ELSEVIER

ISCHEMIA-REPERFUSION INJURY

Effect of Perioperative Infusion of Antioxidants on Neutrophil Activation During Liver Transplantation in Humans

F. Biasi, G. Poli, M. Salizzoni, E. Cerutti, S. Battista, G. Mengozzi, F. Zamboni, A. Franchello, G. Molino, E. Chiarpotto, J.C. Cutrin, D. Zanetti, M. Meurisse, P. Honore, O. Detry, J.-O. Defraigne, and J. Pincemail

DURING ORTHOTOPIC human liver transplantation (OLT), postreperfusion tissue damage is associated with a significant increase in markers of cytolysis and cholestasis.[1,2,3] In the early phases of reperfusion an increase in the steady-state levels of reactive oxygen species (ROS) was observed to correlate with the presence of cytolysis;[2] nevertheless, the role of oxidative stress in causing and/or amplifying postreperfusion damage to human liver grafts has not as yet been conclusively defined. The topology and chronology of the damage in the transplanted liver may be described as an endothelial cell injury that appears in the late stage of cold ischemia, followed immediately after reperfusion by involvement of the whole liver vasculature with concomittant activation of Kupffer cells, which, together with endothelial cells,[4] attract and trigger polymorphonuclear cells (PMNs), the major source of ROS. While oxidative stress occurring in the hepatic vasculature appears to be a primary cause of the reperfusion injury, the role of oxidative reactions in phagocyte recruitment and activation is not yet clear. Hence, patients scheduled for OLT in two different transplantation units were randomly divided into two groups: one group received an intravenous dose of an antioxidant supplement during surgery. During the first 2 hours of reperfusion, we monitored blood indices of oxidative stress and neutrophil activation.

RECRUITMENT OF PATIENTS AND METHODS

Twenty-four adult patients (32 to 65 years old) included: 2 women and 14 men, were from the Transplantation Unit of "S. Giovanni Battista" Hospital of Torino, Italy; and 3 women and 5 men from

From the Department of Clinical & Biological Sciences, ASL S. Luigi Gonzaga, Orbassano, Torino, Italy (G.P., E.C., J.C.C., D.Z.); CNR Center of Immogenetics & Experimental Oncology, Torino, Italy (F.B.); Liver Transplantation Center and Baldi & Riberi Laboratory (M.S., E.C., G.M., F.Z., A.F.), and Division of Medicine A (S.B., G.M.), ASL San Giovanni Battista, Torino, Italy; A.Fa.R. Fatebenefratelli Hospital, S. Maurizio Canavese, Torino, Italy (J.C.C.); Department of Cardiovascular Surgery ULG and Experimental Research Center of Surgery Dept (CREDEC), University of Liège, Sart Tilman, Belgium (J.-O.D., J.P.), and Department of Organ Transplantation, University of Liège, Sart Tilman, Belgium (M.M., P.H., O.D.).

The Italian study was supported in part by National Research Center (CNR) Strategic Project on Cellular and Oxidative Stress and National Target Project on Biotechnology, by Ministry of the University, in the frame of Research Projects of National Interest, by the University of Torino and by the Consorzio Italiano per le Biotechnologie. The Belgian study was supported by grants FNRS n. 3.4544.99. This work was supported with technical assistance of the two transplantation teams.

Address reprint requests to Prof Giuseppe Poli, Dipartimento di Science Cliniche, & Biologiche, Asl S. Luigi Gonzaga, 10043 Orbassano (To), Italy. E-mail: giuseppi.poli@unito.it

0041-1345/02/$–see front matter
PII S0041-1345(01)02903-7

Table 1. Plasma Lipid Peroxides (LPO) and Vitamin E/Total Cholesterol Ratio During the Early Phases of Reperfusion, in Adult Patients Subjected to OLT, Treated or Not With the Antioxidant Mixture

Reperfusion time	Without Antioxidant Mixture	With Antioxidant Mixture
Torino Transplantation Unit[a]		
Basal	2.5 ± 0.6	2.4 ± 0.5
5 min	3.6 ± 1.4	2.8 ± 0.8
60 min	3.1 ± 0.9	3.1 ± 0.8
120 min	6.3 ± 1.2	4.5 ± 1.2
Liège Transplantation Unit[b]		
Basal	9.1 ± 1.4	7.6 ± 2.3
5 min	10.7 ± 1.0	10.3 ± 1.4
60 min	10.5 ± 1.1	13.4 ± 3.2
120 min	9.9 ± 0.6	16.6 ± 6.6

[a]LPO levels (nmoles/mL plasma). Data are means ± S.E. of eight values for each group.
[b]Vitamin E/total cholesterol ratio (mg/g). Data are means ± S.E. of four values for each group.

