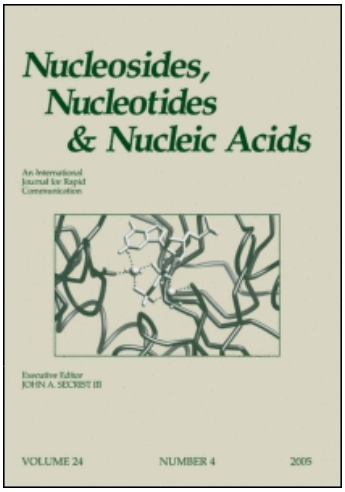

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Liver Transplant: Adenosine Metabolism and Apoptosis

F. Carlucci[a]; E. Marinello[a]; G. Gerunda[b]; D. Neri[b]; F. Rosi[a]; F. Floccari[a]; A. Tabucchi[a]
[a] Dipartimento di Medicina Interna, Sci. Endocrino-Metaboliche e Biochimica, Università di Siena, Italy [b] Istituto di Chirurgia Generale, Università di Padova, Italy

Online publication date: 27 October 2004

To cite this Article Carlucci, F. , Marinello, E. , Gerunda, G. , Neri, D. , Rosi, F. , Floccari, F. and Tabucchi, A.(2004) 'Liver Transplant: Adenosine Metabolism and Apoptosis', Nucleosides, Nucleotides and Nucleic Acids, 23: 8, 1295 — 1299
To link to this Article: DOI: 10.1081/NCN-200027551
URL: http://dx.doi.org/10.1081/NCN-200027551

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 23, Nos. 8 & 9, pp. 1295–1299, 2004

Liver Transplant: Adenosine Metabolism and Apoptosis

F. Carlucci,[1,*] E. Marinello,[1] G. Gerunda,[2] D. Neri,[2] F. Rosi,[1] F. Floccari,[1] and A. Tabucchi[1]

[1]Dipartimento di Medicina Interna, Sci. Endocrino-Metaboliche e Biochimica, Università di Siena, Italy
[2]Istituto di Chirurgia Generale, Università di Padova, Italy

ABSTRACT

Apoptosis and necrosis coexist in ischemia–reperfusion (I/R) injury following organ transplant. During experimental liver transplant we evidenced a deep alteration in energy and antioxidant status. The activity of purine catabolic enzymes was also altered. Caspase-3 (C-3), protein tyrosine phosphatase (PTP) showed significative alterations that lead to DNA fragmentation. These findings could be of interest in new potential strategy to prevent and treat I/R injury.

Key Words: Transplant; Apoptosis; Antioxidant status; Adenosine.

INTRODUCTION

Organ dysfunction secondary to ischemia-reperfusion (I/R) injury still represents a major problem in liver transplant. A potentially critical pathophysiological mechanism of the I/R injury is the development of apoptotic cell death. Apoptosis has been observed in hepatocytes and sinusoidal endothelial cell following ischemia-reperfusion injury and it has been postulated as a contributing factor to ischemia-reperfusion graft

*Correspondence: F. Carlucci, Dipartimento di Medicina Interna, Sci. Endocrino-Metaboliche e Biochimica, Università di Siena, Italy.

DOI: 10.1081/NCN-200027551
1525-7770 (Print); 1532-2335 (Online)

dysfunction. It has also been found that changes of protein tyrosine–kinase phosphorylation are involved in the regulation of apoptosis in various cell types.[1] The distinction between apoptosis and necrosis, which implies different mechanisms of cell death, is blurred in the case of a pathologic insult such as ischemia–reperfusion. It could be of great interest to learn how to differentiate them, fill the gaps in our understanding and exploit the knowledge acquired for clinical benefit.

MATERIALS AND METHODS

We analysed hepatic biopsy specimens from six Landrace pigs (weight 30–35 kg) undergoing experimental liver transplant. Biopsies were obtained before explantation procedure (t1), after cold ischemic period (t2) and 30 min. from reperfusion (t3). Specimens were immediately (within 20 sec.) frozen in liquid nitrogen and stored until analysis. The samples were homogenized at 10% with 0.4 N perchloric acid (PCA) or with lysis buffer containing zwitterionic detergent for nucleotide determination and for enzyme activity evaluation respectively. Aliquots were analysed by capillary electrophoresis (CE) for purine compounds (nucleotides, nucleosides and bases) and reduced/oxidised glutathione, according to Carlucci et al.[2] The same CE method was utilised for the determination of correlated enzyme activities: purine nucleoside phosphorylase (PNP), adenosine deaminase (ADA), and the two soluble isoforms of 5′-nucleotidase (e-Ns and c-N-II) AMP and IMP dependent respectively. We evaluated the substrate disappearance and the product formation in suitable incubation mixtures.[3] The caspase-3 activity was evaluated by the Caspase-3 Assay Kit (Sigma). To study DNA fragmentation, we used a specific kit (ROCHE Diagnostic Corp). The method permitted a rapid and easy identification of apoptotic DNA fragmentation from tissue, with no need for DNA extraction.

