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Normothermic Liver Ischemia and Antioxidant Treatment during Hepatic Resections

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The purpose of our study was to evaluate the clinical impact of reperfusion injury after normothermic ischemia during major liver resections and the effect of an intraoperative antioxidant infusion.

This prospective randomized study comprised 50 patients; half of them (treatment group) were given an antioxidant infusion containing tocopherol and ascorbate immediately prior to reperfusion onset. Venous blood samples for the determination of MDA-TBARS (malondialdehyde-thiobarbituric acid reactive substances) by a HPLC-based test as a marker of lipid peroxidation were taken prior to ischemia, 30 min after reperfusion onset and at the end of the operation.

In the control group there was a significant increase of MDA-TBARS ($p = 0.001$) at 30 min after reperfusion onset. At the end of the operation the values had returned to the initial level. The treatment group showed only a marginal increase (p-value for the difference between the two groups: 0.007). After exclusion of the patients with histologically proven advanced cirrhosis the increase in the control group ($p < 0.001$) and the difference between the increase in the two groups ($p = 0.001$) became more significant. Prothrombin time was also significantly better in the treatment group ($p = 0.003$). Postoperative complications such as prolonged liver failure, bleeding disorders and infections were seen more often in the control group.

In our study MDA-TBARS was increased after liver ischemia, but in patients with advanced cirrhosis the effect was smaller or even absent. This increase and possible clinical consequences of reperfusion injury could be reduced by intraoperative administration of an antioxidant infusion.

Keywords: Ischemia-reperfusion injury, normothermic liver ischemia, lipid peroxidation, antioxidant treatment, liver surgery, malondialdehyde

INTRODUCTION

Many experimental studies have suggested a causative role of free oxygen species and lipid peroxidation for ischemia-reperfusion injury of the liver,[1–11] but there are little data on the consequences of this observation after normothermic liver ischemia used during liver resections in humans.[12,13] In a precursor study we have seen an improved postischemic course of such liver parameters as prothrombin time and transaminases in patients receiving an

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antioxidant cocktail containing tocopherol and ascorbate immediately before the start of reperfusion.[14] The aim of this study was to determine whether this cocktail could decrease the extent of postischemic lipid peroxidation measured as MDA-TBARS (malondialdehyde-thiobarbituric acid-reactive substances) and postoperative complications such as prolonged liver failure, bleeding disorders and infections. Inhibition of the transient increase of lipid peroxidation products during reperfusion had been previously shown with this treatment in kidney transplant recipients, with significant improvement of organ function,[15] and in revascularization operations with significant reduction of postoperative leg edema.[16]

PATIENTS AND METHODS

This prospective, randomized, double-blinded study comprised 50 patients undergoing major liver surgery including normothermic liver ischemia. In order to get comparable conditions liver ischemia was induced by uninterrupted portal triad clamping (Pringle manoeuvre) in all patients. Twenty-five patients (treatment group) received an antioxidant multivitamin infusion using two ampoules of Omnibionta^{R} prior to reperfusion. One ampoule (10 ml) contains 5.5 mg retinol palmitate, 50 mg thiamine chloride-hydrochloride, 10 mg riboflavin 5-phosphate, 100 mg nicotinamide, 25 mg dexpanthenol, 15 mg pyridoxine hydrochloride, 500 mg ascorbate, 5 mg alpha-tocopherol acetate, 150 mg benzyl alcohol, 500 mg polysorbate 80, 1.0 mg DL-alpha-tocopherol, 200 mg propylene glycol, 2500 mg glycerine 85%, 360 mg trometamol and water 10 ml.

Indications for surgical intervention were hepatic metastases (19 patients), hepatocellular carcinoma (13), intrahepatic cholangiocellular carcinoma (3), focal nodular hyperplasia (5), hemangioma (3), liver adenoma (1), hydatid cysts (4) and other liver cysts (2). The mean duration of warm ischemia was 54.3 min in the treatment group and 52.3 min in the control group (median values 55 min in both groups). Preoperative liver function, the extent of the liver resections and the duration of ischemia were comparable in both groups. All the liver resections were performed by the same team and by a standardized procedure employing the Cavitron Ultrasonic Aspirator (CUSA) for the parenchymal dissection.

