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Protective effect of antioxidative vitamins against lipid peroxidation in liver ischemia and reperfusion – an animal experimental study

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ABSTRACT

Ischemia and reperfusion (I/R) leads to oxidative stress with free radical formation. With respect to liver surgery and transplantation this can lead to deleterious clinical effects. Protection of the liver against I/R injury is of major concern. Thus, in this study, we examined the effect of an antioxidant vitamin solution (vitamin E, C and ß-carotene) on warm I/R injury. Twelve pigs of the German landrace (7 animals in the vitamin group and 5 untreated controls) were examined in this animal model. Twenty-four hours before laparotomy, the vitamin group was initiated with a single intravenous infusion of the vitamin cocktail. The duration of complete warm ischemia of the liver was 4 hours. Serum liver enzyme levels (AST and ALT) and with thiobarbituric acid reacting substances (TBARS) in liver tissue were measured. Furthermore, immunohistochemical staining of oxidative products (oxidized proteins and 4-hydroxy-nonenal = 4-HNE) in liver tissue was made. The maximum accumulation of oxidized proteins was seen six days postoperatively in the controls whereas in the vitamin group only small amounts were seen. 4-HNE showed a marked accumulation in the controls but was almost not detectable in the vitamin group. TBARS were lower in the vitamin group compared to controls. Although the emulsifier necessary for the vitamin solution leads to increased liver enzyme levels in the vitamin group, the values returned to normal more rapidly. Antioxidant vitamins are able to improve warm I/R liver injury. Oxidative stress is directly verifiable at the tissue level. Future animal experiments as well as clinical trials are necessary to explore the optimization of the combination of antioxidative vitamins for the maximum protection from I/R injury.

Key words: Ischemia and reperfusion - liver – oxidative stress – antioxidative vitamins**.**

INTRODUCTION

Ischemia and reperfusion (I/R) induces oxidative stress with free radical formation. (Serracino-Inglott et al., 2001, Cutrìn et al., 2002). From clinical and experimental data it is assumed that this leads to an increased consumption of antioxidants resulting in an imbalance of the antioxidant system. (Barsacchi et al., 1992, Biasi et al., 1995, Giakoustidis et al., 2001, Salvemini et al., 2002, Cuzzocrea et al., 2001). In liver surgery I/R induced oxidative stress caused by the temporary cross clamping of the portal vein and hepatic artery is considered to play an important role not only for injury of the remaining liver but also for the clinical outcome of patients. With respect to liver transplantation a marked I/R injury can lead to graft failure. This can result in deleterious clinical problems. Furthermore, free radicals are known to be responsible for the development of postoperative complications. Clinical studies indicate that a loss of antioxidants is associated with an increased risk for sepsis and multi-organ failure (Leff et al., 1993, Metnitz et al., 1999). A major concern in liver surgery and transplantation has been liver protection.

Endothelial cells represent one of the main targets of free radicals. Oxidative stress induces morphological and biochemical alterations of these cells. (Cuzzocrea et al., 2001, Molyneux et al., 2002). This contributes to damage of the entire organ. Administration of vitamins with antioxidative properties are able to minimize or prevent this damage (Marubayashi et al., 1986, lehr et al. 1998). Indeed, experimental and clinical studies have shown positive effects of antioxidant vitamins on I/R injury of the liver (Marubayashi et al., 1989, Rabl et al., 1993, Cerwenka et al., 1998, Bartels et al., 2004).

Vitamin E (= α-tocopherol) is considered to be the most important fat soluble antioxidant in cell membranes. It prevents lipid peroxidation-induced destabilization of the cell membrane by breaking the lipid radical chain reaction. Consequently, high cellular α-tocopherol concentrations might increase the endothelial antioxidant defence and function. Vitamin C was shown to be protective in cardiac surgery with I/R, (Dingchao et al., 1994) and is important for the regeneration of α-tocopherol. In case of vitamin C supplementation, a sufficient concentration of vitamin E is necessary and vice versa. β-carotene is able to inactivate ”singulette” oxygen and peroxyl radicales.

The purpose of the present animal experimental study was to evaluate whether the parenteral administration of an antioxidant solution consisting of α-tocopherol, ascorbic acid, and β-carotene has beneficial effects on warm I/R liver injury.

METHODS

The animal experiments presented were confirmed by the Bezirksregierung Hannover, Germany (TS 94-711).

Twelve female pigs of the German landrace served as experimental animals. All the animals were held in the central animal laboratory of the Hannover University School of Medicine in single boxes (2,7 m^2). The mean body weight was 30-35 kg. The animals were fed with commercially available animal nutrition HG HY (RHG Nord AG, Hannover, Germany). All animals were administered prophylaxis against endoparasites with Rintal®. One week before the intended operation stool specimens were investigated concerning ascarides, oxyures, band worms, hawk worms, and salmonellas. The animals were divided into two study groups. The vitamin group (7 animals) received the vitamin solution i.v. 24 hours preoperatively. The control group (5 animals) was not treated.

