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INFLUENCES OF *GINKGO BILOBA* ON CYCLOSPORIN A INDUCED LIPID PEROXIDATION IN HUMAN LIVER MICROSOMES IN COMPARISON TO VITAMIN E, GLUTATHIONE AND *N*-ACETYLCYSTEINE

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Abstract—The *in vitro* effect of cyclosporin A (CsA) on lipid peroxidation in human liver microsomes was investigated, and efforts were made to prevent the resulting toxic effect of CsA. Microsomes were prepared from human liver resection material and incubated with CsA (0, 10, 30, 100, 300, 1000 μg/mL) for one hour (pH 7.4, 37°, 95% O_2, 5% CO_2). Subsequently the resulting concentrations of malondialdehyde equivalents (MDA) were determined, a breakdown product of lipid peroxidation. Furthermore the duration of incubation was varied (0, 15, 30, 60, 90 min) using a CsA concentration of 300 μg/mL. CsA was shown to stimulate MDA-formation to up to 10-fold of the control value in both a time and concentration dependent manner. The dosage dependent experiment stated above was repeated, adding α-tocopherol (vitamin E, 1 mM), reduced glutathione (GSH, 1 mM), *N*-acetylcysteine (0.1, 0.3, 1, 3 mM), and *Ginkgo biloba* extract (Gbe, 15, 50, 150 μg/mL), respectively, to the medium of incubation. Vitamin E, a potent radical scavenger, proved to inhibit lipid peroxidation almost totally. Both GSH and *N*-acetylcysteine were also able to prevent lipid peroxidation, suggesting that the antioxidant effect of GSH might be caused by its thiol group and does not depend on the integrity of the whole molecule. Gbe inhibited CsA induced lipid peroxidation in a concentration dependent manner. This effect of Gbe was diminished yet not totally abolished when $FeCl_3$ was added to the medium of incubation, whereas *N*-acetylcysteine even slightly enhanced CsA stimulated lipid peroxidation in the presence of iron. These results suggest that Gbe might be able to prevent radical mediated damage to human membranes caused by CsA.

Cyclosporin A (CsA) is a potent and highly specific immunosuppressive drug which is widely used in human bone marrow as well as solid organ transplantation [1, 2]. Its usage, however, is limited by its toxicity, especially to the kidney and liver [3, 4], and its narrow therapeutic range [5]. It is difficult to achieve and maintain a plasma level of CsA that is effective yet not toxic. CsA is absolutely non-soluble in water. It therefore accumulates in the fat tissue of the body as well as in certain organs such as the liver, the adrenal glands, and the pancreas [6]. The amount of CsA which will actually be circulating in the blood varies largely from one individual to another [5]. It therefore would be desirable to find a way of reducing CsA toxicity by investigating the underlying mechanisms, in order to then be able to counteract these reactions in a specific way.

CsA seems to be exerting its immunosuppressive effects through the impairment of T-cell functions resulting in a reduced production of lymphokines [7]. The underlying molecular mechanisms remain still unknown, as do those of the toxic side effects of the drug. Previous investigations showed that CsA is capable of inducing lipid peroxidation in rat liver microsomes [8, 9].

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In the present paper we focused on the question whether lipid peroxidation might be involved in mediating CsA toxicity in the human liver. Lipid peroxidation is thought to be caused by radicals which destroy membrane lipids whereby new radicals are being generated [10]. Thus a chain reaction is being set off which can ultimately lead to substantial defects in these membranes [11]. Especially in microsomal membranes which contain high amounts of polyunsaturated fatty acids this mechanism seems to be playing an important role [12].

Well known radical scavengers such as α-tocopherol (vitamin E) and glutathione (GSH) are able to prevent lipid peroxidation *in vitro* [13, 14]. Thus these substances were tested to determine whether any changes observed here are in fact due to radical mediated lipid peroxidation. Furthermore we wanted to determine whether also *N*-acetylcysteine, an acetylated derivative of the sulfhydral group containing constituent of GSH, alone would serve as a radical scavenging agent.

Until now several yet not all constituents of the extract from *Gingko biloba* leaves (Gbe) have been identified [15]. However, an iron containing superoxide dismutase could be found [16]. This enzyme catalyses the disproportionation of superoxide radicals according to the following equation:

$$2O_2^{-}\cdot + 2H^+ \rightarrow H_2O_2 + O_2.$$

In animal studies it was shown that Gbe reduces

CsA induced nephrotoxicity, whereby the protective effect is thought to be due to a decreased syntheses of platelet activating factor [17]. Our aim now was to investigate whether Gbe would also be able to prevent CsA induced lipid peroxidation in human liver microsomes *in vitro*.

