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The correlation of serum hyaluronan of liver donors with posttransplant liver function

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Abstract Primary organ non-function occurs in 2–17% of patients after liver transplantation (LTX). Liver function tests focus on hepatocytes, underestimating the importance of non-parenchymal cells such as endothelium. Liver endothelium metabolises hyaluronan (HA). The function of endothelium can be estimated by measuring the concentration of HA in serum. We evaluated two organ donor groups, the first with HA levels below 200 μg/l and the second with HA levels above 200 μg/l. Both groups degraded more than 60% of HA during the first 6 h after reperfusion. Determination of hyaluronic acid in patients after LTX gives valuable information about the functional state of liver endothelium. It does not necessarily parallel the release of transaminases. In this study, there was no correlation between the levels of HA in the donor and posttransplant liver function. High HA in organ donors are most likely a consequence of different degrees of trauma and a higher release of HA into the circulation rather than impaired endothelial function.

Key words Hyaluronan · Organ donor · Liver function · Liver transplantation · Primary organ non-function (PNF)

Introduction

During the last 15 years, liver transplantation (LTX) has become an established therapeutic tool to treat patients with end-stage liver disease [1, 2]. Introduction of cyclosporin to the immunosuppressive armoury [3], as well as improved surgical and anaesthetic skills, have contributed to the clinical success rate of liver transplantation [4]. Organ preservation with University of Wisconsin (UW) solution has enabled many centres to extend preservation time and facilitates in logistics [5, 6]. Not much progress has been achieved in the evaluation of donor livers: primary organ non-function (PNF) occurs in 2–17% of transplanted livers [7–9]. PNF is characterised by minimal or no bile production, rapidly rising transaminases, coagulopathy, hepatic encephalopathy and acute renal failure. In most cases, retransplantation is required [10]. The mechanism responsible for PNF remains largely unclear. From different studies it seems likely that donor liver histology [7], the period of cold ischaemia [8], the back table bath [11], as well as other factors [12], play a distinct role.

To evaluate donor livers the transplant surgeon depends on the patients history (if available), transaminases, bilirubin and prothrombin time (PT). Last but not least, the experience of the surgeon in evaluating livers is required to decide if a liver is suitable for LTX or not. If the incidence of PNF is to be reduced, methods of

accurately assessing the suitability of donor livers prior to transplantation are required. More recently, the lignocaine metabolite formation test (MEGX) has contributed to a quantitative approach of liver function assessment [13, 14]. Liver function tests focus on hepatocytes, underestimating the importance of non-parenchymal cells [15]. The endothelium has a number of important functions that, to some extent, hepatocellular function depends on. These functions are maintenance of selective permeability, integration and transduction of blood-borne signals, modulation of leucocyte interactions with tissues, regulation of vascular tone and inflammatory and immune reactions [16]. There is strong experimental evidence that endothelial cells are more sensitive to preservation and reperfusion injury than hepatocytes [17, 18]. To date, it has only been possible to evaluate the viability of liver endothelium by light and electron microscopy. With the discovery that liver endothelium metabolises hyaluronic acid (HA) [19], and with the introduction of a radiometric assay for determination of HA, a new and workable model for estimating endothelial function has become available. The aim of this project was to study, for a period of 6 months, a possible correlation between the levels of HA in the organ donor and liver function after transplantation. We wanted to investigate whether high levels of HA in the organ donor predicted poor graft function.

Materials and methods

Organ donors

Two groups were formed based on serum HA concentration of the organ donor. Group 1 had HA levels in the range of 20 to 196 μg/l with a mean value of 128 μg/l. HA concentration in group 2 was in the range of 295 to 828 μg/l with a mean value of 490 μg/l (Fig. 1). The average age of donors in groups 1 and 2 was 36 and 30 years, respectively. Cause of death in group 1 was attributed to head injury in two cases, head injury in combination with polytrauma in three cases, intracerebral bleeding in three cases and a cerebral tumour in one case. The cause of death for donors in group 2 was head injury in one case and head injury in combination with polytrauma in two cases, and intracerebral bleeding and cerebral tumour in one case. The transaminases before surgery of the organ donors in group 1 were GOT 54 ± 17 U/l and GPT 53 ± 19 U/l. Transaminases in group 2 were: GOT 26 ± 6 U/l and GPT 22 ± 7 U/l.