the Transplantation Unit of Sart Tilman, University of Liège, Belgium. The pretransplant etiology for most of the patients was posthepatitic cirrhosis due to HBV+ or HCV+, or alcoholic cirrhosis. The organs were preserved in University of Wisconsin solution. The Italian transplantation unit followed the surgical procedures of Tzakis et al.[5]; the Belgian one, the procedure of Belghiti et al.[6] which differs from the former based upon unclamping of the suprehepatic veins preceding that of the portal vein. The patients were divided into two randomized groups: one received a commercially available mixture of antioxidants by IV infusion throughout the whole period of warm ischemia (19 to 55 min) and during the first 30 minutes after portal vein declamping. Each ampoule of antioxidants (OMNIBIONTA, from Merck, Darmstadt, Germany) contained (in 10 mL water): 5.5 mg retinol palmitate, 50 mg thiamine chloride-hydrochloride, 10 mg riboflavin-5-phosphate sodium, 100 mg nicotinamide, 25 mg dexpanthenol, 15 mg pyridoxin hydrochloride, 500 mg ascorbate, 5 mg α-tocopherol-acetate, 150 mg benzylalcohol, 500 mg polysorbate, 1 mg DL-α-tocopherol, 200 mg propylene glycol, 2500 mg glycerin 85%, 360 mg trometamol. Two ampoules were diluted to 200 ml with 0.9% NaCl and infused intravenously. The study protocol, in agreement with the ethical guidelines of the 1975 Declaration of Helsinki, was approved by the Ethical Committees of the S.G. Battista Hospital, Torino, Italy, and of the University of Liège, Belgium. Informed consent was obtained from or for all patients. Blood samples were withdrawn from the jugular vein at different times: immediately before general anesthesia, and 5, 15, 60, and 120 min after reperfusion. Cellular redox imbalance was monitored in terms of lipid peroxide plasma levels (LPO)[7] and plasma vitamin E/plasma cholesterol ratio.[8] Neutrophil activation was evaluated by myeloperoxidase plasma levels (MPO) using an ELISA kit procedure (Oxys Int. Inc., Portland, Oregon, U.S.A.). The statistical differences between the two groups were examined using Student's t-test; the differences between basal and corresponding reperfusion times with ANOVA associated with a Student-Newman-Keuls test.

RESULTS

A tissue redox imbalance that favored oxidation appeared to be the trend in liver transplant patients not receiving intrasurgical antioxidant supplementation (Table 1). However, only 2/3 of patients (65%) showed an early response to oxidative stress by virtue of an increased plasma LPO by 5 minutes after reperfusion. The remaining 35% denoted as late "responders", only showed significant increase in LPO plasma levels during later observation periods, explaining the lack of statistically significant LPO mean values except at 120 min post-reperfusion. The group treated with the antioxidant mixture showed an overall lower extent of membrane lipid peroxidation, at least during the first two hours of reperfusion (Table 1). With regard to individual patients, 60% showed lower LPO values than untreated subjects at 5 minutes post-reperfusion; indeed 40% showed, at least at this time point, values that were similar to those measured in the preoperative blood sample. In one of the two centers plasma vitamin E/total cholesterol ratios were determined as a complementary approach to evaluation of redox changes. As reported in Table 1, while untreated patients did not show a change in the relative mean values over 120 minutes, the treated subjects showed increases at 60 and 120 minutes. As shown in Table 2, within 5 minutes of reperfusion, all unsupplemented patients at both centers showed a statistically significant and often marked increase in the plasma concentration of myeloperoxidase compared with that at the basal time; in contrast, the infused patients did not show any significant changes. Of note, 7 of 12 patients infused with antioxidants did not show any increment in this phagocyte activation marker (data not shown). Further, myeloperoxidase mean values remained statistically unchanged over 60 min in the patients infused with antioxidants in Torino and during the whole observation period in those from Liège.

DISCUSSION AND CONCLUSIONS

This pilot study not only provides a further demonstration of an oxidative biochemical imbalance early after human

Table 2. Plasma Myeloperoxidase Levels During the Early Phases of Reperfusion in Adult Patients Subjected to OLT, Treated or not With the Antioxidant Mixture

Reperfusion time	Without Antioxidant Mixture	With Antioxidant Mixture
Torino Transplantation Unit[a]		
Basal	52 ± 10	75 ± 23
5 min	117 ± 25*	90 ± 23**
60 min	138 ± 19*	115 ± 29**
120 min	201 ± 42*	228 ± 52*
Liège Transplantation Unit[b]		
Basal	37 ± 11	37 ± 16
5 min	65 ± 7*	54 ± 6**
60 min	58 ± 9*	41 ± 5**
120 min	48 ± 9**	30 ± 3**