MATERIALS AND METHODS

Human liver resection material of five patients with different past medical histories was frozen down immediately after surgical removement using liquid nitrogen and stored at −80° thereafter. All experimental procedures were performed under aerobic conditions.

Preparation of microsomes. 45 g of the disease-free liver resection material was homogenized (Homogenisator Typ 853202, Braun, Melsungen, F.R.G.) in buffer I (sucrose, 0.25 M; Tris, 10 mM; EDTA, 2 mM; pH 7.4) under ice-cold conditions. The resulting homogenate was then sedimented at 17,000 *g* for 20 min at 4° (Ultrazentrifuge Typ L7-55, Beckmann, München, F.R.G.). The supernatant was diluted with buffer I and centrifugated with 110,000 *g* for 1 hr. The sediment was suspended in buffer II (sucrose, 0.25 M; Tris, 10 mM) and again centrifugated at 110,000 *g* for 1 hr. The new sediment was suspended in KCl, 0.17 mM, and once more centrifugated at 110,000 *g* for 1 hr. The resulting sediment was diluted with sodium phosphate buffer, 0.1 mM. The microsomes were stored at −80°.

Protein content measurements. Protein content measurements were performed using the BIO-RAD-Protein-Assay (BIO-RAD, München, F.R.G.).

We used the dispence for oral application containing olive oil (*oleum olivarum*) and ethanol. This was transformed into a suspension in sodium phosphate buffer, 0.1 mM, with aid of the emulsifier polyethylenglucol (20)-sorbitan-monooleat, 62.5 μL/mg (Sonifier B-12, Bronson Sonic Power Company, Danbury, U.S.A.).

Lipid peroxidation. Lipid peroxidation was measured using the thiobarbituric acid assay (TBA) [10]. An extinction coefficient of 1.56×10^5 l mol^{-1} cm^{-1} was employed in the calculation of the resulting concentration of malondialdehyde equivalents (MDA) (Spektralphotometer Typ PMZK, Zeiss, Hamburg, F.R.G.).

Microsomes were incubated with sodium phosphate buffer, 0.1 mM, plus NADPH, 0.5 mM, pH 7.4 in an atmosphere of 95% oxygen and 5% carbon dioxide at 37° using a metabolic shaker (Küttermann Inkubator, Hänigsen, F.R.G.), the protein concentration being 1 mg/mL. In the concentration dependent experiment the duration of incubation was 1 hr. The following substances were added to the medium of incubation respectively: CsA, 10, 30, 100, 300, 1000 μg/mL alone; CsA, concentrations as above plus either vitamin E, 1 mM; GSH, 1 mM; *N*-acetylcysteine, 0.1, 0.3, 1, 3 mM; or Gbe, 15, 50, 150 μg/mL. Time dependent measurements were made after 0, 15, 30, 60 and 90 min of incubation with a CsA concentration of 300 μg/mL. The influence of iron on lipid peroxidation was investigated using three different media of incubation containing $FeCl_3$ at a concentration of either 0, 25, or 250 μM. The following substances were each added to all three solutions: CsA, 300 μg/mL; CsA, 300 μg/mL, plus *N*-acetylcysteine, 1 mM; CsA, 300 μg/mL, plus Gbe, 150 μg/mL; Ac, 1 mM; Gbe, 150 μg/mL.

Controls. Controls to all series of experiment were performed with microsomes and buffer alone as well as in combination with all solvants used (olive oil, ethanol, and polyethylenglucol (20)-sorbitan-monooleat) in their respective concentrations. Furthermore measurements were carried out with all individual substances in either microsome-containing or microsome-free buffer. Neither vitamin E, GSH, *N*-acetylcysteine, nor Gbe were shown to stimulate any increase in the MDA concentrations.

Chemicals. CsA (Sandimmun®, dispence for oral application) was obtained from Sandoz (Basel, Switzerland). GSH and vitamin E were bought from Sigma-Chemie (Deisenhofen, F.R.G.). *N*-Acetylcysteine (Fluimucil®) was purchased from Inpharzam (Gräfelfing, F.R.G.), Gbe (Tebonin®) from Schwabe (Karlsruhe, F.R.G.), NADPH from Boehringer (Mannheim, F.R.G.), and polyethylenglucol (20)-sorbitan-monooleat (Tween® 80) from Hof-Apotheke (Kiel, F.R.G.). All other substances of high purity were obtained from Merck (Darmstadt, F.R.G.).