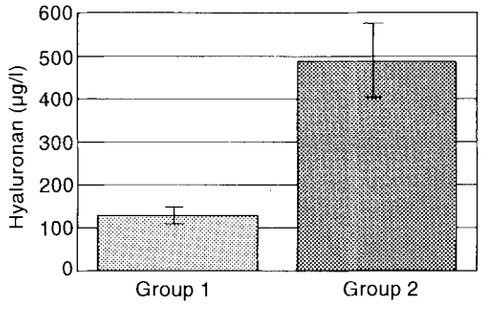

Fig. 1 Hyaluronan (HA) level of organ donors. The average HA level was 128 μ/l in group 1 and 490 μg/l in group 2

Liver recipients

Nine liver recipients in group 1 (three female and six male) and five recipients in group 2 (three female and two male) were similar in age with an average of 44 and 40 years, respectively. The diagnosis for LTX was HBV and HCV cirrhosis, PSC PBC, hepatocellular carcinoma, fulminant hepatitis B and nutritive toxic cirrhosis. The average time of organ preservation in group 1 was 11 h and 53 min and in group 2, 9 h and 55 min. The average time of anastomosis was 86 min in group 1 and 79 min in group 2. The preoperative HA levels in the recipient groups were similar (412 μg/l in group 1 and 493 μg/l in group 2). The average bilirubin level in group 2 was higher than in group 1 (17.5 ± 12.1 mg/dl compared with 3.8 ± 1.34 mg/dl). In large part this difference was due to one patient in group 2 with hepatitis C cirrhosis and a bilirubin level of 64 mg/dl.

Samples

Blood samples were collected from the donor before surgery was started. The blood was allowed to clot. In our clinic, it was centrifuged and the serum was frozen at $-20\,^{\circ}C$ until determination of HA. Blood samples were taken from the recipient before surgery, during surgery, just before reperfusion of the liver, at 30, 60 and 120 min, and 6, 12 and 24 h after reperfusion. Further samples were collected on days 2, 7 and 14, and at 1, 3 and 6 months after transplantation. Samples collected intraoperatively and during the first 12 h after reperfusion were used only for HA determination.

To determine HA in serum we used a radiometric assay, commercially available at KABI Pharmacia, Uppsala, Sweden. The test uses specific HA-binding proteins (HABP), isolated from bovine cartilage. The HA in the patient sample reacts with ^{125}I-labelled HABP in solution. The unbound ^{125}I-HABP is then quantified by incubation with HA covalently coupled to sepharose particles of small size and low density. Separation is performed by centrifugation followed by decanting. The radioactivity bound to the particles is measured in a gamma counter and the result is inversely proportional to the concentration of HA in the sample. Data are expressed as the mean ± SE.

Results

Hyaluronan

HA was metabolised rapidly in both groups (Fig. 2). Preoperatively, the average HA levels in serum were 412 ± 68 μg/l in group 1 and 493 ± 74 μg/l in group 2. Six hours after reperfusion, HA was reduced by 64% in group 1 to a level of 150 ± 23 μg/l and by 61% in group 2 to a level of 194 ± 60 μg/l. HA in group 2 decreased further to 132 ± 31 μg/l on the 2nd day while the con-

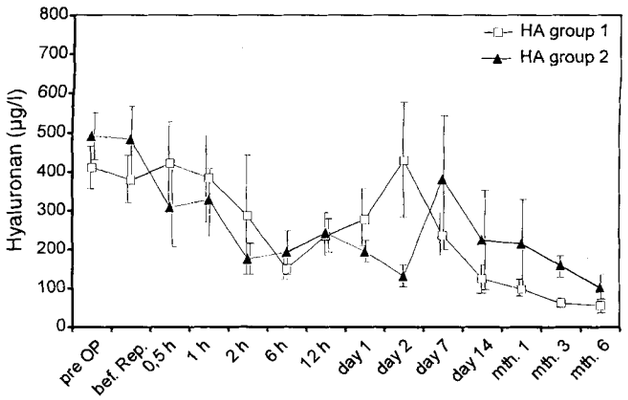

Fig. 2 The course of HA concentration from before LTX until 6 months after LTX. The HA concentrations of the recipients before LTX were 412 µg/l and 493 µg/l for groups 1 and group 2, respectively. At 6 months after LTX, the level of HA decreased to 55 µg/l in group 1 and 102 µg/l in group 2 (*Op* operation, *Rep.* reperfusion)

