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NON-STRUCTURAL LIPIDS AND THEIR DERIVATIVES AS ANTIOXIDANTS

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INTRODUCTION

Lipids are traditionally considered as substrates for lipid peroxidation. This statement refers mainly to structural lipids, constituents of cell membranes. Major subjects of lipid peroxidation in membranes are polyunsaturated fatty acids of phospholipids but other lipid compounds of membranes also undergo peroxidation caused by reactive oxygen species.

While membrane lipids peroxidize, some non-structural lipids and lipid derivatives, such as bile acids with less pronounced hydrophobic properties, eicosanoids – prostaglandins and prostacyclines, show clear antioxidative properties.

Some authors considered cholesterol as an *in vivo* antioxidant compound. Cholesterol is oxidized by reactive oxygen very easily forming oxysterols. It is generally accepted now that not cholesterol but oxysterols cause atherosclerosis and other cardiovascular diseases. Cholesterol protects phospholipid liposomes and erythrocytes against oxidative damage *in vitro*. One of the hypotheses suggest that endogenous cholesterol in blood and tissues acts an interceptor ROS of *in vivo* and protects whole organism against oxidative stress as an antioxidant (Smith, 1991).

Cholesterol is not the only steroid having antioxidant properties. Many natural and semisynthetic steroid compounds are known as inhibitors of lipid peroxidation. The synthetic compounds, 6-methylprednisolone and 21-aminosteroids are potent inhibitors of lipid peroxidation. It is establish that some steroid estrogens are naturally occurring antioxidants. Recently we found antioxidant action *in vivo* for steroid compounds, 17α-estradiol and 17β-estradiol and their D8,9-dehydro derivatives and for a bile acid, namely ursodeoxycholic acid. Furthermore, we studied antioxidant properties of prostaglandins and polyunsaturated phospholipids.

ESTROGENS AND THEIR DEHYDRO DERIVATIVES

It is well established that some estrogens, namely 17β-estradiol and estriol, are naturally occurring antioxidants (Mooradian, 1993). Other studies suggest that dehydroderivatives of estrogens, so-called "scavestrogens" are very effective scavengers of free radicals and antioxidants (Römer, Oettel, Droescher & Schwarz, 1997). In our experiments we studied 17α-estradiol, 17β-estradiol compared to "scavestrogens", (Jenapharm GmbH, Jena, Germany), estra-1,3,5[10],8-tetraene-3,17-diol (J811) and 14a,15a-methylene-8-dehydro-17α-estradiol (J861) to evaluate their ability to scavenge free oxygen radicals (Fig. 1). Using spin trapping for the evaluation of the investigated compounds as scavengers of free oxygen radicals we found that all the tested estrogens were quite effective interceptors of radicals. The decrease in the concentration of spin trap-radical adducts demonstrated that the scavenging efficacy of the estrogens was increased in the following order: 17β-estradiol < 17α-estradiol < J811 < J861 (Fig. 2).

In rabbit experiment the feeding of cholesterol-rich diet developed a prooxidant effect, significantly increasing all the measured radical-related parameters: SOD activity and H_2O_2 production in

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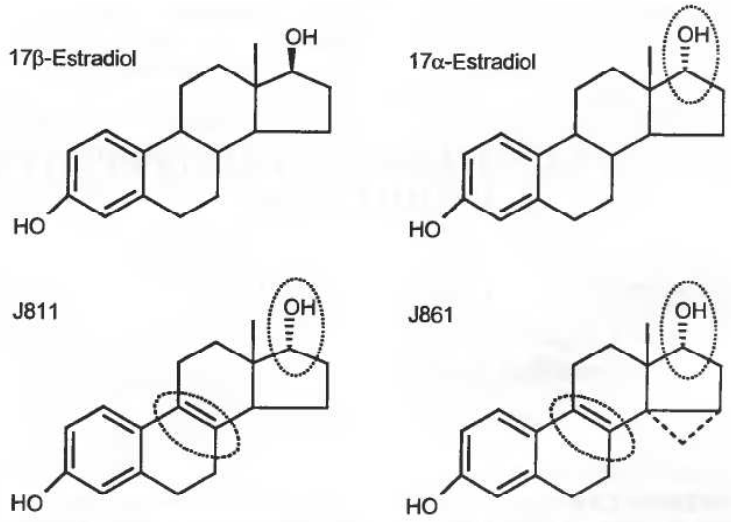

