Prediction of Early Graft Function by Effluent Levels of Hyaluronic Acid in Clinical Liver Transplantation

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IT is estimated that 7% of orthotopic human liver allografts never function properly, with consequent death or retransplantation.[1] Although gross defects of the liver can be identified prospectively with frozen section biopsies, this is an ineffective way to predict preservation injury. Because a selective target of preservation injury is the microvasculature,[2,3] we previously evaluated purine nucleoside phosphorylase (PNP), an endothelial cell-specific enzyme, as a predictor of preservation injury.[4]

Another candidate predictor substance is hyaluronic acid (HA), a high-molecular-weight polysaccharide which is produced in the cell plasma membrane and deposited in the extracellular matrix.[5] When released from the tissues, HA enters the blood via the lymphatics and is transported to the liver where it is catabolized uniquely by the endothelial cells.[6,7] In liver disease serum levels of HA are increased,[8] and elevations of HA have been used to monitor rejection of liver grafts.[9]

METHODS

Thirty-two livers were transplanted orthotopically to randomly selected adult primary recipients with negative lymphocytotoxic crossmatches; 29 were treated with FK 506 and low-dose steroids (20 mg/d) and 3 were treated with cyclosporine and conventional high-dose steroids, starting with 200 mg/d methylprednisolone with taper. During and following procurement, the livers were infused with University of Wisconsin (UW) solution at 4°C. After variable periods of static cold storage (13.3 ± 3.2 SD hours), the livers were unpackaged and reinfused with lactated Ringer's solution (4°C). The first 50 mL of the reperfusion effluent were collected from the infrahepatic vena cava. Twenty-three of the samples were collected at the operating table after the upper caval anastomosis was completed, whereas the other 9 livers were reperfused on the back table before the implantation was started. The latter 9 livers were reflushed with 500 mL of cold UW solution before further storage. The 50-mL effluent collections were centrifuged at 2000 rpm for 10 minutes to remove erythrocytes, and aliquots were analyzed for HA (100 μl) and PNP (20 μL).

The PNP was assayed by the breakdown with xanthine oxidase of inosine to uric acid as measured by the increase in absorbance at 293 nm in a coupled assay system.[4] For HA measurement a radiometric assay was used with kits marketed by Pharmacia Diagnostics (Uppsala, Sweden).[10] All samples including standards were run in duplicate. Aliquots of samples processed in earlier assays were included as quality controls. Interassay variation of results ranged between 1.5% and 2.2%.

The clinical care physicians in the intensive care units and other departments were unaware of the HA and PNP levels, and the laboratory personnel (P.N.R. and J.S.) were not involved in or cognizant of the clinical monitoring, diagnostic tests including biopsies, or treatment of the patients. Graft function after transplantation was stratified by the criteria of Makowka et al.[11] Peak serum aspartate aminotransferase (AST) levels of below 1500

Table 1. Predictive Value of Effluent Hyaluronic Acid and Purine Nucleoside Phosphorylase Levels for Posttransplant Liver Function

Parameter	R² For AST (%)	P	R² For ALT (%)	P
HA	32.2	<.001	41.6	<.001
PNP	18.3	<.02	13.5	<.05

IU/L and peak serum alanine aminotransferase (ALT) levels below 1000 IU/L at any time during the first postoperative week were indications of good grafts vs poor grafts, if the levels were higher.

Using this arbitrary demarcation of acceptable vs unacceptable postoperative hepatocyte necrosis, the mean HA concentration with so-called good grafts was 257.25 ± 142.88 μg/L (range, 100 to 400 μg/L), and the mean PNP level was 92.6 ± 42.21 (SD) U/L (range, 47 to 242 U/L). Putative bad grafts had an effluent concentration of 493.30 ± 143.83 μg/L (range, 440 to 660 μg/L), and PNP of 135.56 ± 55.0 U/L (range, 59.0 to 188.0 U/L). A linear regression analysis[12] to determine the predictive value of HA and PNP for AST and ALT showed that both endothelial markers correlated significantly with the transaminases, with the HA being the more sensitive (Table 1). Based on these observations, we selected HA and PNP levels of 400.0 μg/L and 135 U/L, respectively, as the hypothetical cutoff, above which livers were classified as high risk.