Venous blood samples were taken prior to ischemia, 30 min after reperfusion onset and at the end of the operation. Prior to this study we have compared liver function parameters and MDA-TBARS measured in blood samples taken simultaneously from the central venous line, from the arterial line and from a radiologically placed liver vein catheter in eight patients and no appreciable differences were found (unpublished data). In this study the central venous line was used (after rejecting the first 20 ml of blood) and possible falsification by hemodilution was excluded by determination of the hematocrit values. MDA-TBARS was measured according to the method of Wong *et al.*[17] which is reported elsewhere.[18] Quantification of lipid peroxidation by determination of MDA-TBARS is practicable under clinical conditions and the high pressure liquid chromatography we use is very reliable and quite insensitive to disturbing factors.[19–21] The measurements in the groups were described as mean and standard deviation. Differences between the groups were evaluated by Mann–Whitney test, differences between the measuring points in one patient were evaluated by Wilcoxon Signed Ranks test. A p-value below 0.05 was considered to be statistically significant.

RESULTS

In the control group the mean value for MDA-TBARS was 0.70 nmol/ml (standard deviation, SD = 0.14); this rose to 0.89 (SD = 0.25) during reperfusion. At the end of the operation it had returned to 0.71 (SD = 0.16). The difference between the pre- and postischemic values was

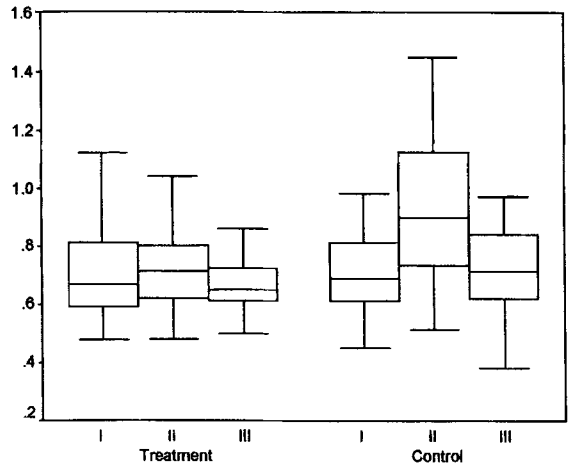

FIGURE 1 Postischemic increase of MDA-TBARS in the treatment (not significant) and in the control groups ($p = 0.001$): (I) pre-ischemic; (II) 30 min after reperfusion onset; and (III) end of operation. p-value for the difference between the increase in the two groups: 0.007.

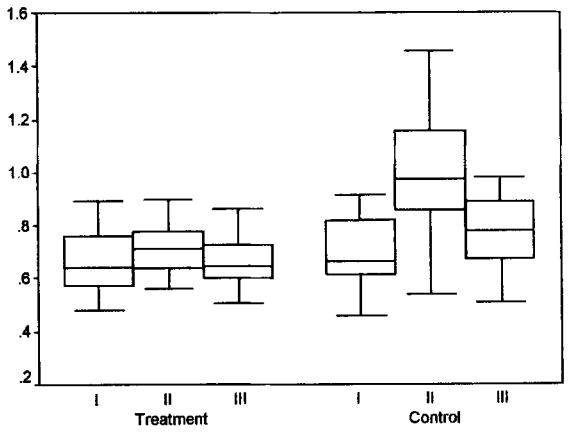

FIGURE 2 Postischemic increase of MDA-TBARS in the treatment (not significant) and in the control groups ($p < 0.001$) after exclusion of patients with advanced cirrhosis. p-value for the difference between the increase in the two groups: 0.001.

