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Decreased Antioxidant Defense Mechanisms in Rat Liver after Methanol Intoxication

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The primary metabolic fate of methanol is oxidation to formaldehyde and then to formate by enzymes of the liver. Cytochrome P-450 and a role for the hydroxyl radical have been implicated in this process. The aim of the paper was to study the liver antioxidant defense system in methanol intoxication, in doses of 1.5, 3.0 and 6.0 g/kg b.w., after methanol administration to rats. In liver homogenates, the activities of Cu,Zn-superoxide dismutase and catalase were significantly increased after 6 h following methanol ingestion in doses of 3.0 and 6.0 g/kg b.w. and persisted up to 2–5 days, accompanied by significant decrease of glutathione reductase and glutathione peroxidase activities. The content of GSH was significantly decreased during 6 hours to 5 days. The liver ascorbate level was significantly diminished, too, while MDA levels were considerably increased after 1.5, 3.0 and 6.0 g/kg b.w. methanol intoxication. Changes due to methanol ingestion may indicate impaired antioxidant defense mechanisms in the liver tissue.

Keywords: Methanol intoxication, antioxidant enzymes, ascorbate, GSH, lipid peroxidation, rat liver

INTRODUCTION

The main pathway for methanol degradation involves hepatic cytosolic alcohol dehydrogenase, the microsomal oxidizing system and catalase, which catalyses the conversion of methanol to formaldehyde. Subsequently aldehyde dehydrogenases and oxidases, including xanthine oxidase, metabolize formaldehyde to formate. These processes are accompanied by formation of superoxide anion that may be involved in lipid peroxidation.[1]

Methanol intoxication is associated with mitochondrial damage, especially with regard to the respiratory chain; partial inhibition may cause increased autoxidation of a redox carrier, resulting in increased production of oxygen radicals.[2,3] Increased microsomal proliferation with elevated NADPH-cytochrome P-450 reductase activity could yield increased superoxide anion and H_2O_2. Moreover, oxidation of methanol can be followed by released leukotrienes, resulting in PMN infiltration of the liver, presumably associated with an oxidative burst and production of reactive oxygen mediators. These factors with the excess of aldehydes formed during acute methanol intoxication are of importance in lipid peroxidation, too. Products of this process are

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harmful to the liver cell.[4] The toxicity of methanol after oral intake is well documented, and accidental intoxication in human with this compound still take place.

The aim of this work is to evaluate the antioxidant defense status of the liver consisting of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione reductase (GSSG-R) and glutathione (GSH) and ascorbate concentration in methanol intoxication in doses of 1.5, 3.0 and 6.0 g/kg b.w. in rats. In addition, total antioxidant status (TAS) and the level of MDA, as a major index of lipid peroxidation were also measured.

MATERIALS AND METHODS

Chemicals

Superoxide dismutase (SOD) from bovine erythrocytes, catalase (CAT), glutathione reductase (GSSG-R), epinephrine, nicotinamide adenine dinucleotide phosphate reduced sodium salt (NADPH), reduced and oxidized glutathione, natrium wolframate and butylated hydroxytoluene (BHT)(Sigma Chemical Co., St. Louis Mo, USA); GSH-400 test (Bioxytech S.A., France); total antioxidant status kit (RANDOX Laboratories, Aldmore, UK). All other chemicals of the highest quality were from POCH (Gliwice, Poland). Deionized water was used throughout.

Animals

Young male Wistar rats (230–250 g body wt) fed on a standard diet were used. All procedures were in strict accordance with the guide lines for the care and use of laboratory animals and were approved by the local Animal Care Committee. Animals were given (by the intragastric route) 50% methanol (sixty each received 1.5, 3.0 or 6.0 g/kg b.w.) in isotonic NaCl solution by syringe through a plastic tube. These quantities of methanol are used in the experimental acute methanol intoxication.[5] An equivalent volume of saline was given to ten control rats. The rats intoxicated with one dose of methanol were divided into six groups (ten rats in each).