Vitamin solution

The vitamin solution was designed in cooperation with Prof. Dr. Biesalski, Department of Biological Chemistry and Nutritional Sciences, University of Hohenheim, Stuttgart, Germany:

100 mg α-tocopherol / kg BW	10 mg β-carotene / kg BW	200 mg ascorbate / kg BW

According to these recommendations the solution was prepared by BASF AG, Ludwigshafen, Germany:

2,5 %	α-Tocopherol
0,25 %	β-Carotene (0,08 % all trans and 0,15 % cis Isomere)
5 %	Ascorbic acid
26 %	Solutol HS 15 (emulsifier)
2,7 %	Miglycol 812
0,005 %	2-Ethylmercurimercaptobenzoe acid (preservation)
1,3 %	NaOH (for pH-adjustment: pH = 6)

The solution was mixed with H_2O 1:2 resulting in an osmolarity of 304 mosmol/kg. 120 ml vitamin solution with 240 ml H_2O was administered. Thus, each animal received 3000 mg α-tocopherol, 300 mg β-carotene, and 6000 mg ascorbic acid. Because of severe side effects, i.e. tachycardia and hyperthermia, when giving the solution without vitamins, it was decided to perform the study with an untreated control group rather than a control group administered solution without vitamins.

Anaesthesia

Premedication was carried out by the i.m. administration of 10 mg/KG body weight Azaperon (Stresnil®) and 0.025 mg Atropinsulfate (Atropinsulfat Braun®). After 15 minutes the intubation anaesthesia was infused via an intravenous line: 0.005 mg/KG body weight Fentanyldihydrogencitrate (Fentanyl Janssen®) and 12.5 mg/KG body weight Thiopentalsodium (Trapanal®). Following this animals were orally intubated. The animals were mechanically ventilated using a mixture of N_2O:O_2 (2:1; frequency 12/min., inspiration volume 400 ml). The maximal airway pressure was 20 mm Hg. The arterial pCO2 was 36 mm Hg during mechanical ventilation. Additionally, Isofluran was administered using a concentration of 1 to 2 %. Analgesia during the operative procedure was achieved by the application of Fentanyl in proportions of 0.0025 mg/kg body weight. 1000 ml Ringer´s solution and 2000 ml HAES 6 % were infused via a central venous line. These amounts were necessary to avoid circulatory depression during hilus occlusion. Catecholamines were not administered in order to maintain microcirculation. Acidosis was treated by $NaHCO_3$ and by increasing the respiratory frequency.

Operative Procedure

According to the literature pigs are able to survive hilus occlusion only for a maximum of 60 minutes (Battersby et al., 1974, Harris et al., 1982, Bredt et al., 1997). Therefore, an extracorporal active shunt was created in order to achieve longer periods of hilus occlusion, and to aggravate ischemia-reperfusion injury (fig. 1).