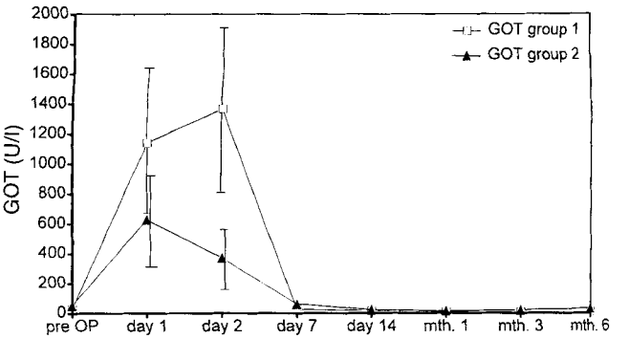

Fig. 3 The course of GOT (U/l) release from before LTX until 6 months after LTX

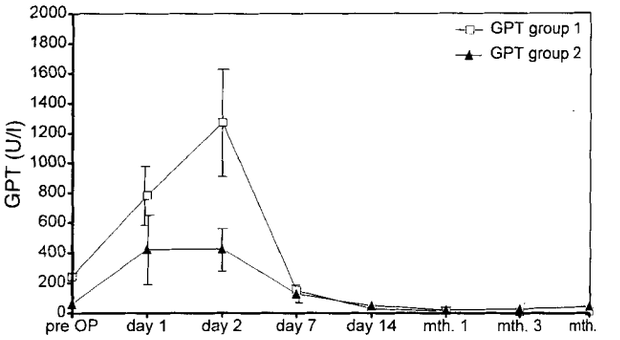

Fig. 4 The course of GPT (U/l) release from before LTX until 6 months after LTX

centration of HA in group 1 increased to 429 ± 166. On day 7 after LTX, the concentration of HA in group 1 was 236 ± 58 µg/l and in group 2, 381 ± 180 µg/l. Two weeks after transplantation, the level of HA in group 1 was 125 ± 26 µg/l and in group 2, 225 ± 136 µg/l. One month after LTX, HA reached normal values in group 1 (98 ± 11 µg/l). It was somewhat elevated in group 2 (215 ± 122 µg/l). During the next 2 months, there was a further decrease in HA concentrations: 62 ± 7.2 µg/l in group 1 and 159 ± 33 µg/l in group 2. Six months after LTX, all but two patients in group 1 had a HA below 100 µg/l with an average of 55 ± 7.4 µg/l. In group 2, three patients out of five were below 100 µg/l with an average of 102 ± 33 µg/l.

GOT

The average GOT level in group 1 was 29 ± 5 U/l before transplantation. It increased to 1140 ± 507 U/l on the 1st day and to 1366 ± 530 U/l on the 2nd day after transplantation. It then decreased to 28 ± 4.3 U/l on day 7 and reached normal levels, below 23 U/l, after 2 weeks.

The average GOT level in group 2 was 53 ± 12 U/l preoperatively. It increased to 630 ± 291 U/l on the 1st day after LTX and decreased to 370 ± 150 U/l on the 2nd postoperative day. One week after LTX, GOT levels in group 2 reached 61 ± 17 U/l, and further decreased to 22 ± 4 U/l after 2 weeks. The average GOT level in group 2 remained in the normal range 1, 3 and 6 month after LTX (Fig. 3).

GPT

The average GPT level in group 1 was 24 ± 4 U/l preoperatively. It increased to 783 ± 206 U/l on the 1st day and to 127 ± 335 U/l on the 2nd day after LTX. One week after LTX, the GPT concentration decreased to 150 ± 29 U/l. The HA concentration was 33 ± 8 U/l after 2 weeks and reached normal values below 21 U/l after 1, 3 and 6 months.