Calculations. Percentual increase of lipid peroxidation relative to the control value of the individual patient were calculated. Statistical analysis was carried out using Friedmann as well as Wilcoxon and Wilcox tests for significance [18], the level of probability applied being that of 0.05. All graphics shown represent the median values of all livers (N = 5). The respective maxima and minima are indicated wherever this did not impair the clearness of the figure.

RESULTS

Cyclosporin A (300 μg/mL) caused a significant increase of lipid peroxidation, expressed formation of MDA equivalents, to up to 660% (min: 588%, max: 752%) after 60 min of incubation. This increase was time dependent and reached the level of significance already after 15 min of incubation. Both controls, containing either microsomes only or microsomes and all solvents used, did not show any significant increase of lipid peroxidation. The median concentration of TBA reactive substances in the controls at $t = 0$ min was 0.77 [nmol/mL] (min: 0.27 [nmol/mL], max: 0.87 [nmol/mL]) and at $t = 60$ min it lay at 0.85 [nmol/mL] (min: 0.25 [nmol/mL], max: 1.62 [nmol/mL]). In the concentration dependent experiment (incubation time = 60 min) stimulation of lipid peroxidation was found to be significant from a CsA concentration of 10 μg/mL upwards. No significant changes were to be seen in the controls performed. At the highest CsA concentration of 1000 μg/mL lipid peroxidation was increased to 1049% (min: 993%, max: 1215%), whereas the control values were elevated to 246% (min: 160%, max: 268%).

Vitamin E (1 mM) was capable of totally inhibiting CsA induced lipid peroxidation (Fig. 1). Glutathione

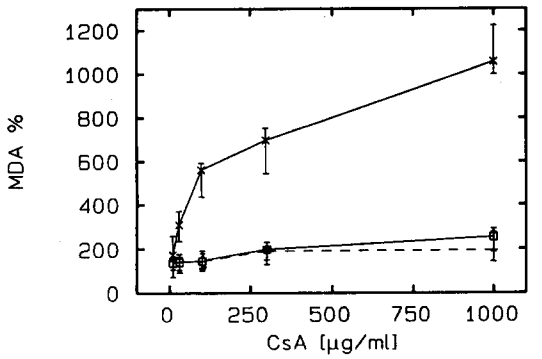

Fig. 1. Concentrations of malondialdehyde (MDA) equivalents in liver microsome preparations upon incubation with Cyclosporin A in various concentrations. Influence of vitamin E. Time of incubation 60 min. Data are given as percentage-differences in comparison to controls (100%) of microsome incubations without additives. Median values, maxima and minima (N = 5). (×) CsA; (+) CsA plus vitamin E (1 mM); (□) controls.

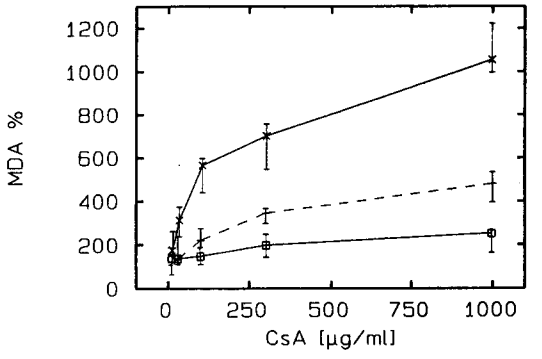

Fig. 2. Concentrations of malondialdehyde (MDA) equivalents in liver microsome preparations upon incubation with Cyclosporin A in various concentrations. Influence of glutathione. Time of incubation 60 min. Data are given as percentage-differences in comparison to controls (100%) of microsome incubations without additives. Median values, maxima and minima (N = 5). (×) CsA; (+) CsA plus GSH (1 mM); (□) controls.