Tissue Preparation

6, 12, 24 h and 2, 5 and 7 days after alcohol administration the animals were sacrified under ether anaesthesia, then livers were removed quickly and placed in iced 0, 15 M NaCl, perfused with the same solution in order to remove the blood cells, then blotted on filter paper, weighed and homogenized in 9 vol of ice-cold 0.25M sucrose and 0.15 M NaCl with addition of 6 µl of 250 mM BHT in ethanol to prevent formation of peroxides during the assay. The homogenization procedure was performed under standardized conditions. 10% Homogenates were centrifuged at 10,000 × g for 15 min at 4°C, and the supernatant was kept on ice until was assayed.

Biochemical Analyses

Cu,Zn-SOD activity (EC.1.15.1.1) was assessed by the method of Misra and Fridovich[6] as modified by Sykes *et al.*[7] which measures the activity of cell cytosolic SOD. Manganese SOD of the mitochondria is known to be removed during the procedure. One unit of SOD activity was defined as the amount of the enzyme required to inhibit the oxidation of epinephrine to adrenochrome by 50% during 1 min and the activity is expressed in U per mg of protein.

CAT activity (EC.1.11.1.6) was measured in the initial supernatant after adding Triton X-100, a 30 min preincubation and centrifugation at 9,000 g for 30 min at 4°C. The activity was assayed as described by Aebi.[8] Rates were determined at 25°C using 10 mM hydrogen peroxide, and one unit of activity is defined as the amount of the enzyme catalysing the decomposed of 1 µmole of H_2O_2/min and the activity expressed in U per mg of protein.

GSH-Px activity (EC.1.11.1.9) was measured spectrophotometrically using a technique described by Paglia and Valentine,[10] wherein GSSG formation is assayed by measurement of conversion of NADPH to $NADP^+$. One unit of activity is defined as the amount of the enzyme catalysing the conversion of 1 µmol of NADPH/min and the activity is expressed in U per mg of protein at 25°C at pH 7.4.

GSSG-R activity (EC.1.6.4.2) was determined using the method of Mize and Langdon.[9] The reaction was initiated with the addition of NADPH to a final concentration of 0.1 mM. One unit of GSSG-R oxidizes 1 nmol of NADPH/min and the activity is expressed in U per mg of protein at 25°C at pH 7.0

Glutathione (GSH) was measured using Bioxytech GSH-400 test. Ascorbate was measured according to Kyaw.[11]

Total antioxidant status (TAS) was measured with ABTS reagent (2,2'-azino-di-[3-ethylbenzthiazoline sulphonate]) that is incubated with a peroxidase (metmyoglobin) and H_2O_2 to produce the radical cation $ABTS^+$, measured spectrophotometrically at 660 nm. Antioxidants in the added sample cause suppression of the colour production. The total antioxidant capacity concentration is compared to equivalent antioxidant capacity of Trolox and is expressed in µmoles of Trolox/g of tissue. This method was elaborated by Miller *et al.*[12] Lipid peroxidation products was measured as a MDA by HPLC according to Esterbauer *et al.*[13] Protein was determined in diluted aliquots of the tissue homogenates by the method of Lowry *et al.*[14] The diagnostic Cormway test was used for assessment of blood serum aspartate aminotransferase (AST) activity.

The results were expressed as means ± S.D (n = 6). Data analysis was performed with standard statistical methods (analysis of variance ANOVA) and values less than 0.05 were considered significant.

RESULTS

Table I shows the antioxidant parameters of the liver after methanol intoxication at a dose of 1.5 g/kg b.w. The activity of Cu,Zn-SOD was not changed during the 7 days of observation, while the activity of CAT was significantly increased after 6 and 12 hours and then returned to normal values. GSH-Px and GSSG-R activities were significantly decreased after 6 to 24 hours. The GSH level was significantly lowered after 12 hours to 2 days. The level of the ascorbate was also diminished after 12 hours and then restored to normal values. Total antioxidant status was lowered from 12 h to 5 days, while the level of MDA increased considerably.

After methanol intoxication at 3.0 g/kg b.w. the activity of Cu,Zn-SOD and CAT was increased from 6 h to 2 days and then restored to normal (Table II). GSH-Px and GSSG-R activities were

TABLE I Anioxidant Parameters in the Liver from Control Rats and Animals Treated with Methanol (1.5 g/kg b.w.)