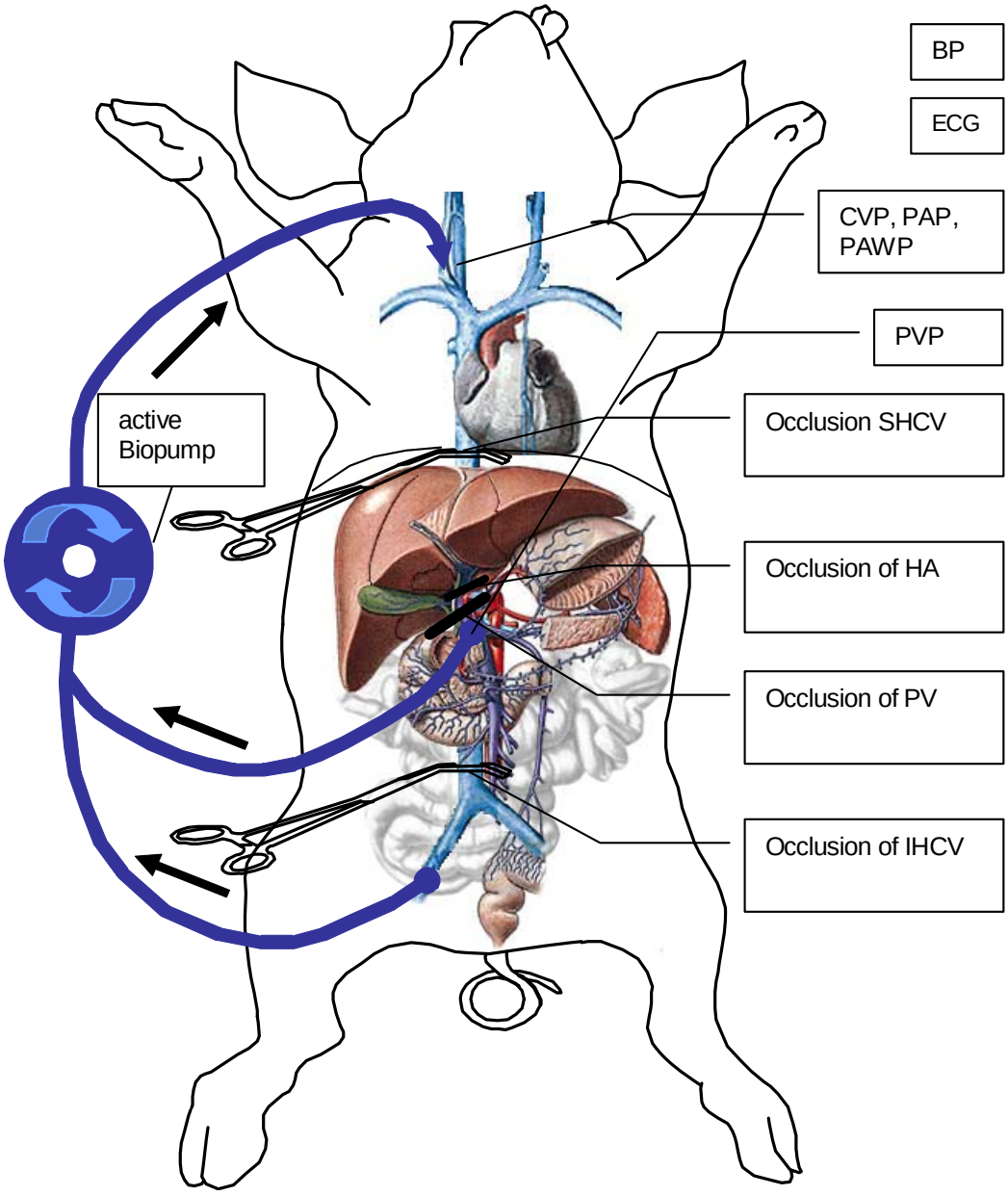


Fig. 1 Experimental design. Total vascular occlusion of the liver. Extracorporal bypass. (BP=blood pressure, ECG=electro cardio graphie, CVP=central venous pressure, PAP=pulmonal arterial pressure, PAWP=Pulmonal arterial wedge pressure, PVP=portal venous pressure, SHCV=suprahepatic caval vein, HA=hepatic artery, PV= portal vein, IHCV=infrahepatic caval vein)

In a first step the internal carotid artery, internal and external jugular veins were prepared. Blood pressure was continuously monitored via a carotid arterial catheter. The external jugular vein was used for the venous part of the shunt. Via the internal jugular vein a pulmonary artery catheter was introduced for measurement of central venous pressure, pulmonal artery pressure and pulmonal artery wedge pressure.

In a second step the animals were laparotomized. The hepatic artery and branches thereof, the portal vein as well as the supra- and infrahepatic caval vein were prepared. The portal part of the shunt was introduced into the portal vein. A further

shunt was created between the common iliac vein and the internal jugular vein for standardization of the hemodynamic situation (fig. 1). Thereafter, the hepatic artery, the portal vein as well as the supra- and infrahepatic caval vein were selectively cross-clamped in order to achieve a total vascular occlusion of the liver. For the extracorporal circulation a Gott aneurysma shunt and a so-called biopump were used (Biomedicus, Minneapolis, Minnesota). The flow was 30 ml/min/kg body weight. Under these conditions the portal venous pressure was 2-5 mm Hg corresponding to physiological values in pigs. After an ischemic period of four hours the clamps as well as the shunt were removed. The abdomen and cervix were closed. The animals were extubated. Analgesia was performed with Piritramid 5 mg (Dipidolor®) whenever necessary. After a period of six days the animals were sacrificed using T61 ad us vet.® (0.2 g Embutramid, 0.05 g Mebezoniumjodid, 0.005 g Tetracainhydrochlorid / ml).

Blood and tissue sampling

Blood samples for serum analyses and wedge liver biopsies (0.5x0.5cm) for the measurement of TBARS as well as for histological and immunohistochemical evaluation were taken at defined time points. (Table 1) Serum values of different enzymes AST, ALT, GLDH) as well as liver function parameters (serum bilirubin, coagulation parameters) were measured.

	Preoperatively	Before ischemic periodOP	End of ischemic period	5 minutes after reperfusion	30 min after reperfusion	1 hour after reperfusion	3 hours after reperfusion	1 day postoperatively	3 days postoperatively	6 days postoperatively
AST	X		X	X	X	X	X	X	X	X
ALT	X		X	X	X	X	X	X	X	X
GlDH	X		X	X	X	X	X	X	X	X
Histology/ TBARS		X	X		X	X				X

Table 1: Time points of blood and tissue sampling.

Histology / Immunohistochemistry in liver tissue

Oxidized proteins

Protein oxidation is characterized by the insertion of additional carbonyl groups into the molecule. The molecule 2.4-dinitrophenylhydrazin (2.4-DNPH) is able to bind to protein bound carbonyl groups. 2.4-dinitrophenylhydrazon (DNPH) emerges and can be visualized with an anti-DNPH-antibody by indirect immunhistological staining.

Frozen sections of liver tissue (5µm) were fixed in a mixture of diethylether and ethanol (1:1) at room temperature. Incubation followed in 2.4-DNPH solution (300 mg DNPH in acid 95% ethanol over night at room temperature). Following the incubation the sections were washed in 95% acid ethanol and rehydrated in decreasing concentrations of alcohol. After this, ligands of possible cross reaction were blocked with sheep's serum (1:10). The incubation with a canine-anti-dinitrophenyl-antibody (1:200, DAKO) was performed at 4°C for two hours. Following this, the sections were incubated with a peroxidase conjugated sheep-anti-canine-antibody (1:100, Boehringer) for one hour at room temperature. After each

antibody incubation the sections were washed with Tris/NaCl buffer (pH=7.4). Aminoethylcarbazol (Sigma) in N,N-dimethylformamide and sodium acetate (0,1 m) served as substrate for the peroxidase (15 min). Finally, the sections were stained with Mayr's haemalaun (Merck). The negative control was not incubated with the primary antibody.

4-hydroxy-nonenal

4-hydroxynonenal (4-HNE) represents a metabolite developing as a consequence of lipid peroxidation and is regarded as a specific marker of oxidative stress.

Frozen sections (5µm) were fixed in cold acetone, washed with PBS-solution and incubated with sheep's serum. The sections were incubated with the primary monoclonal mouse 4-HNE-antibody 1g4h7 (1:50, Dr. Waeg, Institute for Biochemistry, University of Graz, Austria) for 1 hour at 37°C. Incubation with a peroxidase conjugated sheep anti-mouse-antibody followed (1:25, Boehringer) for 1 hour at room temperature. The sections were washed with PBS-solution (pH:7.4). Corresponding to the method described above aminoethylcarbazol served as substrate for the peroxidase. Finally, the sections were stained with Mayr's haemalaun. The negative control was not incubated with the primary antibody.