(1 mM) also significantly reduced the lipid peroxidation stimulated by CsA (Fig. 2), as did *N*-acetylcysteine (0.1, 0.3, 1, 3 mM) in a concentration dependent manner (Fig. 3). Here *N*-acetylcysteine at a concentration of 1 mM significantly reduced the formation of MDA equivalents with all CsA concentrations, whereas at a CsA concentration of 10 and 30 µg/mL a concentration of *N*-acetylcysteine of 0.3 mM was sufficient to significantly reduce lipid peroxidation. When *Ginkgo biloba* extract was added to the medium of incubation in different concentrations (15, 50, 150 µg/mL), the CsA induced lipid peroxidation was totally inhibited at a Gbe concentration of 50 µg/mL (Fig. 4). At a CsA concentration of 10 µg/mL formation of MDA equivalents was already significantly reduced by Gbe at a concentration of 15 µg/mL.

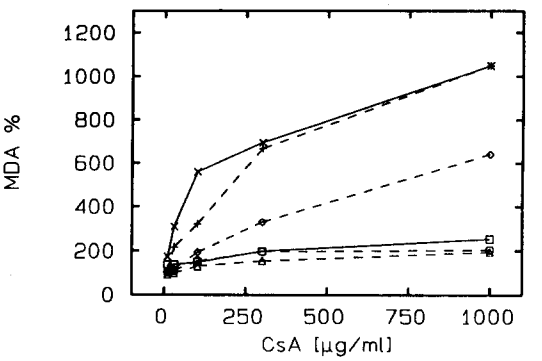

Fig. 3. Concentrations of malondialdehyde (MDA) equivalents in liver microsome preparations upon incubation with Cyclosporin A in various concentrations. Influence of *N*-acetylcysteine. Time of incubation 60 min. Data are given as percentage-differences in comparison to controls (100%) of microsome incubations without additives. Median values, maxima and minima (N = 5). (×) CsA; (+) CsA plus *N*-acetylcysteine (0.1 mM); (◇) CsA plus *N*-acetylcysteine (0.3 mM); (○) CsA plus *N*-acetylcysteine (1 mM); (△) CsA plus *N*-acetylcysteine (3 mM); (□) controls.

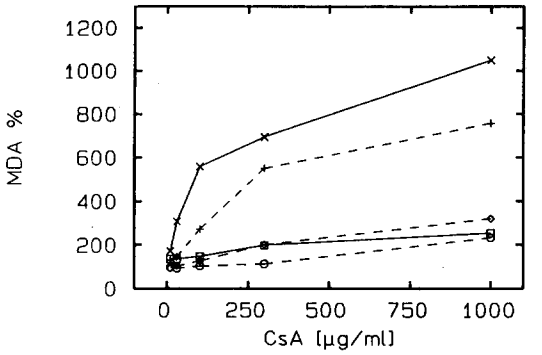

Fig. 4. Concentrations of malondialdehyde (MDA) equivalents in liver microsome preparations upon incubation with Cyclosporin A in various concentrations. Influence of *Ginkgo biloba* extract. Time of incubation 60 min. Data are given as percentage-differences in comparison to controls (100%) of microsome incubations without additives. Median values, maxima and minima (N = 5). (×) CsA; (+) CsA plus Gbe (15 µg/mL); (◇) CsA plus Gbe (50 µg/mL); (○) CsA plus Gbe (150 µg/mL); (□) controls.

$FeCl_3$ (250—data not shown—mM) proved to stimulate lipid peroxidation in human liver microsomes *in vitro* and also significantly enhanced the CsA induced lipid peroxidation (Fig. 5). When *N*-acetylcysteine was added to the medium of incubation it was able to significantly inhibit the lipid peroxidation which was caused by $FeCl_3$ alone whilst it had no protective effect on the lipid peroxidation induced by $FeCl_3$ and CsA (300 µg/mL) together. Gbe also significantly inhibited the $FeCl_3$ mediated lipid peroxidation but in contrast to *N*-acetylcysteine it also caused a reduction in the rate of lipid