Parameters analysed	Intoxication time						
	control	6h	12h	24h	2 days	5 days	7 days
Cu,Zn-SOD U/mg of protein	12.0 ± 0.5	12.4 ± 0.7	12.3 ± 0.8	11.9 ± 0.7	11.9 ± 0.7	11.8 ± 0.7	11.6 ± 0.6
CAT U/mg of protein	232 ± 16	275 ± 15*	260 ± 23*	231 ± 22	233 ± 19	230 ± 18	226 ± 17
GSH-Px, U/mg of protein	133 ± 11	133 ± 12	122 ± 12	103 ± 10*	114 ± 12*	122 ± 11	131 ± 6
GSSG-R, U/mg of protein	38.3 ± 3.1	33.7 ± 3.6*	32.4 ± 3.4*	29.0 ± 3.4*	35.8 ± 3.4	37.4 ± 3.2	38.1 ± 3.1
GSH, µmol/g of tissue	4.37 ± 0.23	4.07 ± 0.35	3.80 ± 0.32*	3.62 ± 0.30*	4.04 ± 0.27*	4.21 ± 0.25	4.33 ± 0.24
Ascorbate, µg/g of-tissue	81.0 ± 4.7	79.0 ± 6.9	71.6 ± 7.5*	76.3 ± 6.1	79.0 ± 6.2	79.7 ± 5.4	80.4 ± 6.3
TAS, µmol/g of tissue	117.9 ± 7.7	112.9 ± 8.0	108.1 ± 7.1*	99.1 ± 7.2*	91.1 ± 7.1*	100.5 ± 6.8*	114.9 ± 6.6
MDA, nmol/g of tissue	62.3 ± 3.8	66.9 ± 4.3	69.8 ± 4.7*	72.0 ± 4.7*	70.9 ± 4.5*	67.4 ± 4.2	61.9 ± 4.0

* Significantly different from control value ($p < 0.05$).

TABLE II Antioxidant Parameters in the Liver from Control Rats and Animals Treated with Methanol (3g/kg b.w.)

Parameters analysed	Intoxication time						
	control	6h	12h	24h	2 days	5 days	7 days,
Cu,Zn-SOD U/mg of protein	12.0 ± 0.5	12.6 ± 0.7	13.2 ± 0.7*	13.0 ± 0.6*	12.3 ± 0.7	11.8 ± 0.5	11.9 ± 0.6
CAT U/mg of protein	232 ± 16	297 ± 15*	311 ± 20*	290 ± 19*	256 ± 18*	234 ± 19	230 ± 15
GSH-Px, U/mg of protein	133 ± 11	128 ± 14	115 ± 12*	92 ± 8*	93 ± 10*	112 ± 12*	128 ± 9
GSSG-R, U/mg of protein	38.3 ± 3.1	32.7 ± 3.7*	26.9 ± 2.8*	20.9 ± 2.4*	22.4 ± 3.0*	28.8 ± 3.3*	37.1 ± 3.2
GSH, µmol/g of tissue	4.37 ± 0.25	3.80 ± 0.27*	3.24 ± 0.28*	3.35 ± 0.25*	3.53 ± 0.23*	4.02 ± 0.24*	4.32 ± 0.25
Ascorbate, µg/g of tissue	81.0 ± 4.7	80.8 ± 6.0	75.1 ± 7.0*	72.2 ± 6.4*	72.3 ± 6.0*	74.2 ± 6.6	78.6 ± 6.0
TAS, µmol/g of tissue	117.9 ± 7.7	105.6 ± 7.9*	100.1 ± 7.3*	84.7 ± 6.8*	63.7 ± 5.9*	88.7 ± 5.2*	106.8 ± 7.3*
MDA, nmol/g of tissue	62.3 ± 3.8	66.7 ± 4.1	71.4 ± 4.5*	79.8 ± 4.7*	82.6 ± 4.9*	73.4 ± 4.2*	63.9 ± 4.0

* Significantly different from control value ($p < 0.05$).

significantly decreased during 5 days after intoxication. GSH level was reduced at 12 h to 5 days after methanol ingestion. The concentration of ascorbate was also diminished significantly after 24 h and 2 days of intoxication. Total antioxidant status of the liver was diminished for all the time after methanol intoxication. The concentration of liver MDA was increased during the 7 days of methanol intoxication.

