2) it explains why the edema of grafted tissue undergoing rejection is resistent to diuretics and calls for other procedures, e.g. HA degradation in a connective tissue to decrease the edema and thereby improve graft function.

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