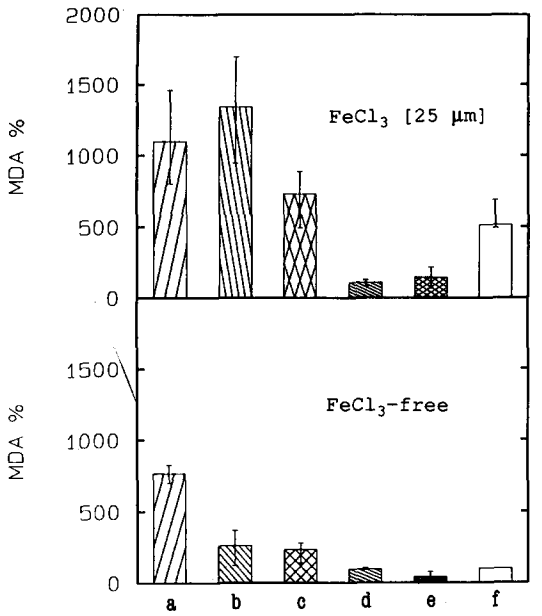

Fig. 5. Concentration of malondialdehyde (MDA) equivalents in human liver microsomes under the influence of Cyclosporin A, *N*-acetylcysteine, and *Ginkgo biloba* extract in both iron-free and iron-containing medium of incubation. Incubation time 60 min. Data are given as % difference to controls (100%) of microsome incubations without additives. Median values, maxima and minima (N = 5). (a) CsA (300 µg/mL); (b) CsA (300 µg/mL) plus *N*-acetylcysteine (1 mM); (c) CsA (300 µg/mL) plus Gbe (150 µg/mL); (d) *N*-acetylcysteine (1 mM); (e) Gbe (150 µg/mL); (f) controls.

peroxidation stimulated by $FeCl_3$ plus CsA (300 µg/mL) in all examined liver microsome preparations. This reduction was not statistically significant but the effect of Gbe on CsA induced lipid peroxidation in the presence of iron was shown to be clearly different from that of *N*-acetylcysteine.

DISCUSSION

The usage of Cyclosporin A is limited by its toxic side effects. Its immunosuppressive properties seem to be mediated by the cytoplasmatic receptor protein cyclophiline [19, 20]. Little is known about the molecular reactions underlying the mode of action of CsA, but there does not seem to be a direct relationship between the immunosuppressive mechanisms and the toxicity of CsA [21]. The aim of the present study was to investigate whether radical mediated lipid peroxidation might be involved in causing toxic side effects of CsA in the human organism.

The concentrations of CsA used (10 to 1000 µg/mL) are high compared to the therapeutic plasma levels of the drug (150–1000 ng/mL). Due to its high lipophilic property, however, CsA vastly accumulates in certain tissues of the body, such as the liver, whereby concentrations are reached which are up to 50 times higher than those in the plasma [5]. Thus a CsA tissue concentration of 50 µg/mL can be considered to be a therapeutic one. Nevertheless it remains yet unknown what exact concentration of CsA are present at the microsomes themselves.

On incubation with human liver microsomes CsA caused a time and concentration dependent increase of lipid peroxidation. This effect of CsA was shown to be inhibited by potent radical scavengers such as vitamin E and glutathione as well as by *N*-acetylcysteine and *Ginkgo biloba* extract. In the presence of iron the protective function of Gbe was diminished whilst *N*-acetylcysteine did no longer have any antioxidant properties.

Studies have shown that vitamin E administration counteracts lipid peroxidation when a vitamin deficiency is present within the organism [13, 22]. The present data suggest that also the toxicity of CsA might be enhanced in vitamin E deficient patients, or even that vitamin E in general might be useful in the protection against lipid peroxidation caused by CsA.

Glutathione cannot be used to prevent lipid peroxidation at the microsomal membranes within the liver cell *in vivo* because it cannot pass through the plasma membrane. It has been suggested that the protective properties of GSH would be due to an enzymatic reaction because *N*-acetylcysteine was not shown to have any effect on lipid peroxidation [23]. In contrast to these findings our results suggest a non-enzymatic influence of GSH on CsA induced lipid peroxidation, probably due to its SH-group. Also the acetylated sulfhydryl group containing the amino acid cysteine was able to inhibit lipid peroxidation which agrees with the findings of other authors describing a direct radical scavenging property of *N*-acetylcysteine [24, 25].

It was shown that *N*-acetylcysteine inhibits lipid peroxidation caused by doxorubicin *in vivo* [26] whereas it enhances lipid peroxidation in the presence of iron *in vitro* [27, 28]. These seemingly contradictory results agree with our findings that *N*-acetylcysteine slightly increases CsA induced lipid peroxidation when iron is added to the medium of incubation. The reason for this observation probably lies in an oxidation of the thiol group contained in the *N*-acetylcysteine molecule whereby new radicals are being generated [29, 30]. The protective effect of *N*-acetylcysteine against lipid peroxidation *in vivo* thus could be explained by the fact that iron is held within complexes by intracellular components so that not enough free Fe^{3+} is present to oxidize the sulfhydral group of *N*-acetylcysteine. In contrast to *N*-acetylcysteine, Gbe leads to a visible yet not statistically significant reduction in the CsA induced lipid peroxidation even in the presence of iron.