As would be expected, the most pronounced changes in the liver antioxidant parameters were observed after methanol intoxication at 6.0 g/kg b.w. Table III shows that activity of SOD and CAT was significantly increased after 6 h following methanol ingestion and maintained through 2–5 days after intoxication. At the same time GSH-Px and GSSG-R activities were significantly decreased. The liver GSH level was significantly lowered after 6 h to 5 days following acute methanol ingestion, while the concentration of ascorbate was also diminished significantly from 24 h to 5 days. Total antioxidant status was significantly diminished throughout. An significant increase in hepatic MDA was noted in the intoxicated rats.

The AST activities, as marker of liver damage were moderately increased after 1,5 3.0 and 6.0 g/kg b.w. methanol intoxication (Fig. 1).

DISCUSSION

In rats, the catalase-peroxidase system is considered to be responsible for oxidizing methanol to formaldehyde.[15] The latter is then oxidized by formaldehyde dehydrogenase, an enzyme that

TABLE III Antioxidant Parameters in the Liver from Control Rats and Animals Treated with Methanol (6g/kg b.w.)

Parameters analysed	Intoxication time						
	control	6h	12h	24h	2 days	5 days	7days
Cu,Zn-SOD U/mg of protein	12.0 ± 0.5	14.4 ± 0.6*	13.8 ± 0.6*	13.2 ± 0.5*	12.3 ± 0.5	12.0 ± 0.5	12.0 ± 0.5
CAT U/mg of protein	232 ± 16	324 ± 14*	320 ± 16*	311 ± 13*	283 ± 13*	260 ± 14*	235 ± 14
GSH-Px, U/mg of protein	133 ± 11	119 ± 11*	85 ± 13*	74 ± 10*	88 ± 8*	108 ± 9*	117 ± 7*
GSSG-R, U/mg of protein	38.3 ± 3.1	28.0 ± 3.3*	24.2 ± 3.2*	17.1 ± 2.3*	22.0 ± 2.6*	33.1 ± 2.9*	34.8 ± 2.7*
GSH, µmol/g of tissue	4.37 ± 0.25	3.61 ± 0.29*	3.02 ± 0.28*	2.83 ± 0.27*	3.21 ± 0.24*	3.68 ± 0.26*	4.02 ± 0.24*
Ascorbate, µg/g of tissue	81.0 ± 4.7	80.6 ± 4.6	78.2 ± 5.0	71.1 ± 4.8*	70.5 ± 4.6*	73.5 ± 4.3*	78.9 ± 4.7
TAS, µmol/g of tissue	117.9 ± 7.7	99.1 ± 8.0*	91.5 ± 7.9*	77.8 ± 7.7*	58.3 ± 6.2*	77.4 ± 6.6*	97.8 ± 7.0*
MDA, nmol/g of tissue	62.3 ± 3.8	71.0 ± 4.4*	79.9 ± 4.8*	88.0 ± 5.3*	89.8 ± 5.2*	83.4 ± 5.3*	73.4 ± 4.8*

* Significantly different from control value ($p < 0.05$).

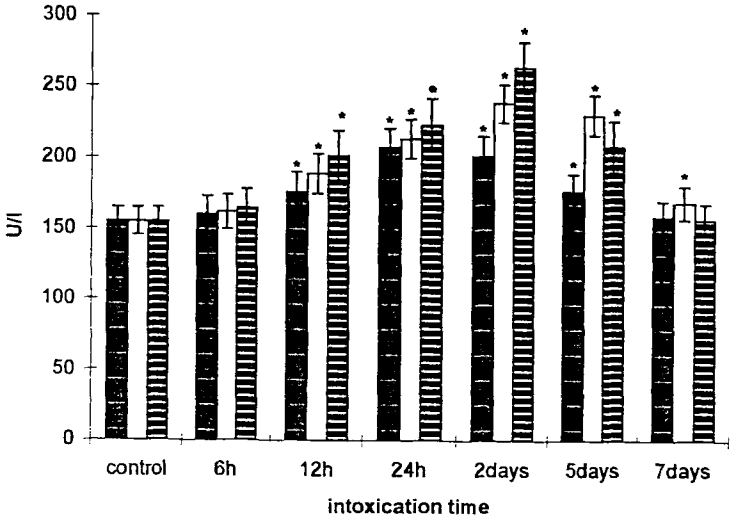

FIGURE 1 Serum aspartate aminotransferase (AST) activities in control rats and animals treated with different doses of methanol, ■ 1.5, □ 3.0, and ⊟ 6.0g/kg b.w. * Significantly different from control value ($p < 0.05$).