The average GPT level in group 2 was 61 ± 21 U/l preoperatively. It increased to 424 ± 215 U/l on the 1st day and to 427 ± 148 U/l on the 2nd day. The HA concentration decreased to 128 ± 27 U/l 1 week after LTX. One week later, the HA concentration reached a level of 50 ± 7 U/l. One month after LTX serum GPT was as low as 22 ± 14 U/l without a significant change 3 and 6 months later (Fig. 4).

Bilirubin

Before transplantation, the average serum bilirubin in group 1 was 3.8 ± 1.3 mg/dl. The bilirubin increased to

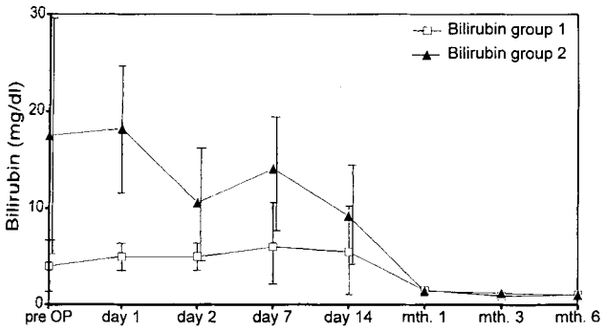

Fig. 5 The concentration of bilirubin mg/dl reached, on average, normal values between the 2nd and 4th. The high level of bilirubin in the group 2 was in large part due to a patient with a bilirubin of 64 mf/dl

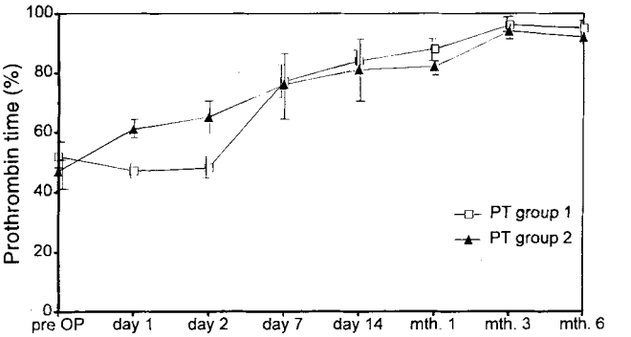

Fig. 6 Prothrombin time increased continuously after LTX in both groups

5.0±0.8 mg/dl 24 and 48 h after LTX. One week after LTX serum bilirubin was 6.0±2.1 mg/dl and reached 5.4±3 mg/dl after 2 weeks. After 1 month, the bilirubin concentration fell to 1.5±0.3 mg/dl. Normal values were reached by the 3rd and 6th months: 0.9±0.2 mg/dl and 1.0±0.2 mg/dl, respectively.

The average serum bilirubin level in group 2 was 17.5±12 mg/dl before surgery. The difference between the two groups was due to one patient in group 2 with a serum bilirubin of 64 mg/dl. The bilirubin was 18.2±8 mg/dl 24 h after transplantation and decreased by the following day to 11±6 mg/dl. One week after LTX, the bilirubin increased to 14.1±6 mg/dl. Two weeks after LTX, the bilirubin was 9.2±5 mg/dl. The bilirubin concentration decreased to 1.4±0.3 mg/dl, 1.2±0.3 mg/dl and 1.0±0.2 mg/dl at 1, 3 and 6 months post LTX, respectively (Fig. 5).

Prothrombin time

Before LTX, the average prothrombin time (PT) in group 1 was 52±7%. One the 1st and 2nd day, the PT was 47±4% and 48±6%. The PT increased to 77±6% and 84±4% 1 and 2 weeks after LTX. The values 1, 3 and 6 months after LTX were 88±4%, 96±2.5% and 95±4%, respectively.

In group 2, the PT was 47±5% before transplantation. It increased to 61±4% and 65±5% on the 1st and 2nd days. After 7 and 14 days, values of 76±10% and 81±9% were reached. One, 3 and 6 months after LTX, the PT increased to 82±2.6%, 94±3% and 92±3%, respectively (Fig. 6).