The results of the present paper give rise to the following conclusions concerning the antioxidant properties of Gbe: First of all the mechanism of action seems to be a different one than that of *N*-acetylcysteine. It is possible that the superoxide dismutase (SOD) contained in the Gbe [16] is capable of capturing the evolving radicals. Secondly the protective properties of Gbe against CsA induced lipid peroxidation was diminished in the presence of free iron. These results imply that the radical scavenging capacity of Gbe is exceeded by the combined effects of CsA and $FeCl_3$.

The present findings of a radical scavenging property of Gbe agree well with the similar results of Pincemail *et al.* [31, 32]. Furthermore it was shown that Gbe inhibits formation of MDA equivalents in the brain of the rat as well as it causes a reduction in doxorubicin toxicity which is also thought to be due to a radical mediated mechanism [33]. It remains however unclear whether therapeutic dosages of Gbe result in a tissue concentration within the liver which would be sufficiently high enough to prevent CsA induced lipid peroxidation. Under the assumption that merely the SOD contained in the Gbe is responsible for the observed radical scavenging effect it would seem to be more appropriate to administer the purified enzyme alone. In the model of artificially induced ischemia of the rat ileum it was shown that intravenous application of SOD resulted in a significant protection against lipid peroxidation [34]. From the data of the present paper, however, it cannot be decided whether indeed the SOD itself leads to the inhibition of CsA induced lipid peroxidation or whether it is a so far unidentified constituent of the Gbe which is responsible for this effect.