requires glutathione as a cofactor.[16] Other dehydrogenases play also a role in oxidation of aldehydes in the liver cytosol and mitochondria.[2] This process involves the augmentation of NADH and leads to manifesting oxidase activity, including xanthine oxidase, which is principal source of superoxide anion formation. When the amount of superoxide produced overwhelms the SOD/catalase system, the superoxide can damage cell membranes, DNA and cell proteins.[17,18] The superoxide can cross membranes via anion channels and may assume a protonated form, the peroxyl radical, in a more acid environment. The peroxyl radical is more reactive than the superoxide and is particularly dangerous to membrane proteins and lipids. A high concentration of lipid peroxides is toxic to cells.[19]

The damage to liver cells is confirmed by histological examination of liver specimens, showing diffuse microvascular fatty infiltration with swollen hepatocytes. After application of 6.0 g of methanol/kg b.w. these changes were more intense (unpublished data).

Production of H_2O_2 in methanol-induced liver injury results from increased dismutation of superoxide. The removal of H_2O_2 is mainly performed by CAT. The liver of rats after methanol ingestion shows increased CAT activity. The mechanism of increasing CAT and Cu,Zn-SOD activities is not understood. Oxidants such as hydrogen peroxide activate gene expression through the antioxidant responsive element.[20]

GSH-Px and GSSG-R were diminished in the liver of rats treated with methanol. Formaldehyde readily reacts with the alpha-amino, epsilon-amino, hydroxyl, sulfhydryl, guanidyl, imidazole and amide groups of proteins,[21,22] leading to hydroxymethyl derivatives and intra- and intermolecular methylene bridges in proteins. Superoxide anions and hydroxyl radicals formed during formaldehyde oxidation to formate can oxidatively modify amino acids residues of proteins, aromatic and sulfhydryl amino acids.[23] Oxygen radicals can also cause formation of the protein peroxides.[24] These changes may result in denaturation, aggregation and fragmentation of protein,[25] altering physico-chemical properties[25,26] and potentially losing in enzymes activity.[27,28] Lipid peroxidation products, such as malondialdehyde and 4-hydroxynonenal can also modify proteins,[2] e.g. cathepsin B.[29]

Changes in enzymatic activities are different for methanol with ethanol intoxication. During acute ethanol intoxication in rats a decrease in SOD, CAT, GSH-Px and GSSG-R activities takes place[30] while methanol ingestion causes significant loss only in GSH-Px and GSSG-R activities. In addition to their involvement in oxidative metabolism, depression of liver GSH and or SH groups are observed, enhancing the susceptibility to peroxidative injury.[31] Fifty to eighty percent of endogenous formaldehyde occurs as adduct with glutathione.[32] Glutathione is the cofactor of formaldehyde dehydrogenase and is responsible for formaldehyde metabolism.[33] A decrease in the glutathione level reduces the formaldehyde metabolism, thereby increasing its toxicity. GSH plays a major role in the cellular defense against endogenous and exogenous oxidant.[34]

The combined deficiency of intracellular GSH and GSH-Px may potentiate methanol toxicity by simultaneously increased oxidative stress, while decreasing oxidative defenses. The hydroxyl, superoxide, and peroxyl radicals and hydrogen peroxide in anaerobic conditions can transform formate ions into further radicals, which, in turn, react with proteins and finally lead to membrane lipid peroxidation and to non-protein and protein sulfhydryl compounds oxidation.[35,36]

