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Lipid Peroxidation in the Reperfusion Injury of the Liver

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INTRODUCTION

A net overproduction of reactive oxygen species (ROS) consistently occurs in the organs undergoing ischemia followed by reoxygenation.[1] In fact, an extensive reduction of molecular oxygen takes place after reperfusion by the reducing equivalents accumulated in the tissue during the hypoxic state. In turn, ROS are mainly oxidant species recognizing unsaturated membrane lipids among their major cellular targets.

The analysis of the pathogenetic role of membrane lipid peroxidation in the tissue damage which follows ischemia-reperfusion has been one of the projects promoted by the intensive collaboration and constant scientific discussion carried on with Hermann Esterbauer and his group. We started monitoring together aldehydic products of lipid peroxidation in blood samples from human patients with a pathology characterized by a dramatic condition of ischemia reperfusion, namely the circulatory shock syndrome; that line of research led to one of the first demonstrations of actual steady-state increase of 4-hydroxy-2,3-nonenal in a human disease state.[2]

Soon we drew our attention on lipid peroxidation and tissue damage following orthotopic organ transplantation in humans. Hermann Esterbauer was involved in kidney transplantation studies with the excellent surgical group of Rabl in Graz, while we remained more traditionally linked to the liver and related transplantation. Quite recently, this Austrian group provided clear evidence in support of a causative role of lipid peroxidation in human kidney transplantation. They demonstrated a significant improvement of transplantation performance, in terms of quicker normalization of creatinine clearance after surgery, following the parenteral pre-infusion of the kidney recipients with a defined antioxidant mixture.[3] Hereafter we

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review the recent reports on evidence and role of lipid peroxidation in the reperfusion damage following human liver transplantation.

ENHANCED LIPID PEROXIDATION SOON AFTER LIVER TRANSPLANTATION

Increased ROS generation and, in particular, stimulated membrane lipid peroxidation in reperfusion injury due to organ transplantation have also been demonstrated in the rat liver.[4,5] As regards human liver transplantation, Serino *et al.*[6] recorded increased levels of malonaldehyde and 9–11 linoleic acid conjugated dienes in the right atrial blood early after portal vein and hepatic artery connection and declamping. Later on, consistent data were provided by our group, which showed increased malonaldehyde and lipid peroxide levels in post-hepatic blood of the large majority of 19 adult patients, monitored for oxidative damage since portal vein declamping up to 120 min post-reperfusion.[7] At the latter time point, usually corresponding to the very end of the intraoperative period, the different routine indices of lipid peroxidation, i.e. red cell malonaldehyde, plasma (or serum) lipid peroxides and plasma vitamin E consumption well correlated each other in statistical terms,[8] by this way showing to be reliable and easily achievable markers of the specific phenomenon. Actually, the oxidative stress triggered by ischemia-reperfusion in the transplanted human liver keeps evident for more than few hours. Bzeizi and colleagues showed two peaks of 9–11 linoleic acid conjugated dienes after 1 and 12 h from liver reperfusion in a cohort of 21 patients.[9] Biasi *et al.*, monitoring lipid peroxidation indices in the blood of 19 liver transplanted patients for three weeks after surgery, clearly showed a peak of malonaldehyde and lipid peroxide levels between 12 and 36 h from portal vein declamping.[8] Of note, the all three markers employed in the three weeks survey reentered the normal range well within the first week.[8]

The occurrence of an oxidative biochemical imbalance of the redox equilibrium in the transplanted hepatic tissue was further supported by direct detection of free radicals using electron spin resonance (ESR) combined to spin trapping technique. Again Bzeizi *et al.*, reported a two–three fold increase of phenyl butyl nitrone (PBN)-free radical adduct in the patient's right atrial blood after 12 h liver reperfusion as to pre-transplant levels.[9] Very recently, by using phenyl butyl nitrone as a spin trap, Tomasi obtained in similar blood samples an ESR signal strongly suggestive for a lipid derived free radical which was already intense after 30–60 min of reperfusion.[10]

All together, the so far available data confirm the occurrence in humans as well as in the experimental animal of an oxidative stress condition with a significant membrane oxidative breakdown soon after liver reperfusion. Lipid peroxidation is a post-reperfusion event already significant in the large majority of the patients before the end of surgery. During the first post-operation day, phospholipid conjugated dienes, lipid peroxides and aldehydic end-products showed differentiated concentration peaks in the patient's blood. Whatever was the marker adopted, its normalization was reached within 12–48 h.

One cannot exclude that tissues other than liver contribute to the increased levels of these "peripheral" markers. While surgical trauma does not appear involved (Biasi, Poli *et al.*, unpublished observation), the interaction between blood cells and endothelial cells in the hepatic vasculature could in theory amplify, for instance, the post-reperfusion increase of red cell malonaldehyde. Certainly in the liver, and probably in various other tissues, microvascular endothelial cells appear as the initiators of free radical-mediated reperfusion injury, being a great source of xanthine oxidase-generated superoxide radical.[11] Superoxide free radical can initiate red cell membrane lipid peroxidation. Any way, according to the work performed

on experimental animal, ischemia-reperfusion of the liver triggers first of all a marked oxidative stress in the hepatic tissue itself, as detectable in terms of enhanced chemiluminescence,[12] or increased malonaldehyde steady-state levels (Cutrin, unpublished observation).

ROLE OF LIPID PEROXIDATION IN THE REPERFUSION INJURY OF TRANSPLANTED LIVER

Once obtained clear evidence of enhanced membrane lipid peroxidation soon after liver reperfusion, the second step was to clarify the possible relationship between such event and the liver injury which consistently occurs in the transplanted organ. In fact, while of variable intensity in the single patients, cytolysis and cholestasis are typical sequelae of liver transplantation.