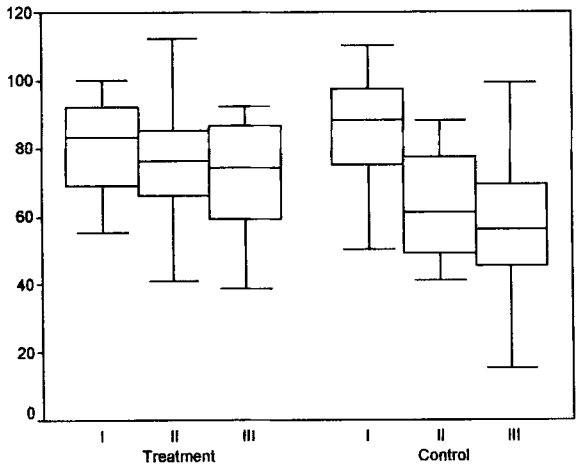

FIGURE 3 Postischemic decrease of prothrombin time (%) in the treatment ($p = 0.01$) and in the control group ($p < 0.001$). p-value for the difference between the decrease in the two groups: 0.003.

statistically significant ($p = 0.001$, Figure 1). In contrast, the treatment group showed only a marginal increase from 0.71 (SD = 0.18) to 0.72 (SD = 0.19) after ischemia ($p = 0.7$:n.s.); at the end of the operation the values were slightly lower than initial (0.68, SD = 0.20).

The difference between the increase in the treatment and in the control group was significant ($p = 0.007$). The exclusion of patients with advanced cirrhosis (histologically proven diagnosis in six patients in the treatment and seven patients in the control group) made the increase in the control group more obvious (from 0.70 (SD = 0.14) to 0.98 (SD = 0.21), $p < 0.001$). The differences in the increase of MDA-TBARS between the control and the treatment group then became even more statistically significant ($p = 0.001$, Figure 2). Interestingly, in patients with advanced cirrhosis the postischemic increase of MDA-TBARS was reduced or even absent.

The clotting factors turned out to be a very efficient parameter for the assessment of acute liver cell damage. The prothrombin time (activity of clotting factors I, II, V, VII, X) showed significantly better postischemic values in the treatment group. Whereas in the control group the mean values fell from 84.5% (SD = 16.4) to 62.9% (SD = 15.2, 30 min after reperfusion onset) and to 58.5% (SD = 19.1, end of the operation), in the treatment group they only decreased from 81% (SD = 13.1) to 75.6% (SD = 16.6) and 71.2% (SD = 15.1). The p-value for the difference in the decrease between the two groups was <0.001 at 30 min after reperfusion onset and 0.003 at the end of the operation (Figure 3).

As for the transaminases, there was an increase of AST (aspartate transaminase) from 11.8 U/L (SD = 9.8) to 270 U/L (SD = 234) and 369 U/L (SD = 351) in the control group as compared to 9.5 U/L (SD = 4.3) to 204 U/L (SD = 238) and 261 U/L (SD = 302) in the treatment group. For ALT (alanine transaminase) it was 13.2 U/L (SD = 9.9) – 297 U/L (SD = 309) – 415 U/L (SD = 457) and 11.3 U/L (SD = 9.1) – 221 U/L (SD = 233) – 276 U/L (SD = 311) respectively. As in our previous study,[14] the differences were not significant ($p = 0.14$ and 0.26, respectively). The mean values for hematocrit were 34% (SD = 5.4) – 31% (SD = 4.6) – 35% (SD = 4.0) in the control group and 33% (SD = 3.6) – 32% (SD = 4.4) – 35% (SD = 3.4) in the treatment group.

There were also some differences in the clinical course between the two groups attributable to differences in the synthetic capacity of the liver: In the control group more patients had postoperative infections (five patients, including one subphrenic abscess) and bleeding disorders associated with coagulopathy (one reoperation for diffuse bleeding, one large hematoma of the abdominal wall and one patient with relapsing epistaxis). In contrast, no infections or bleeding problems were seen in the treatment group. Prolonged postoperative recovery of liver function with bilirubin above 5 mg/dl for more than one week was seen in four patients of the control group and in one patient of the treatment group. No side effects of the antioxidant infusion were found.