The effect of ascorbate is complex and the relative contribution of antioxidant and prooxidant effects may depend on a number of factors, including transition metal status.[37,38] Ascorbate restores the antioxidant properties of fat-soluble α-tocopherol. Therefore, it interrupts the radical chain reaction of lipid peroxidation. The decrease of ascorbate concentration observed in our study can diminish the cellular resistance of liver cells to oxidative damage. This phenomenon is associated with parallel increase in lipid peroxidation products. These data are in agreement with results of other authors on the interrelationship between ascorbate and lipid peroxidation products.[39]

Our results suggest that intoxication in rats with one dose of methanol leads to some impairment of the antioxidant defense system in the liver. In conclusion, we hypothesized that oxidative stress induced by metabolism of methanol in the liver creates changes in the liver leading to the impairment of some enzymatic and nonenzymatic antioxidant defense system. During these processes it comes to the lipid peroxidation causing attenuation of membrane functioning, decreased fluidity and increased nonspecific permeability to ions.

References

[1] Poli, G. (1993). Liver damage due to free radicals. *British Medical Bulletin*, **49**, 604–609.

[2] Liesivuori, J. and Savolainen, H. (1991). Methanol and formic acid toxicity: biochemical mechanisms. *Pharmacology* and Toxicology, **69**, 157–163.

[3] Tephly, T. R. (1991). The toxicity of methanol. *Life Science*, **48**, 1031–1041.

[4] Esterbauer, H., Gebicki, J., Puhl, H. and Jürgens, G. (1992). The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radical Biology and* Medicine, **13**, 341–390.

[5] McMartin, K. E., Makar, A. B., Martin-Amat, G., Palese, M. and Tephly, T. R. (1975). Methanol poisoning. I. The role of formic acid in the development of metabolic acidosis in the monkey and the reversal by 4-methylpyrazole. *Biochemical Medicine*, **13**, 319–333.

[6] Misra, H. P. and Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutation. *Journal of Biological Chemistry*, **247**, 3170–3174.

[7] Sykes, J. A., McCormac, F. X. and O'Brien, T. J. (1978). Preliminary study of the superoxide dismutase content of some human tumors. *Cancer Research*, **38**, 2759–2762.

[8] Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology*, **105**, 121–126.

[9] Mize, C. E. and Langdon, R. G. (1962). Hepatic glutathione reductase. I. Purification and general kinetic properties. *Journal of Biological Chemistry*, **237**, 1589–1595.

[10] Paglia, D. E. and Valentine, W. N. (1967). Studies on the quqntitative and qualitative characterization of erythrocyte glutathione peroxidase. *Journal of Laboratory and Clinical Medicine*, **70**, 158–169.

[11] Kyaw, A. (1978). A simple colorimetric method for ascorbic acid determination in blood plasma. *Clinica Chimica Acta*, **86**, 153–157.

[12] Miller, N. J., Rice-Evans, C., Davies, M. J., Gopinathan, V. and Milner, A. (1993). A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clinical Science*, **84**, 407–412.

[13] Esterbauer, H., Lang, J., Zadravec, S. and Slater, T. F. (1984). Detecion of malonaldehyde by high-performance liquid chromatography. *Methods Enzymology*, **105**, 319–328.

[14] Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J. (1951). Protein measurement with Folin phenol reagent. *Journal of Biological Chemistry*, **193**, 265–275.