The few available observations about the involvement of lipid peroxidation in the pathogenesis of liver damage after transplantation are supporting a role of free radical reactions in the injury's expression. Indeed, a statistically significant correlation has been proved in the single patients between routine markers of cytolysis and oxidative damage early after liver reperfusion. In fact, Biasi *et al.*[8] showed a direct relationship between plasma transaminases or lactic dehydrogenase and red cell malonaldehyde or plasma lipid peroxides in all but one patient, 19 adults in total, 120 min after reperfusion.

With regard to the onset of the reperfusion damage of bile canaliculus, a contribution of free radical-driven reactions has also been proposed, yet only in terms of hypothesis.[13] It seems likely that the derangement of intracellular calcium homeostasis which occurs during prolonged tissue hypoxia becomes rapidly worsened at the time of blood flow restauration. As a consequence, the calcium-dependent functionality of the complex network of microfilaments regulating structure and activity of biliary canaliculi would be heavily impaired. In support of this hypothesis, besides the electron microscopy evidence of microfilament disfunction in the ischemic then reperfused human transplanted liver, there is the demonstration in rat isolated hepatocytes that lipid peroxidation can markedly affect membrane calcium transport.[14] Still in relation to the oxidative damage at the level of the bile canaliculus, much attention is now payed to possible variations in the activity of gamma-glutamyl transpeptidase during ischemia-reperfusion, based on the possible generation of ROS by this enzyme[15] mainly expressed on the biliary pole of the hepatocyte.

Even if standardized criteria are not yet adopted to assess early postoperative liver graft function, the indexes of cytolysis, cholestasis and haemostasis appear the key markers in the case of liver transplantation. Bzeizi *et al.*, employed these three parameters to evaluate the graft outcome in 21 adult transplanted patients. They have actually found a significant inverse correlation between extent of ROS formation and graft outcome.[9]

Conclusive proof of a pathogenetic role of ROS and related radical species will be eventually obtained in the case patient's supplementation with free radical scavengers and antioxidants will achieve prevention or amelioration of reperfusion syndrome.

While a randomized trial employing a trade registered antioxidant mixture is now in progress in our University, hereafter we report on very preliminary data obtained following the IV administration of a galenic antioxidant preparation to liver transplant recipients in the last hour preceeding reperfusion. The antioxidant mixture diluted up to 250 ml of 0.9% NaCl contained: 10 mg retinol palmitate, 1 g ascorbic acid, 50 mg DL-alpha-tocopherol. As reported in Figure 1, the intravenous infusion during warm ischemia of such antioxidant preparation in four not randomized patients led to a marked quenching of serum transaminase increase already in the intraoperative period.

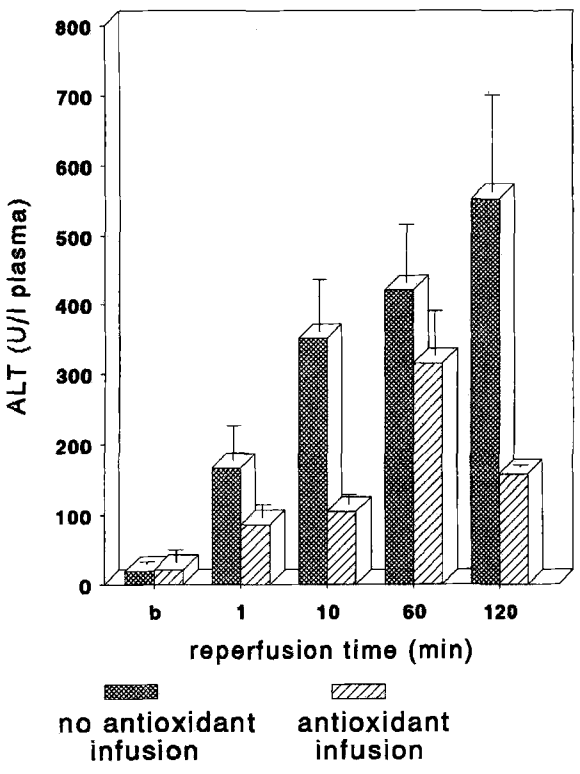

FIGURE 1 Lower increase of serum alanine amino transferase (ALT) in the early reperfusion of human transplanted liver following the infusion of an antioxidant mixture. Internal control was represented by 19 adult patients not treated with the antioxidant mixture. Four not randomized patients were intravenously infused with the mixture (see text) during warm ischemia until portal vein declamping. b: basal time.

CONCLUSIONS

In the transplanted human liver an increased steady-state level of reactive oxygen species is detectable early after reperfusion. In particular, the enhanced oxidative breakdown of membrane polyunsaturated fatty acids generates after blood flow restauration excessive amounts of diffusible toxic aldehydes. An increasing body of evidence supports a major role for these and other products of lipid peroxidation in the pathomechanisms of the so called ischemia-reperfusion syndrome leading, in the case of the hepatic graft, to cytolysis, cholestasis and sometimes to primary non function. However, still little is known about topology and chronology of oxidative damage in the transplanted liver. Is the endothelial cell the cytotype in which ROS overproduction is initiated? When polymorphonuclear cells (PMNs) are actually invading the graft and which are the factors influencing the extent of PMN's recruitment? How ischemia-reperfusion can influence the gene transcription of inflammatory cytokines? Future research will likely focus on these and other crucial points. Easy to forecast that the reactive aldehydes which became of deep biological interest over the last years, mainly thanks to Hermann Esterbauer, will be further considered in these studies as well.

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