DISCUSSION

Formation of reactive oxygen species and peroxidation of phospholipid fatty acids constitute the starting point of a cascade of cytotoxic reactions[22–29,12] and in clinical life the patient is often seen at the end point of this cascade. In contrast to oxidative stress induced by toxic agents, drugs, hemorrhagic shock or arterial occlusive disease, liver ischemia during hepatic resections is one of the rare clinical situations in which the duration of ischemia is exactly known and the beginning of reperfusion is clearly defined. Reactive oxygen species interact with polyunsaturated fatty acids[30,31] at the cell membrane level, resulting in the formation of several oxygenated compounds (lipoperoxides) whose hydrolysis produces several aldehydes, the best represented being MDA, a degradation product of peroxidized fatty acids.[32] Our previous studies on revascularization operations had shown that a peak of lipid peroxidation products such as MDA is to be expected shortly after reperfusion onset.[16,18]

During liver resections we demonstrated that 30 min after reperfusion onset the MDA values were significantly higher than prior to ischemia. In contrast, in the treatment group the MDA values remained stable, showed only a small increase or even decreased. Omnibionta® was chosen because it was already in clinical use, its antioxidant effect had been proven in other clinical conditions,[15,16] and it contains the synergistic vitamins tocopherol and ascorbate.[31,33–39] The antioxidant function and requirement for vitamin E seem to be related to the status of vitamin C. It appears that ascorbic acid may react directly with free radicals as well as intermediates of tocopherol oxidation and may thus spare the oxidative degradation of vitamin E.[34,35,40–43] Of course we cannot exclude that other components of this cocktail play an important role as well.

We believe that the accurate timing of administration of the antioxidant infusion is essential. The transient time dependent increase in MDA after reperfusion onset led us to assume that the therapeutic window would be limited to a short time prior to or just upon the start of reperfusion.[16] The increase of MDA in the control group was associated with a higher number of clinical postoperative complications (attributable to the synthetic capacity of the liver) as compared to the treatment group, although there were no differences in preoperative liver

function, in the extent and in the technique of the resections.

Fluctuations of MDA may be explained by difficulties in measuring the extent of lipid peroxidation under clinical conditions. Only part of the lipid hydroperoxides are converted into MDA,[44–46] the conversion of lipid hydroperoxides to MDA varies depending on conditions like acidity or temperature and lipid hydroperoxides are not the only source of MDA in biological systems.[47] Last but not least, lipid hydroperoxides and their secondary products such as aldehydes can readily be metabolized,[48,49] so they may be present only for a very short period of time.

Interestingly, the increase of MDA was smaller or even missing in patients with advanced cirrhosis. Increased lipoperoxide levels have been reported in patients with various liver diseases,[50] but normal lipoperoxide levels have been shown in patients with advanced liver cirrhosis[51] and may be explained by polyunsaturated fatty acid deficiency[52,53] reducing the substrate availability for lipoperoxide formation as well as to a reduction of superoxide dismutase[54] in these patients. Under these circumstances, these patients may be described as "burnt out"; a similar observation has been made in patients with severe chronic peripheral arterial occlusive disease.[16]

We conclude that in our study lipid peroxidation assessed by MDA-TBARS was less pronounced, liver function parameters were better and postoperative complications were seen more rarely in patients receiving the antioxidant cocktail and that ischemia-reperfusion injury could be alleviated in this way. In patients with advanced cirrhosis, however, the mechanisms of lipid peroxidation seem to be reduced. Of course, tolerance of the operation also depends on other factors, such as blood loss and functional capacity of the liver remnant, especially in cirrhotic patients. Although our experiences are very promising so far, much work remains to be done to find out if the protective effect could be strengthened by variation of dosage, multiple administration or other antioxidants.

References

[1] M. Younes and O. Strubelt (1988) The involvement of reactive oxygen species in hypoxic injury to rat liver. *Research Communications in Chemical Pathology and Pharmacology*, **59**, 369–381.