TBARS (Thiobarbituric acid reacting substances)

In addition to malondialdehyde (MDA), 4-HNE and other metabolites develop as a consequence of lipid peroxidation. These metabolites bind to thiobarbituric acid, and are therefore called thiobarburic acid reacting substances (TBARS). The products can be quantitatively measured by fluorescence spectrometry and are considered to correspond to the amounts of protein measured in the same tissue: µmol MDA equivalents /g protein.

20 frozen sections (20 µm) were incubated in 250 µl lyse buffer for two hours on ice. Lysis buffer consisted of 10 mM tris buffer (pH7.4), 0.9% Nonidet P-40 (Sigma) 0.1% Sodium Dodecyl Sulfate (Sigma) and benzonase (0.25 U/µl buffer, Sigma). Subsequently, the samples were centrifuged at 4°C for 5 minutes (10000xg). 40 µl and 200 µl were taken for measurement of protein concentration and for TBARS, respectively.

200 µl of the lysate or of MDA-standard was mixed with 10 µl hydroxytoluol in ethanol (0.227 M) and 200 µl ortho-phosphoric acid. Incubation with 25 µl thiobarbituric acid reagent (800 mg thiobarbituric acid in 50 ml 0.1 M NaOH) at 90°C for 45 minutes followed. TBA-adducts were extracted with 1-butanole and 50 µl saturated NaCl solution and centrifuged for 1 minute (12000 rounds/min). 200 µl of the above butanole phase was introduced in 96 well flat-bottom microtiter plate. TBARS emission was measured at 590 nm in a fluorescence-ELISA-reader (Titertek Fluoroscan II).

A commercially available colourimetric assay was used for the measurement of protein concentration (Bio-Rad DC Protein Assay, Bio-Rad).

Statistical analysis

The Wilcoxon-Mann-Whitney-U-test was used for statistical data analysis. Statistical significance was reached at values for $p<0.05$.

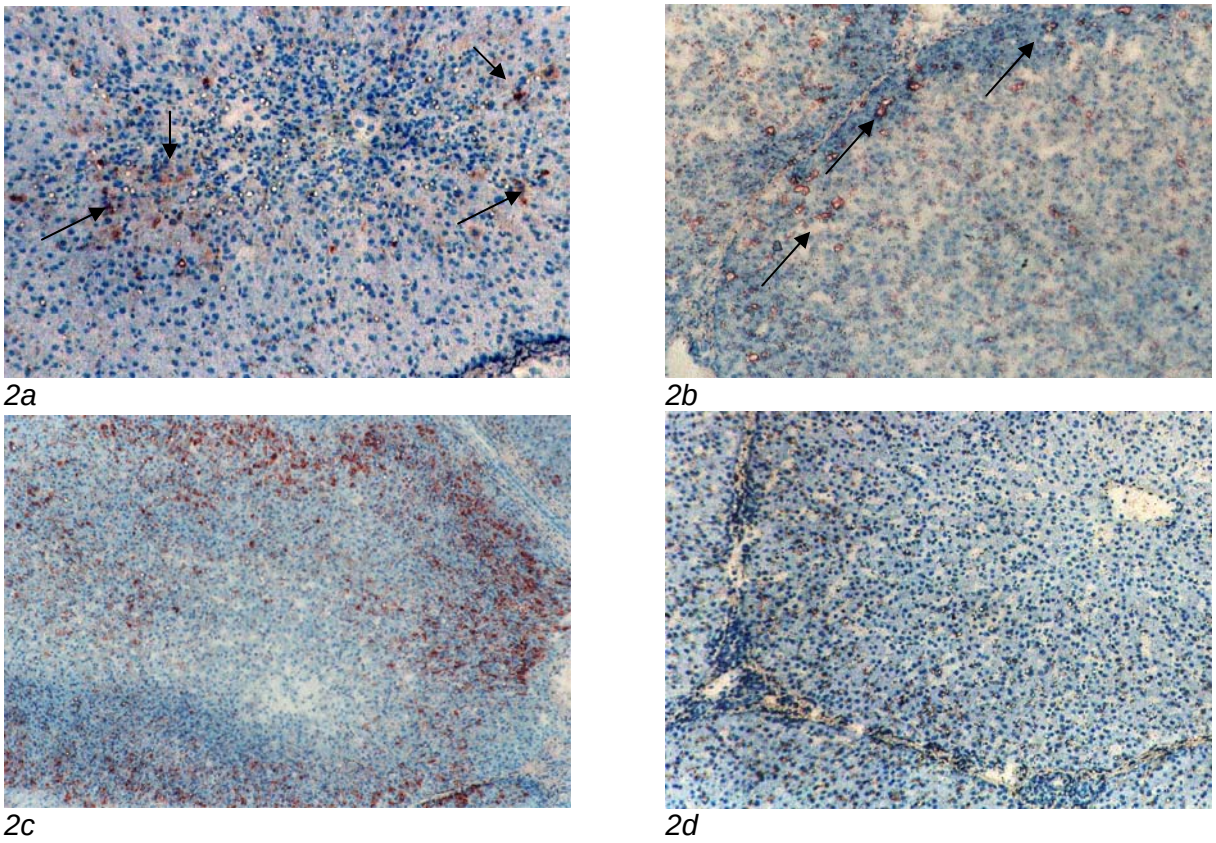
RESULTS

Immunohistochemistry

Oxidized proteins

Control group: An intracellular accumulation with periportal accentuation was seen at the preischemic time point with no change during the ischemic period. In the reperfusion period the intracellular accumulation increased predominantly confined to the central vein after 30 minutes (fig. 2a). After 60 minutes the accumulation increased with involvement of the periportal region as well. The maximum accumulation occurred after 6 days (fig. 2b).