[15] Heck, H. D'A., Casanova, M. and Starr, T. B. (1990). Formaldehyde toxicity—new understanding. *Critical Reviews in Toxicology*, **20**, 397–426.
[16] Koivula, T. and Koivusalo, M. (1975). Different forms of rat liver aldehyde dehydrogenase and their subcellular distribution. *Biochimica et Biophysica Acta*, **397**, 9–23.
[17] Blum, J. and Fridovich, I. (1985). Inactivation of glutathione peroxidase by superoxide radical. *Archives of Biochemistry and Biophysics*, **240**, 500–508.
[18] Davies, M. J., Fu, S. and Dean, R. T. (1995). Protein hydroperoxides can give rise to reactive free radicals. *Biochemical Journal*, **305**, 643–649.
[19] Weglicki, W. B., Dickens, B. F. and Tong Mak, I. (1984). Enhanced lysosomal phospholipid degradation and lysophospholipid production due to free radicals. *Biochemical and Biophysical Research Communications*, **124**, 229–235.
[20] Rushmore, T. H., Morton, M. R. and Pickett, C. B. (1991). The antioxidant response element. *Journal of Biological Chemistry*, **266**, 11632–11639.
[21] Skrzydlewska, E. (1994). Interaction of formaldehyde with amino acids, peptides and proteins. *Polish Journal of Environmental Studies*, **2**, 13–20.
[22] Tome, L., Rusnak, L., Tyihak, E., Szarvas T. and Szende B. (1983). Spontanous N-methylation and N-formylation reaction between L-lysine and formaldehyde inhibited by ascorbic acid. *Biochemical Journal*, **25**, 258–292.
[23] Kleinveld, H. A., Swaak, A. J. G., Hack, C. E. and Koster, J. F. (1989). Interactions between oxygen free radicals and proteins. *Scandinavian Journal of Rheumatology*, **18**, 341–352.
[24] Gebicki, S. and Gebicki, J. M. (1993). Formation of peroxides in amino acids proteins exposed to oxygen free radicals. *Biochemical Journal*, **289**, 743–749.
[25] Davies, K. J. A. (1987). Protein damage and degradation by oxygen radicals. I. General aspects. *Journal of Biological Chemistry*, **262**, 9895–9901.
[26] Galembeck, F., Ryan, J. R. and Feeney, R. E. (1977). Reaction of proteins with formaldehyde in the presence and absence of sodium borohydride. *Journal of Agricultural of Food Chemistry*, **25**, 238–245.
[27] Jentoft, J. E., Jentoft, N., Gerken, T. A. and Dearborn, D. G. (1979). ^{13}C NMR studies of ribonuclease A methylated with [^{13}C]formaldehyde. *Journal of Biological Chemistry*, **254**, 4366–4370.
[28] Martin, C. J. and Oza, N. B. (1972). Formaldehyde as an active site label of α-chymotrypsin. *Canadian Journal of Biology*, **50**, 1114–1121.
[29] Hoppe, G., O'Neil, J., Hoff, H. F. (1994). Inactivation of lysosomal proteases by oxidized low density lipoprotein is partially responsible for its poor degradation by mouse peritoneal macrophages. *Journal of Clinical Invetigation*, **94**, 1506–1512.
[30] Farbiszewski, R., Holownia, A. and Chwiecko, M. (1987). The changes in sulhydryl compounds in plasma, liver and brain after acute and chronic ethanol administration in rats. *Drug and Alcohol Dependence*, **20**, 129–134.
[31] Lieber, C. S. and Savolainen, M. (1984). Ethanol and lipids. Alcoholism (NY). **8**, 409–423.
[32] Heck, H. D'A., White, E. L., Casanova-Schmitz, M. (1982). Determination of formaldehyde in biological tissue by gas chromatography/mass spectrometry. *Biomedical Mass Spectrometry*, **9**, 347–353.
[33] Heck, H. D'A., Casanova, M., Starr, T. B. Formaldehyde toxicity—new understanding. *Critical Reviews in Toxicology*, **20**, 397–426.
[34] Meister, A. (1983). Selective modification of glutathione metabolism. *Science*, **220**, 471–477.
[35] Fridovich, I. and Freeman, B. (1986). Antioxidant defenses in the lung. *Annual Review of Physiology*, **48**, 693–702.
[36] Simpson, J. A., Gieses, S. P. and Dean, R. T. (1993). Free radical and enzymatic mechanisms for the generation of protein bound reducing moieties. *Biochimica et Biophysica Acta*, **1156**, 190–196.
[37] Minotti, G. and Aust, S. I. (1987). The requirement for iron (III) in the initiation of lipid peroxidation by iron (II) and hydrogen peroxide. *Journal of Biological Chemistry*, **262**, 1098–1104.
[38] Young, I. S., Torney, J. J. and Trimble, E. R. (1992). The effect of ascorbate supplementation on oxidative stress in the streptozotocin diabetic rat. *Free Radical Biology and Medicine*, **13**, 41–46.
[39] Cadenas, S., Rojas, C., Perez-Campo, R., Lopez-Torres, M. and Barja, G. (1994). Effect of dietary vitamin C and catalase inhibition on antioxidants and molecular markers of oxidative damage in guinea pigs. *Free Radical Research*, **21**, 109–118.