[a]Myeloperoxidase (ng/mL plasma); Data are means ± S.E. of eight values for each group.
[b]Myeloperoxidase (ng/mL plasma); Data are means ± S.E. of four values for each group.
*Significantly different from the corresponding basal value ($P < 0.05$).
**Not significant.

donor liver reperfusion, but also provides original evidence that IV infusion of a moderate amount of antioxidants during the most critical period of transplantation surgery: i) attenuates oxidant damage and importantly ii) significantly delays and even attenuates a likely major feature of early postreperfusion damage, i.e. neutrophil activation, monitored as plasma myeloperoxidase levels, an enzyme almost exclusively contained in the azurophilic granules of PMNs.[9] Increased phagocyte activation to this degree, had not yet been described in human OLT. However, studies on ischemia-reperfusion injury to the rat liver showed a precocious accumulation of neutrophils in the hepatic vasculature after transplantation[10] or after microvascular clamping.[11] The vascular injury seems to involve endothelial cells, beginning in the ischemic period and then Kupffer cells during the postreperfusion period; both effects cause a continuous recruitment and activation of neutrophils.[4,12,13] Accordingly a PMN-dependent increase in plasma myeloperoxidase has been observed and correlated with generation of oxidants. In fact, the present study shows that intrasurgical treatment with antioxidants markedly delays neutrophil activation.

The increased ROS generated during reperfusion may favour early neutrophil rolling on the sinusoidal surface through oxidative changes in membrane fluidity. Little is known about the up-regulation of adhesion molecules on the luminal surface of endothelial cells, and of complementary ligands on PMNs both of which occur during the early phases of human liver graft reperfusion. Thiel and colleagues recently reported the activation of β_2-integrins [CD18] in a subgroup of patients undergoing OLT,[14] but they did not investigate the effect of antioxidants. Measurement of serum soluble forms of ICAM-1 in OLT patients during and after operation has been performed in our laboratory; however, a significant increase in the content of this molecule was evident only 1 or 2 days following surgery.[15] In addition, intra-operative treatment with antioxidant, did not produce any significant change in serum ICAM-1 levels compared to those in unsupplemented patients.[15]

In conclusion, three theoretical possibilities may explain the observed increase in the steady-state levels ROS in human liver grafts: 1) just an epiphenomenon, 2) the sole cause of reperfusion injury or 3) one of various mechanisms of tissue damage. The third possibility appears to be the most likely one. In support of this conclusion is the reported effect of antioxidant infusion prevent or delay PMN activation during reperfusion. Another supportive finding is the significant inhibition of postreperfusion hypertransaminasemia consistently found in liver transplanted patients intraoperatively infused with a galenic preparation of antioxidants.[16] Finally, one should consider that the intraoperatively infused antioxidants reach the liver only after blood reflow. Thus, in the future, this therapeutic strategy might be better implemented by increasing the dose and/or optimizing the mixture of antioxidants and, last but not least, by improving the antioxidant status of the recipient before transplantation.

REFERENCES

1. Bzeizi HI, Dawkes R, Dodd NJF, et al: J Hepatol 26:69, 1997
2. Biasi F, Bosco M, Chiappino I, et al: Free Radic Biol Med 19:311, 1995
3. Cutrin JC, Cantino D, Biasi F, et al: G. Hepatology 24:1053, 1996
4. Jaeschke H: Proc Soc Exp Biol Med 209:104, 1995
5. Tsakis A, Todo S, Starlz TE: Ann Surg 210:649, 1989
6. Belghiti J, Noun R, Sauvenet A: Am J Surg 169:277, 1995
7. Tateishi T, Yoshimine N, Kuzuya F: Exp Gerontol 22:103, 1987
8. Bieri JG, Tolliver T, Cattignani GL: Am J Clin Nutr 32:2143, 1979
9. Kettle AJ, Winterbourn CC: Redox Report 3:3, 1997

10. Teramoto K, Bowers JL, Khettry U, et al: Transplantation 55:737, 1993
11. Komatsu H, Koo, A, Ghadishah E, et al: Am J Physiol 262:G669, 1992
12. Bulkley GB: Lancet 344:934, 1994
13. Jaeschke H, Farhood A: Am J Physiol 260:G355, 1991
14. Thiel M, Imendörffer S, Chouker A, et al: Hepatology 28:1538, 1998
15. Battista S, Bar F, Mengozzi G, et al: Hepatology 26:233A, 1997
16. Poli G, Cutrin JC, Biasi F: Free Rad Res 28:547, 1998