Protein–tyrosine–Phosphatase (PTP) activity was evaluated by PTP assay kit (Sigma Company) measuring the free phosphate generation in the dephosphorylation reaction of phosphotyrosine peptide substrate.

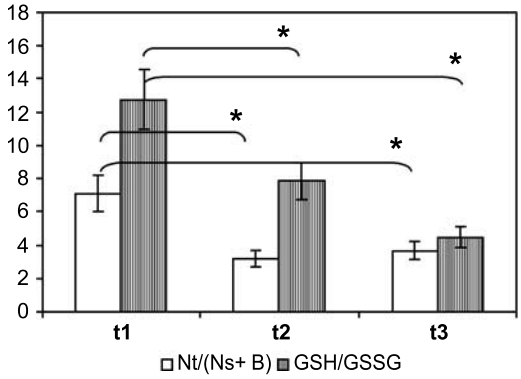

Figure 1. Mean nucleotides/(nucleosides + bases) and GSH/GSSG ratios during the time course of transplant. Bars indicate St. dev. * = $p \leq 0.05$ with respect to values at t1 according to Kruskall–Wallis test.

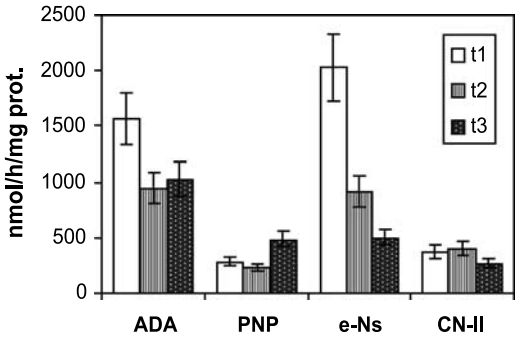

Figure 2. Behaviouor of purine catabolic enzympe, bars as in Fig. 1, significance reported in text.

RESULTS

During ischemic phase (t2) Adenosine triphosphate (ATP) levels decreased from 24.59 ± 6.56 to 15.20 ± 4.27 nmol/mg of protein, GSH/GSSG ratio also significantly decreased (Fig. 1). The basal levels of adenosine, adenosine monophosphate (AMP) and inosine monophosphate (IMP) (0.91 ± 0.25, 6.83 ± 1.21 and 3.15 ± 0.78 nmol/mg of protein respectively) increased 34, 55, and 49% respectively. At 30 min from reperfusion (t3), ATP and IMP levels inverted the trend evidenced at t2 (data not shown). ATP/ADP ratio was greatly reduced at t2 and remained stable during the reperfusion. The behaviour of Energy Charge resembled that of ATP/ADP ratio. Nucleotide/(nucleoside + base) ratio decreased during the ischemic period (t2), after 30 min from reperfusion this tendency was poorly reversed (Fig. 1). GSH/GSSG ratio dramatically decreased in the time-course of transplantation phases. Adenosine deaminase (ADA) and soluble AMP-ase (e-Ns) were reduced both at t2 and t3, purine

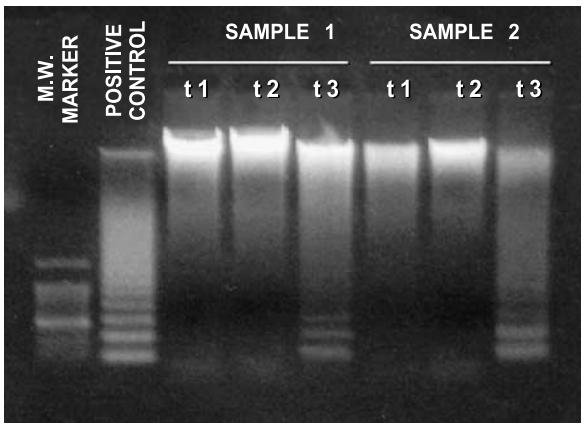

Figure 3. Gel electrophoresis of DNA in two typical experiments, it is evident the DNA fragmentation at t3.