Episodes of acute rejection

Seven days after LTX, every patient in our programme had a liver biopsy. Biopsies were graded from 0–3 [20, 21]:

0. No evidence of rejection
1. Mild periportal mononuclear infiltrate with minimal endothelitis and minimal bile duct injury, and without hepatocyte necrosis
2. Moderate periportal mononuclear infiltrate extending beyond the limiting membrane of the portal field, marked endothelitis, marked bile duct injury and single-cell hepatocyte necrosis
3. The same alterations as described in 2 plus severe injuries and massive confluent hepatocyte necroses

Six patients in group 1 had no or only very discreet histological signs of acute rejection. Three biopsies were classified as grade 1 or 1–2. Two patients in group 2 had no signs of AR while three biopsies were classified as grade 1 or 1–2.

Discussion

To minimise the risk of hepatic failure during the postoperative period, selection of organs and their preservation is decisive. The transplant surgeon's decision is based on biochemical analysis of liver function, such as bilirubin levels, GOT and GPT release, and the MEGX test, as well as an impression of the texture, colour and perfusion pattern of the liver. PNF occurs in 2–17% of transplanted livers [7–9]. Biochemical analysis of liver

function focuses exclusively on hepatocyte function. Non-parenchymal cells are important for organ preservation and seem more susceptible to preservation injury than parenchymal cells [15, 17, 18]. Analysis of HA levels allows on estimation of liver endothelial function.

Our hypothesis was that if HA levels of the organ donor were within the physiological range, the endothelium of these donor livers was in better shape than those with high HA levels. We postulated that this would be manifested in the recipient by a faster rate of HA degradation. Taking the role of endothelium for overall liver function into account, low levels of HA in the organ donor should correlate with a better performance of the transplanted liver.

In a retrospective study, we formed two groups: one with HA below 200 μg/l with an average of 128 ± 23 μg/l and the other above 200 μg/l with an average of 490 ± 97 μg/l. As in recent reports of elevated levels of HA in patients with acute and chronic liver failure, the average concentrations of HA in the serum of patients before LTX were 412 ± 68 and 493 ± 74 μg/l in groups 1 and 2, respectively. These levels offered enough "substrate" to be metabolised. As illustrated in Fig. 2, HA levels after LTX decreased immediately. Six hours after LTX, the level of HA was less than half the pre-LTX level in both groups. This indicated that liver endothelium was functioning equally well in both groups immediately after liver perfusion. It seems rather unlikely that the endothelium of livers in group 2 was injured and responsible for the high HA levels. As to where the high levels of HA in group 2 came from remains unclear. One could speculate that the concentration of HA was increased in some organ donors due to tissue damage (joint and bone fractures etc.) as a consequence of trauma leading to death. It is our opinion that this issue deserves further study.

After the 2nd day, the course of HA levels differed in both groups. HA in group 1 increased while HA in group 2 was still falling. Between the 2nd and 7th days, both trends changed directions. From previous studies we know that 1 week after LTX, cellular-mediated immunological reactions occur. Three out of five patients in group 2 had grade 1–2 or grade 2 rejection but only three out of nine patients in group 1 experienced acute rejection. Since endothelium is the first target of acute rejection, the consequence is an increasing HA level [22, 23]. In the long-term, no significant change was seen in the average decrease in HA levels (Fig. 2). After 6 months, most patients reached normal levels of HA in both groups.

The release of transaminases during the first 2 days was higher in group 1 than in group 2, despite very similar demographic data of the organ donors, cold preservation time, similar time of anastomosis and no other apparent differences in the quality of the donor organ. The increase in HA in group 1 on the 2nd day paralleled the higher release of transaminases. Since there was no difference between both groups in the rate of HA metabolism during the first 24 h, one might conclude that endothelial function in group 1 was effected by less well-functioning hepatocytes between the 1st and 2nd days after LTX. In the long-term, hepatocyte and endothelial function was recovered in both groups.

Bilirubin levels and PT during the 1st week after LTX are considered to be less valuable parameters for comparing liver function since the first depends to some degree on the volume of bile lost through the t-tube and the second parameter is influenced by the amount of fresh frozen plasma and clotting factors administered. Usually, after 1 week, the *t*-tube is closed and patients don't require any more clotting factors. Thereafter, there were no significant differences in bilirubin and PT in both groups.

Determination of HA in patients after LTX gives valuable information about the functional state of liver endothelium. It does not necessarily parallel the release of transaminases. In this study we did not detect a correlation between the levels of HA in the donor and posttransplant liver function.

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