Vitamin group: At the preischemic time point an extracellular accumulation was seen predominantly confined to the central vein. Intracellular accumulation of oxidized proteins was not observed. No change occurred during the ischemic period. 30 minutes after reperfusion, extracellular accumulation increased, and occasionally intracellular accumulation was observed, predominantly confined to the periportal region (fig. 2b). The maximum accumulation became visible 60 minutes after reperfusion with homogenous extracellular accumulation in the sinusoidal spaces. After six days only small extracellular periportal amounts of oxidized proteins were detectable. There was a marked difference compared to the controls after 6 days (fig. 2d)



2a *2b* *2c* *2d*

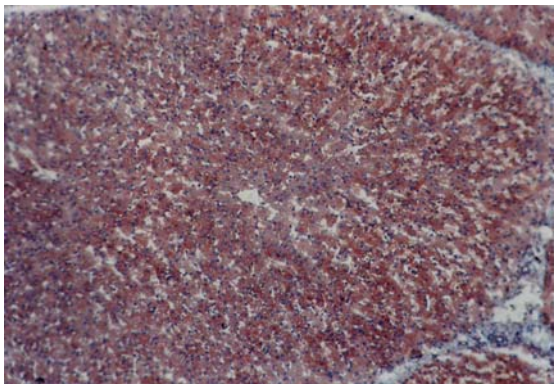
Fig. 2 a-d Vitamin group and controls. Oxidized proteins. Indirect peroxidase staining. Counter-staining with Mayer`s Hämalaun. **30 minutes after reperfusion:** In the control group an intracellular accumulation of oxidised proteins is visible predominantly in the central region *(2a, arrows, x100)*, whereas in the vitamin group oxidized proteins only are seen at the extracellular level in the periportal region *(2b, arrows, x100)*. **6 days postoperatively**: In controls oxidized proteins are markedly pronounced in the cytoplasm of hepatocytes predominantly in the periportal but also in the central region *(2c, x100)* In the vitamin group only small amounts of oxidized proteins are visible at the extracellular level in the periportal region *(2d, x100)*.

4-Hydroxynonenal

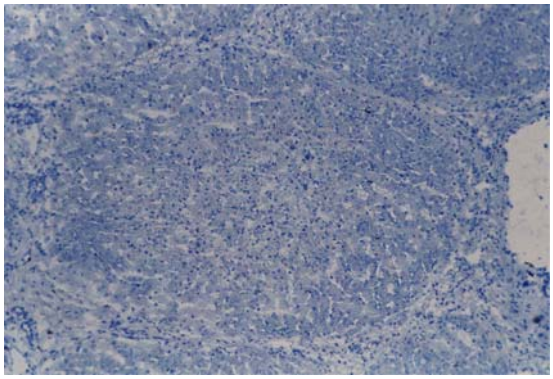
Control group: At the preischemic time point a slight periportal accumulation was seen. This increased during the ischemic period. Cytosolic granulations occurred in the periportal region. 30 minutes after reperfusion these granulations increased rapidly (fig. 3a) reaching a maximum after 60 minutes After 6 days the accumulation decreased, the granulations disappeared (fig. 3c).

Vitamin group: Before and after reperfusion no 4-HNE was detectable using the monoclonal antibody (fig. 3b). After 6 days

only very small amounts of 4-HNE were seen (fig. 3d).



3a



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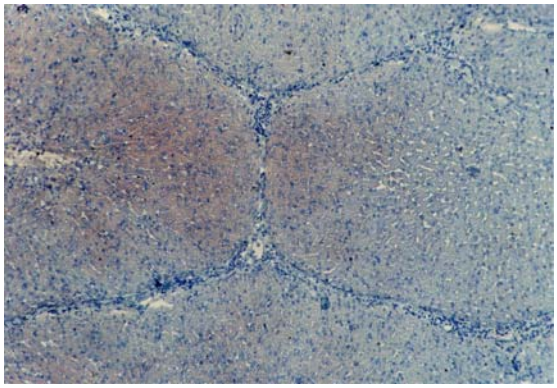
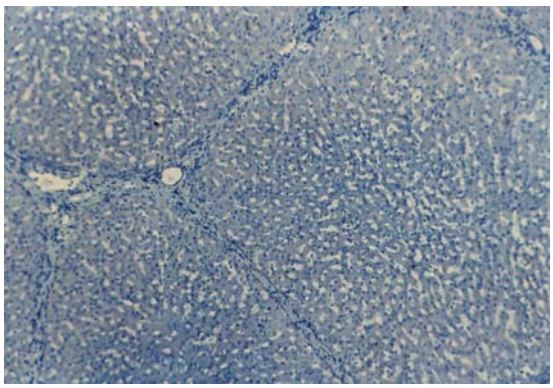


3c



3d

Fig. 3 a-d Vitamin group and controls. Indirect peroxidase staining of protein-bounded 4-HNE, counter-staining with Mayer`s Hämalaun. **30 minute after reperfusion:** In the control group an increasing accumulation of 4-HNE in hepatocytes occured. *(3a, x100)*. In contrast 4-HNE was not detected in the vitamin group *(3b, x100).* **6 days postoperatively:** Small amounts of 4-HNE were visible in the periportal region in the control group (3c, x100) with a clear difference to the vitamin group*. (3d, x100)*

TBARS

Compared to the vitamin group the control animals had higher TBARS concentrations in the liver tissue (fig. 4).