RESULTS

The hypothesis that the cutoffs described above could predict graft quality was tested with the clinical results in 32 patients of whom 27 (84.3%) are alive after 8 to 13 months. Primary graft survival was the principal criterion. Twenty-one of the 32 grafts had HA values below 400 μg/L and 11 were above this. All of the 21 livers with low HA values functioned adequately (Table 2) although 3 recipients died after 2½, 2¾, and 4 months because of sepsis, graft-versus-host disease, and rejection, respectively. Of the 11 grafts with high HA values, 5 failed from primary

Table 2. Hyaluronic Acid and Purine Nucleoside Phosphorylase vs Primary Nonfunction

Parameter	No. of Grafts	No. of Cases With Primary Nonfunction	False-Positive*	False-Negative†	Predictive Value (%)
HA ≤ 400.0 μg/L	21	0	—	0	100
HA > 400.0 μg/L	11	5	6	0	45
PNP < 135.0 U/L	24	3	—	3·	87.5
PNP > 135.0 U/L	8	2	6	—	25

*False-positive: Graft survival with transplant HA value > 400 μg/L and PNP value > 135.0 U/L.
†False-negative: Graft failure due to preservation injury with HA level ≤ 400.0 μg/L and PNP ≤ 135.0 U/L.

nonfunction, leading to death in 14 to 58 days (n = 2) or successful retransplantation within 4 to 10 days (n = 3). Of the 6 grafts which survived despite high HA values, 2 had subsequent biopsy evidence of moderate to severe preservation injury (Table 2). Nevertheless, all 6 of these grafts were considered false-positives (Table 3). All of these 6 patients have satisfactory results after follow-ups of 8 to 13 months.

The PNP was less discriminating. Twenty-one of the 24 grafts (87.5%) with PNP values below 135.0 survived (Table 1), but the other 3 failed from primary nonfunction. Of the 21 surviving grafts, 3 more were lost after 2½ to 4 months from nonpreservation complications; these patients were the same 3 patients who had late deaths despite low HA livers (see above). Six of the 8 grafts (75%) with high PNP values survived and were classified as false-positive; the other 2 failed from primary nonfunction.

DISCUSSION AND CONCLUSIONS

Thus, in contrast to PNP, the HA concentration in the reperfusion effluent was unfailingly predictive of good graft quality if it was below 400 μg/L and predictive of a high (45%) immediate graft failure if it exceeded this cutoff. The washout had to be with lactated Ringer's solution. In pilot experiments with animals and human livers (data not shown), the HA test lost all discrimination if the reperfu-

Table 3. List of False-Positive Surviving Grafts With High Hyaluronic Acid (> 400.0 μg/L)

Patient	HA (μg/L)	Peak* AST (U)	Peak* ALT (U)	Postoperative Biopsy Results (90 min to 10 d)
RH	500.0	2614	3265	Mild ischemia
KYM	600.0	11,343	8090	Severe ischemic injury
FL	660.0	6037	6257	Unremarkable
TR	500.0	282	178	Unremarkable
DW	510.0	—	—	Unremarkable
DV	565	897	661	Unremarkable

*In first week.

sion was with UW solution. The explanation for this is not known.

The failure of effluent PNP concentration to reliably predict either acceptable or unacceptable grafts was disappointing because of the expectation from earlier work that it would do so.[4] The performance of the PNP may have been methodologic. The assay for PNP is a spectrophotometric one, subject to interference by allopurinol, a hypoxanthine analogue and a critical component of the UW solution, whereas the radiometric HA assay is free of these interferences.

In conclusion, the HA test may contribute to the safety and predictability of liver transplantation. The test was reliable with no false-negatives. The assay, which can be performed in 3 hours, is economical and easily learned.

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