[2] S.L. Atalla, L.H. Toledo-Pereyra, G.H. MacKenzie and J.P. Cederna (1985) Influence of oxygen-derived free radical scavengers on ischemic livers. *Transplantation*, **40**, 584–590.

[3] S. Marubayashi, K. Dohi, K. Ochi and T. Kawasaki (1986) Role of free radicals in ischemic rat liver cell injury: prevention of damage by alpha-tocopherol administration. *Surgery*, **99**, 184–192.

[4] I. Hamamoto, E.T. Nemoto, R.W. Evans, H.J. Mischinger, S. Fujita, N. Murase and S. Todo (1993) Rat liver lipids during *ex vivo* warm and cold ischemia and reperfusion. *Journal of Surgical Research*, **55**, 382–389.

[5] S.J. Ontell, L. Makowka, J. Trager, V. Mazzaferro, P. Ove and T.E. Starzl (1989) Pharmacologic modulation of experimental postischemic hepatic function. *Annals of Surgery*, **209**, 200–210.

[6] H.J. Mischinger, P.N. Rao, S. Todo, J.T. Snyder, F. Quehenberger, N. Murase and T.E. Starzl (1991) Levels of purine nucleoside phosphorylase (PNP) as a viability marker of nonparenchymal cells in cold preserved livers. *Transplantation Proceedings*, **23**, 222-225.

[7] H. Kobayashi, T. Nonami, T. Kurokawa, Y. Takeuchi, A. Harada, A. Nakao and H. Takagi (1995) Role of endogenous nitric oxide in isehemia-reperfusion injury in rat liver. *Journal of Surgical Research*, **59**, 772–779.

[8] B. Koneru, M.C. Reddy, A.N. de la Torre, D. Patel, T. Ippolito and R.J. Ferrante (1995) Studies of hepatic warm ischemia in the obese Zucker rat. *Transplantation*, **59**, 942–946.

[9] B. Gonzales-Flecha, J.C. Cutrin and A. Boveris (1993) Time course and mechanism of oxidative stress and tissue damage in rat liver subjected to *in vivo* ischemia-reperfusion. *Journal of Clinical Investigation*, **91**, 456–464.

[10] T. Nishimura, M. Nakahara, S. Kobayashi, I. Hotta, S. Yamawaki and Y. Marui (1992) Ischemic injury in cirrhotic livers: an experimental study of the temporary arrest of hepatic circulation. *Journal of Surgical Research*, **53**, 227–233.

[11] H. Kobayashi, T. Nonami, T. Kurokawa, S. Sugiyama, T. Ozawa and H. Takagi (1991) Mechanism and prevention of ischemia-reperfusion-induced liver injury in rats. *Journal of Surgical Research*, **51**, 240–244.

[12] U. Rauen, R. Viehbahn, W. Lauchart and H. de Groot (1994) The potential role of reactive oxygen species in liver ischemia/reperfusion injury following liver surgery. *Hepato-Gastroenterology*, **41**, 333–336.

[13] C. Niederau (1994) Free Radical Science. The long road from basic science to clinical medicine. *Hepato-Gastroenterology*, **41**, 308.

[14] H. Cerwenka, H. Bacher, G. Werkgartner, A. El-Shabrawi, F. Quehenberger, H. Hauser and H.J. Mischinger (1998) Antioxidant treatment during liver resections for alleviation of ischemia-reperfusion injury. *Hepato-Gastroenterology*, **45**, 777–782.