Enzymes

Preoperatively, at the end of the ischemic period and until 6 hours after reperfusion the vitamin group showed higher activities of AST and ALT than control animals (figs. 5,6). Between 3 and 6 hours after reperfusion the enzyme activity in controls nearly doubled, whereas in the vitamin group the activities showed only a slight increase (figs.5,6). From postoperative day 1 until postoperative day 6 the enzyme activities of AST and ALT were higher in the control animals (figs. 5,6). The serum activity of GLDH was higher until postoperative day 1 in the vitamin group (fig. 7). Between postoperative day 1 and 2 the serum activity of GLDH rapidly decreased in the vitamin group whereas the GLDH activity in controls continued to increase (fig.7). The GLDH activity in controls was five-fold higher at postoperative days 2 and 3 compared to the vitamin group (fig. 7).

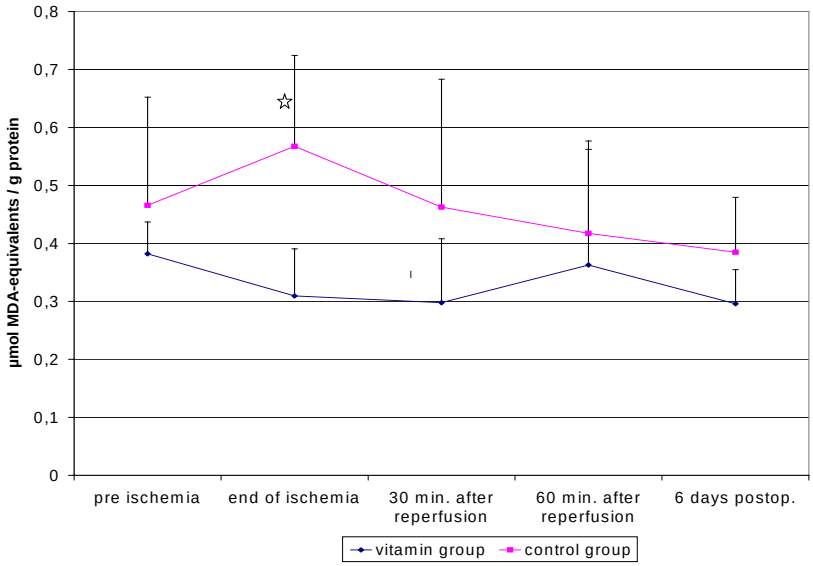


Fig. 4 TBARS in liver tissue. The controls had significantly higher TBARS at the end of the ischemic period. The vitamin group demonstrated lower mean TBARS values until the end of the experiment. * $p < 0,05$

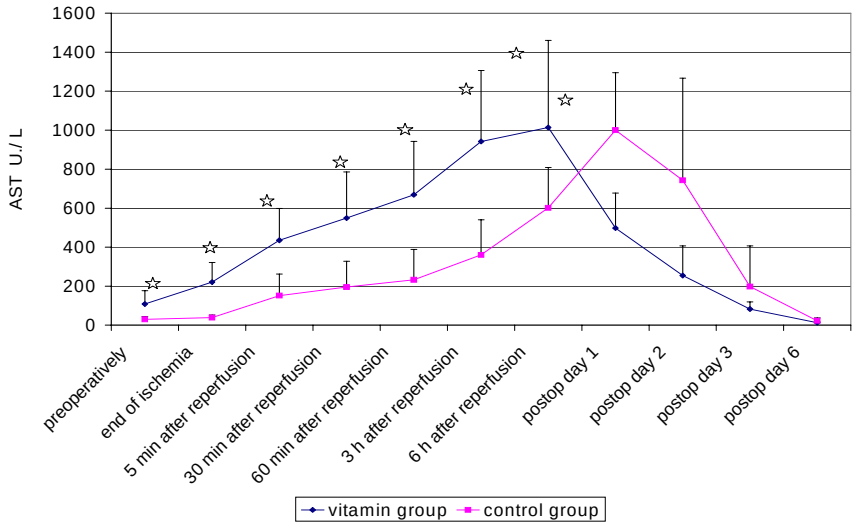


Fig. 5 Serum activity of AST. The vitamin group values returned to normal more rapidly. * $p < 0,05$