REFERENCES

1. Rotolo FS, Branum GD, Bowers BA and Meyers WC, Effect of Cyclosporine on bile secretion in rats. *Am J Surg* **151**: 35–40, 1985.
2. Starzl ET, Iwatsuki S, Shaw BW, Gordon RD and Esquivel C, Liver transplantation in the Ciclosporin era. *Prog Allergy* **38**: 366–394, 1986.
3. Lorber MI, Van Buren CT, Flechner SM, Williams C and Kahan BD, Hepatobiliary and pancreatic complications of cyclosporine therapy in 466 renal transplant recipients. *Transactions* **43**: 35–40, 1987.
4. Thomson AW, Whiting PH and Simpson JG, Cyclosporine: immunology, toxicity and pharmacology in experimental animals. *Agents Actions* **15**: 306–327, 1984.
5. Lemaire M, Maurer G and Wood AJ, Pharmacokinetics and metabolism. *Prog Allergy* **38**: 93–107, 1986.
6. Kahan BD, Individualization of cyclosporine therapy using pharmacokinetic and pharmacodynamic parameters. *Transplantation* **40**: 457–476, 1985.
7. Harris DT, Kozumbo WJ, Cerutti PA and Cerottini JC, Effects of CsA on T-cell activation. *Cell Immunol* **109**: 104–114, 1987.
8. Inselmann G, Blank M and Baumann K, CsA induced lipid peroxidation in microsomes and effect on active and passive glucose transport by brush border membrane vesicles of rat kidney. *Res Commun Chem Path Pharmacol* **62**: 207–220, 1988.
9. Inselmann G, Hannemann J and Baumann K, Cyclosporine A induced lipid peroxidation and influence on glucose-6-phosphatase in rat hepatic and renal microsomes. *Res Commun Chem Path Pharmacol* **68**: 189–203, 1990.
10. Buege JKA and Aust SD, Microsomal lipid peroxidation. *Methods Enzymol* **52**: 302–310, 1978.
11. Younes M and Siegers CL, Interrelation between lipid peroxidation and other hepatotoxic events. *Biochem Pharmacol* **33**: 2001–2003, 1984.
12. Högberg J, Bergstrand A and Jakobsson SV, Lipid peroxidation of rat-liver microsomes. *Eur J Biochem* **37**: 51–59, 1973.
13. Hill KE and Burk RF, Influence of vitamin E and selenium on glutathione-dependent protection against microsomal lipid peroxidation. *Biochem Pharmacol* **33**: 1065–1068, 1984.
14. Mimnaugh EG, Trush MA and Gram TE, Stimulation by Adriamycin of rat heart and liver microsomal NADPH-dependent lipid peroxidation. *Biochem Pharmacol* **30**: 2797–2804, 1981.
15. Michel PF, Hosford D and Braque P (Eds.), From living fossil to modern therapeutic agent. In: *Ginkgolides—Chemistry, Biology, Pharmacology and Clinical Perspectives*, Vol. 1, pp. 1–8. J. R. Prous Science Publishers, S.A., Barcelona, 1988.
16. Duke MV and Salin ML, Purification and characterization of an iron-containing superoxide dismutase from a eucaryote, ginkgo biloba. *Arch Biochem Biophys* **243**: 305–314, 1985.
17. Pirotzky E, Colliez P, Hosford D, Guilmard C, Egido J, Chabrier E and Braquet P (Eds.), Effect of BN 52063 on alterations in blood pressure and renin activity induced by cyclosporine. In: *Ginkgolides—Chemistry, Biology, Pharmacology and Clinical Perspectives*, Vol. 1, pp. 641–647. J. R. Prous Science Publishers, S.A., Barcelona, 1988.
18. Sachs L, *Angewandte Statistik*, Vol. 6. Aufl., Springer, Berlin, 1984.
19. Cittrio F and Kahan BD, The inhibition of cyclosporine on the nuclear proliferative response to a variety of T-cell activators. *Transplantation* **47**: 334–338, 1989.
20. Harding MW and Handschuhmacher RE, Cyclophilin, a primary molecular target for Cyclosporine. *Transplantation* **46**: 29S–35S, 1988.
21. Ryffel B, Foxwell BM, Gee A, Greiner B, Woerly G and Mihatsch MJ, Cyclosporin, relationship of side effects to mode of action. *Transplantation* **46**: 90S–96S, 1988.
22. Leonard PJ and Losowsky MS, Effect of α-tocopherol administration on red cell survival in vitamin-E-deficient human subjects. *Am J Chem Nutr* **24**: 388–393, 1971.
23. Wefers H and Sies H, The protection by ascorbate and glutation against microsomal lipid peroxidation is dependent on vitamin E. *Eur J Biochem* **174**: 353–357, 1988.
24. Bernard GR, Lucht WD, Niedermeyer ME, Snapper JR, Ogletree ML and Brigham KL, Effect of *N*-acetylcysteine on the pulmonary response to endotoxin in the awake sheep and upon *in vitro* granulocyte function. *J Clin Invest* **73**: 1772–1784, 1984.
25. Kharazmi A, Nielsen H and Schiøtz PO, *N*-Acetylcysteine inhibits human neutrophil and monocyte chemotaxis and oxidative metabolism. *Int J Immunopharmac* **10**: 39–46, 1988.
26. Olson RD, MacDonald JS, Van Boxtel CJ, Boerth RC, Harbison RD, Slonin AE, Freeman RW and Oates JA, Regulatory role of glutathion an soluble sulfhydryl grouds in the toxicity of Adriamycin. *J Pharmacol Exp Ther* **215**: 450–454, 1980.
27. Stoo WE, Olson RD and Boerth RC, Efficacy of sulfhydryl compounds as inhibitors of iron-dependent Doxorubicin-enhanced lipid peroxidation. *Res Commun Chem Path Pharmacol* **48**: 291–303, 1985.
28. Tien M, Bucher JR and Aust SD, Thiol-dependent lipid peroxidation. *Biochem Biophys Res Commun* **107**: 279–285, 1982.
29. Fantone JC and Ward PA, Role of oxygen-derived free radicals and metabolites in leukocyte-dependent inflammatory reactions. *AJP* **3**: 395–418, 1982.
30. Mimnaugh EG, Potentiation by reduced glutathion of Adriamycin-stimulated lipid peroxidation in kidney microsomes. *Biochem Pharmacol* **35**: 4337–4339, 1986.
31. Pincemail J, Thirion A, Dupuis M, Braquet P, Drieu K and Deby C, *Ginkgo biloba* extract inhibits oxygen species production generated by phorbol myristate

acetate stimulated human leukocytes. *Experientia* **43**: 181–184, 1987.
32. Pincemail J and Deby C, Propriétés antiradicalaires de l'extrait de *Ginkgo biloba. Presse Méd* **15**: 1475–1479, 1986.
33. Chatterjee SS and Gabard B, Protection of doxorubicin toxicity by an extract of *Ginkgo biloba. Arch Pharm* **312**: Abstr. 57, 1982.
34. Otamiri TH, Oxygen radicals, lipid peroxidation, and neutrophil infiltration after small-intestinal ischemia and reperfusion. *Surgery* **105**: 593–597, 1988.