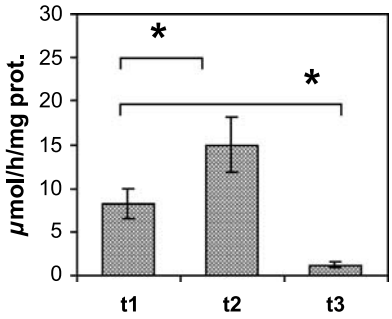

Figure 4. Behaviour of PTP activity, symbols as in Fig. 1.

nucleoside phosphorylase (PNP) was increased at t3; no variation was evident for IMP-ase (cN-II) (Fig. 2). Caspase-3 activity showed a progressive increase during transplant time-course, especially one day after implantation (t4, single case). The activity in the control (t1) was 17.33 ± 6.23 nmol/h/mg of protein, and reached the level of 57.40 ± 9.93 at t3. In three cases, an increase in caspase-3 activity over the level of 60 nmol/h/mg of protein was associated with a sustained DNA fragmentation as reported in Fig. 3. PTP activity was increased during ischemic period (t2) and decreased after 30 min from implantation and reperfusion with blood (Fig. 4), in line with the results reported by other Authors during human lung transplant.[2] No correlation was evident neither with caspase-3 activity nor with DNA fragmentation.

DISCUSSION

Data on purine compounds evidenced an active degradation of ATP to AMP, adenosine and IMP during ischemic time, a further degradation seems to be, however, slowed down by the poor increment of inosine and hypoxanthine levels (not statistically significative). Besides the catabolic rate of AMP via adenosine pathway is reduced by the negative imbalancement of ADA and expecially e-Ns activities. This behaviour could be related to the preservation of AMP and adenosine, precursors of ATP via adenosine kinase. The behaviour of PNP at t3 seems more difficult to interpret but it could be related to an attempt of purine ring salvage through HPRT reaction. Intracellular levels of purine nucleotides may play an important role in the modulation of apoptotic and necrotic cell death signals,[4] our results show a precise behaviour of adenosine metabolism following transplant procedure. Data on glutathione pointed out a deep imbalancement of the antioxidant status and even if this is not surprisingly in the I/R sequence some authors reported that oxidants at low concentrations can induce a low level of apoptosis, but at higher concentrations cause necrosis.[5] In our study we also clearly evidenced some biochemical events associated with apoptosis wich include caspase-3 activation, DNA fragmentation and PTP reduction during the reperfusion. Dynamic alteration in PKC/PTP balance could be related to cell death through p38 protein (mitogen-activated protein kinas or MAPK) triggering as reported by

Mackay et al.[6] The present study is aimed to clarify the role of ischemic insult in liver transplant procedure and the contribution of hepatocyte necrosis and/or apoptosis to this process. Independently from the relationships beethween all the alterations observed, these preliminary data clearly indicate that during liver transplant, they may lead to apoptotic cell death. These findings may be of interest in new potential strategy to prevent and treat ischemia/reperfusion injury.

REFERENCES

1. Keshavjee, S.; Zhang, X.M.; Fischer, S.; Liu, M. Ischemia reperfusion-induced dynamic changes of protein tyrosine phosphorylation during human lung transplantation. Transplantation **2000**, *70*, 525–531.
2. Carlucci, F.; Tabucchi, A.; Biagioli, B.; Sani, G.; Lisi, G.; Maccherini, M.; Rosi, F.; Marinello, E. Capillary electrophoresis in the evaluation of ischemic injury: simultaneous determination of purine compounds and glutathione. Electrophoresis **2000**, *21*, 1552–1557.
3. Carlucci, F.; Rosi, F.; Di Pietro, C.; Marinello, E.; Pizzichini, M.; Tabucchi, A. Purine nucleotide metabolism: specific aspects in chronic lymphocytic leukemia lymphocytes. Biochim. Biophys. Acta **1997**, *1360*, 203–210.
4. Marton, A.; Mihalik, R.; Bratincsak, A.; Adleff, V.; Petak, I.; Vegh, M.; et al. Apoptotic cell death induced by inhibitors of energy conservation-Bcl-2 inhibits apoptosis downstream of a fall of ATP level. Eur. J. Biochem. **1997**, *250*, 467–475.
5. Lee, Y.J.; Shacter, E. Hydrogen peroxide inhibits activation, not activity, of cellular caspase-3 in vivo. Free Radic. Biol. Med. **2000**, *29*, 684–692.
6. Mackay, K.; Mochly-Rosen, D. Involvement of a p38 mitogen-activated protein kinase phosphatase in protecting neonatal rat cardiac myocytes from ischemia. J. Mol. Cell. Cardiol. **2000**, *32*, 1585–1588.