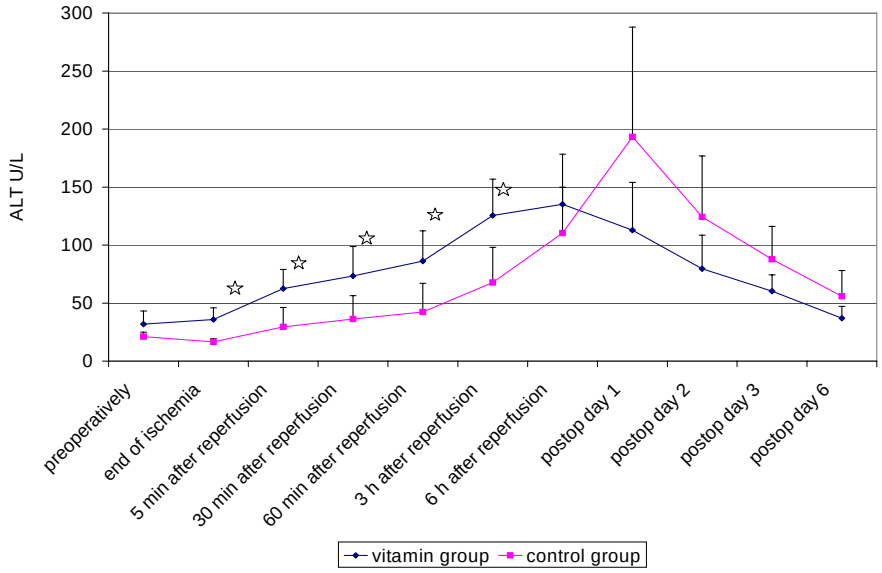


Fig. 6 Serum activity of ALT. The controls demonstrate a higher peak 6 hours after reperfusion. ALT levels are falling in the vitamin group, while in the control group, levels are rising. * $p < 0,05$

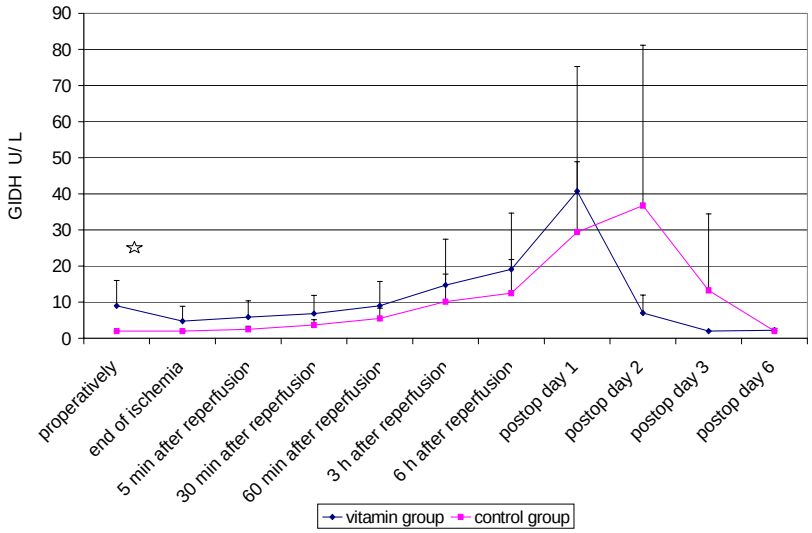


Fig. 7 Serum activity of GLDH. In the vitamin group levels of GLDH returned to normal more rapidly. Between postoperative day 1 and 2 the serum activity rapidly decreased in the vitamin group whereas the GLDH activity in controls continued to increase. * $p < 0,05$

No differences were seen concerning hemodynamic parameters, or liver function tests, e.g. serum bilirubin level and coagulation parameters.

DISCUSSION

In the present animal experimental study, we demonstrate the positive effects of an antioxidant vitamin solution on hepatic ischemia and reperfusion injury. These beneficial effects are shown through both histological methods and by liver enzyme levels. Histologically, peroxidation products were seen at the intracellular level in the control group whereas the vitamin group showed these products predominantly at the extracellular level. Due to the experimental design in our study using an extracorporal

bypass it was possible to investigate the hepatic I/R injury excluding the influence of hemostasis and disturbed circulation in the entire gastrointestinal tract. The results clearly demonstrate that oxidative stress is directly verifiable at the tissue level.

Less oxidative stress in animals treated with Antioxidative Vitamins

The cytotoxic effects of oxidative stress are caused by the peroxidative degradation of membrane lipids, which results in the accumulation of conjugated aldehydes. Among the most abundant and damaging of these is 4-hydroxynonenal (4-HNE). 4-HNE accumulation has been implicated in several chronic disorders including Alzheimer's and Parkinson's disease as well as alcoholic liver disease, preeclampsia and cancer (Uchida 2003, Sampey et al. 2003). 4-HNE therefore, also usefully serves as a specific biomarker for oxidative stress and may accumulate in concentrations of 10 μM – 5 mM in response to oxidative insult (Uchida 2001). Histological sections stained with 4-HNE lend a dramatic visual representation of the protective effects of antioxidative vitamins. Thirty minutes after reperfusion, accumulation of oxidized proteins is clearly present in the central vein of control animals, while at the same time in vitamin treated animals, oxidized proteins are found only in the periportal region. (fig. 2a,b). The most remarkable difference is visible by six days post-operatively. In control animals, numerous large blotches of oxidized proteins can be seen compared to none in the vitamin treated cohort (fig. 2 c,d). This is a clear visual representation of the protective effects of antioxidative vitamins in treated animals.

Liver Enzyme Levels Demonstrate Benefit of Antioxidative Vitamins

TBARS provides a measure of the amount of lipid peroxides formed in a sample of LDL cholesterol, or the amount of LDL oxidation. Since a loss of antioxidants is associated with organ failure, supplementation with antioxidant vitamins such as vitamin C and vitamin E, which can inhibit the oxidation of LDL cholesterol, should prove protective for oxidative stress. This is borne out in fig. 4, which demonstrates that animals that received infusions of antioxidant vitamins had significantly lower TBARS at the end of the ischemic period and that there was a clear protective or beneficial trend over the entire experiment in which antioxidant vitamins demonstrated lower mean TBARS values. Interestingly, in the control animals, the TBARS levels *increase* between the pre-ischemic and end of ischemia period while in the same period, in the experimental animals TBARS decrease.