[15] H. Rabl, G. Khoschsorur, T. Colombo, P. Petritsch, M. Rauchenwald, P. Koltringer, F. Tatzber and H. Esterbauer (1993) A multivitamin infusion prevents lipid peroxidation and improves transplantation performance. *Kidney International*, **43**, 912–917.
[16] H. Rabl, G. Khoschsorur and W. Petek (1995) Antioxidative vitamin treatment: effect of lipid peroxidation and limb swelling after revascularisation operations. *World Journal of Surgery*, **19**, 738–744.
[17] S.H. Wong, J.A. Knight, S.M. Hopfer, O. Zaharia, C.N. Leach, Jr. and F.W. Sunderman, Jr. (1987) Lipoperoxides in plasma as measured by liquid-chromatographic separation of malondialdehyde-thiobarbituric acid adduct. *Clinical Chemistry*, **33**, 214–220.
[18] H. Rabl, G. Khoschsorur, T. Colombo, F. Tatzber and H. Esterbauer (1992) Human plasma lipid peroxide levels show a strong transient increase after successful revascularisation operations. *Free Radicals in Biology and Medicine*, **13**, 281–288.
[19] R.P. Bird, S.S. Hung, M. Hadley and H.H. Draper (1983) Determination of malonaldehyde in biological materials by high-pressure liquid chromatography. *Analytical Biochemistry*, **12**, 240–244.
[20] J.M. Gutteridge and B. Halliwell (1990) The measurement and mechanism of lipid peroxidation in biological systems. *Trends in Biochemical Sciences*, **15**, 129–135.
[21] R.P. Bird and H.H. Draper (1984) Comparative studies on different methods of malonaldehyde determination. *Methods in Enzymology*, **105**, 299–305.
[22] T.F. Slater (1984) Free radical mechanisms in tissue injury. *Biochemical Journal*, **222**, 1–15.
[23] G. Poli (1993) Liver damage due to free radicals. *British Medical Bulletin*, **49**, 604–620.
[24] H. de Groot (1994) Reactive oxygen species in tissue injury. *Hepato-Gastroenterology*, **41**, 328–332.
[25] R.S. Britton and B.R. Bacon (1994) Role of free radicals in liver diseases and hepatic fibrosis. *Hepato-Gastroenterology*, **41**, 343–348.
[26] B. Halliwell and J.M.C. Gutteridge (1989) *Free Radicals in Biology and Medicine* (2nd edn.), Claredon Press, Oxford.
[27] H. Sies (1991). *Oxidative Stress. Oxidants and Antioxidants*. Academic Press, London and New York.
[28] B. Halliwell and J.M. Gutteridge (1984) Lipid peroxidation, oxygen radicals, cell damage, and antioxidant therapy. *Lancet*, **1** (8391), 1396–1397.
[29] B. Halliwell (1987) Oxidants and human disease: some new concepts. *FASEB Journal*, **1**, 358–364.
[30] B. Halliwell and S. Chirico (1993) Lipid peroxidation; its mechanism, measurement, and significance. *American Journal of Clinical Nutrition*, **57**, 715–724.
[31] B. Halliwell (1991) Reactive oxygen species in living systems: source, biochemistry, and role in human disease. *American Journal of Medicine*, **91**, 14S–22S.
[32] C.E. Cross, B. Halliwell, E.T. Borish, W.A. Pryor, B.N. Ames, R.L. Saul, J.M. McCord and D. Harman (1987) Oxygen radicals and human disease. *Annals of Internal Medicine*, **107**, 526–545.
[33] C. Rousseau-Richard, C. Richard and R. Martin (1991) Synergy effects of vitamin C and amino acids on the antioxidant properties of vitamin E. *New Journal of Chemistry*, **15**, 283–291.
[34] C.K. Chow (1991) Vitamin E and oxidative stress. *Free Radicals in Biology and Medicine*, **11**, 213–232.
[35] J.E. Packer, T.F. Slater and R.L. Willson (1979) Direct observation of a free radical interaction between Vitamin E and Vitamin C. *Nature*, **278**, 737–738.