Although both groups of animals had the same maximal rise of AST, around 1000 U/L, and despite the higher than normal values in the vitamin group, the experimental group values returned to normal sooner indicating a speedier recovery, and this recovery occurred at more rapid rate as compared to the control group (fig. 5). Furthermore, despite initial higher values in the experimental group, antioxidant treatment had a clear beneficial effect; in that serum activity of ALT (fig. 6) in control animals demonstrate a higher peak indicating a greater degree of liver damage. Six hours after reperfusion in the vitamin treated group, ALT levels are falling, indicating that cells are recovering, while in the control group, levels are rising, indicating that the injury is continuing. These tests taken together, show that vitamin treated animals were protected, as compared to control animals.

Serum levels of GLDH (fig. 7) demonstrate again, a rapid return to normal levels of GLDH in antioxidant treated animals in comparison to control animals between post-op day one and two. This indicates a restoration of functional recovery and reveals the beneficial effects of pre-ischemic antioxidant treatment.

The Solubility Problem

Preoperatively and in the first hours after reperfusion, serum activity of liver enzymes was higher in the vitamin treated group. The higher enzyme concentrations preoperatively until postoperative day 1 can be explained

through the administration of the emulsifier Solutol HS 15 which may be toxic to liver tissue. Although the non-ionic surfactant Solutol HS 15 is an excipient approved for use in parenteral formulations in humans, detailed studies on the biodistribution of Solutol HS 15 in the literature remain incomplete (Bravo Gonzelez et al. 2004). At the time this study was conceived, Solutol HS 15 was a recommended excipient for the solubilization of lipid vitamins such as α-tocopherol, furthermore, complications involving liver (toxic) effects of Solutol were not widely described. Significantly, according to a 2003 publication, Solutol HS 15 cannot be considered to be an "inert formulation ingredient". Rather, in clinical formulations, Solutol may increase the plasma exposure of a co-administered compound (increasing its toxicity) especially in regards to liver function (Bittner et al. 2003). The use of the novel soybean lipid emulsion in a pilot study in humans demonstrates that these negative effects can be avoided, such that a patient receiving the vitamin infusion experiences the beneficial effects of the antioxidants without the concurrent increase in liver enzyme levels observed in this study, presumably caused by the solvent (Bartels et al. 2004). Any future study, looking at the effects of combined or separate vitamin antioxidants should avoid use of surface active potentially liver-damaging excipients.

Several attempts to use Solutol HS 15 without supplemental vitamins in this extracorporal bypass experimental design as controls, resulted in marked side effects. This fact alone indicates the protective effects of antioxidative vitamins, nevertheless, concerns regarding side-effects lead us to carry out this experiment using untreated animals as controls.

Protective Spectrum of Antioxidative Vitamins

Oxidative stress is characterized by an imbalance of the antioxidant system with increased lipid peroxidation products and decreased antioxidants. This phenomenon can be observed in major surgery, and particularly in ischemia and reperfusion (I/R) injury which represents a major clinical problem (Muzokova et al. 2001., Ninimiya et al., 2004). The I/R injury may have deleterious effects on the clinical outcome of patients undergoing hepatic surgery or transplantation.

Substances that scavenge or remove hydroxyl radicals including MCI-186, Trolox, Tempol and edaravone are among the promising new agents that have been shown to have positive outcomes in ameliorating the effects of oxidative stress in ischemia/reperfusion models (Eum and Lee 2004, Sepodes et al., 2004, Abe et al., 2004, Cerwenka et al. 1999). However, antioxidant vitamins which are immediately available, relatively safe and inexpensive have not been thoroughly explored as regards to their protective benefits in this respect. Only limited data exist concerning the influence of high doses of antioxidant vitamins on I/R injury of the liver under normothermic conditions. A positive effect on postischemic liver functions was shown by the administration of a multivitamin infusion with minor doses of α-tocopherol and ascorbate (Rabl et al., 1995). Additionally, several experimental studies revealed a positive impact on I/R injury by antioxidant vitamins (Rhoden et al., 2001, Yoshimura et al., 2002, Wijnen et al., 2002). Specifically, vitamin C was found to be protective for liver injury in the rat in lower doses (Seo and Lee 2002). Similar effects were seen for dehydoascorbate, for α-tocopherol and its combination with gadolinium chloride (de Tata et al., 2005, Lee and Lee 2005, Giakoustidis et al. 2006). Furthermore, antioxidant vitamins E and C in critically ill surgical patients reduce the incidence of organ failure and the length of stay in ICU (Nathens et al. 2002). These results support our placebo controlled double blind pilot study (Bartels et al. 2004) in which patients undergoing partial liver resection were administered infusions containing vitamin E one day before surgery. This study is the first demonstration of the beneficial effects of pre-operative vitamin E administration for patients. We describe

patient benefits including a significantly shorter intensive care-unit stay, and a swifter return to normal levels of ALT and AST levels. The trend towards more rapid recovery is similar in both our human and swine studies.

The results from these studies lay a positive foundation on which to further explore the protective effects of antioxidant vitamins on ischemia and reperfusion. Furthermore, the availability of a non-liver-toxic excipient for lipid soluble antioxidative vitamins will support future clinical trials as well as animal based experiments exploring the optimization of the combination of antioxidative vitamins for the maximum protection from ischemia-reperfusion injury.

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