[36] M. Scarpa, A. Rigo, M. Mairino, F. Fulvio and C. Gregolin (1984) Formation of alpha-tocopherol radical and recycling of alpha-tocopherol by ascorbate during peroxidation of phosphatidyl-choline liposome. An electron paramagnetic resonance study. *Biochimia and Biophysica Acta*, **801**, 215–219.
[37] W. Stahl and H. Sies (1997) Antioxidant defense: vitamins E and C and carotenoids. *Diabetes*, **46**(suppl. 2), S14–18.
[38] H. Sies and W. Stahl (1995) Vitamins E and C, beta-carotene, and other carotenoids as antioxidants. *American Journal of Clinical Nutrition*, **62**(suppl. 6), 1315S–1321S.
[39] H. Sies, W. Stahl and A.R. Sundquist (1992) Antioxidant functions of vitamins. Vitamins E and C, beta-carotene, and other carotenoids. *Annals of the New York Academy of Sciences*, **669**, 7–20.
[40] A. Bendich, L.J. Machlin, O. Scandurra, G.W. Burton and D.D.M. Wayer (1986) The antioxidant role of vitamin C. *Advances in Free Radical Biology and Medicine*, **2**, 419–444.
[41] P.T. Chou and A.U. Khan (1983) L-Ascorbic acid quenching of singlet delta molecular oxygen in aqueous media. Generalized antioxidant property of vitamin C. *Biochemical and Biophysical Research Communications*, **115**, 932–937.
[42] H. Wefers and H. Sies (1988) The protection by ascorbate and glutathione against microsomal lipid peroxidation is dependent on vitamin E. *European Journal of Biochemistry*, **174**, 353–357.
[43] P.B. McCay (1985) Vitamin E: interactions with free radicals and ascorbate. *Annual Review of Nutrition*, **5**, 523–540.
[44] H. Esterbauer and H. Zollner (1989) Methods for determination of aldehydic lipid peroxidation products. *Free Radicals in Biology and Medicine*, **7**, 197–203.
[45] H. Scherz (1970) Formation of deoxycompounds and malonaldehyde in irradiated aqueous solutions of carbohydrates and related compounds. *Radiation Research*, **43**, 12–24.
[46] E.N. Frankel (1987) Biological significance of secondary lipid oxidation products. *Free Radical Research Communications*, **3**, 213–325.
[47] D.R. Janero (1990) Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radicals in Biology and Medicine*, **9**, 515–540.
[48] C.K. Chow (1979) Nutritional influence on cellular antioxidant defense systems. *American Journal of Clinical Nutrition*, **32**, 1066–1081.
[49] R.O. Recknagel, E.A. Glende, R.L. Waller and K. Lowrey (1982) Lipid peroxidation: Biochemistry, measurement and significance in liver cell injury. In *Toxicology of the Liver* (Eds., G. Plaa and W.R. Hewitt), Raven Press, New York, pp. 213–241.
[50] T. Suematsu, T. Kamada, H. Abe, S. Kikuchi and K. Yagi (1977) Serum lipoperoxide levels in patients suffering from liver diseases. *Clinica Chimica Acta*, **79**, 267–270.
[51] G. Bianchi, G. Marchesini, A. Fabbri, M. Ronchi, R. Chianese and G. Grossi (1997) Lipoperoxide plasma levels in patients with liver cirrhosis. *Hepato-Gastroenterology*, **44**, 784–788.

[52] E. Cabré, J.L. Periago, A. Abad-Lacruz, A. Gil, F. Gonzales-Huix, F. Sanchez de Medina and M.A. Gassull (1988) Polyunsaturated fatty acid deficiency in liver cirrhosis: its relation to associated protein-energy malnutrition (preliminary report). *American Journal of Gastroenterology*, **83**, 712–717.

[53] P. Muriel and M. Mourelle (1992) Characterization of membrane fraction lipid composition and function of cirrhotic rat liver. Role of S-adenosyl-L-methionine. *Journal of Hepatology*, **14**, 16–21.

[54] T. Inagaki, K. Katoh, S. Takiya, K. Ikuta, S. Kobayashi, M. Suzuki, Y. Fukuzawa, T. Ayakawa, K. Shimizu, T. Tagaya and K. Katoh (1992) Relationship between superoxide-dismutase (SOD) and viral liver diseases. *Gastroenterologia Japonica*, **27**, 382–389.