Fig. 1. Chemical structures of 17-estradiol, 17-estradiol, estra-1,3,5(10),8-tetraene-3,17-diol (J811) and 14,15-methylene-estra-1,3,5(10),8-tetraene-3,17-diol (J861)

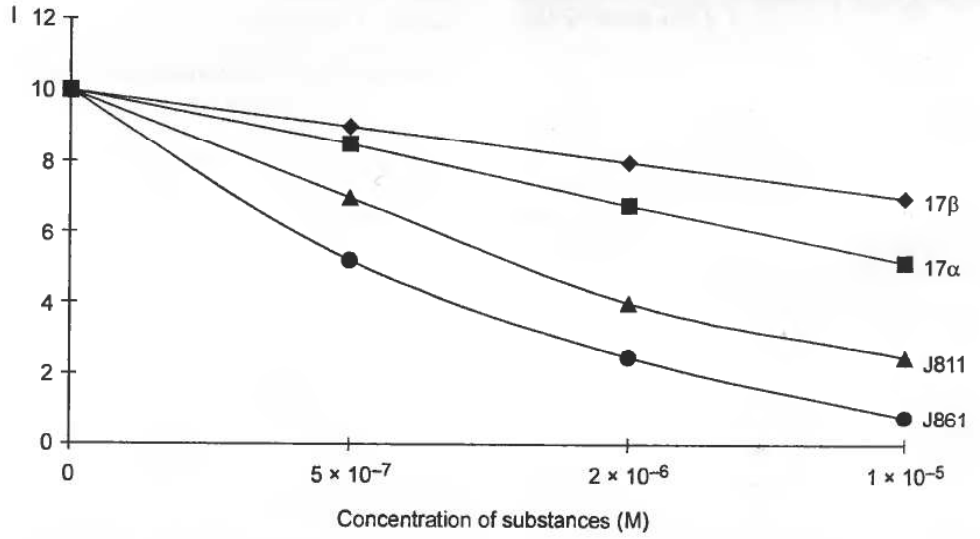

Fig. 2. ESR signal intensity (I) of Tiron radical in liver microsomes preincubated with different concentrations of estrogens. 1 – 17-estradiol; 2 – 17-estradiol; 3 – J811; 4 – J861. The incubation mixture containing 10 mM Tiron, 0.05 mM $FeSO_4$, 0.5 mM ascorbate and 2 mg/ml microsomal protein. Estrogens at different concentrations were added and the mixtures were incubated at 24°C for 15 min

the liver and serum as well as NADPH-induced chemiluminescence, enhanced by lucigenin, superoxide anion content and lipid peroxidation evaluated as TBARS formation in liver microsomes.

The *in vivo* data obtained confirmed the efficacy of the investigated compounds as antioxidants and the highest free radical scavenging and antioxidative effectiveness of "scavestrogens" compared with 17α-estradiol and 17β-estradiol. All the estrogens tested decreased the NADPH-induced chemiluminescence of liver microsomes enhanced by lucigenin, superoxide anion content and TBARS formation in liver microsomes where the effects of J811 and J861 were especially pronounced.

Ever since it was shown that estrogens could attenuate the atherosclerotic lesions in rabbits, monkeys and rats, much interest has been focused on

so called scavestrogens in preventing the oxidation of LDL and retarding the atherosclerotic process. Recently we found the antiatherogenic effect of J861 and J811 which was relatively higher compared to 17α-estradiol and 17β-estradiol (Buko, Chirkin, Lukivskaya, Naruta, Chirkina, Popov, Oettel & Hübler, in press). We propose that the higher antiatherogenic effect of "scavestrogens" can explain by their marked scavenging and antioxidative effect.

URSODEOXYCHOLIC ACID

Ursodeoxycholic acid (UDCA) widely use as hepatoprotector improving liver structure and functions in chronic cholestatic liver diseases (James, 1990).

The data on antioxidant effect of UDCA was sporadically seen in the current literature during the last decade. These data on this effect of UDCA are controversial in experiments *in vitro* (DeLange & Glazer, 1990; Sipos, Bhatu, You, Güldutuna & Leuschner, 1995; Rodrigues, Fan, Ma, Kren & Steer, 1998). The first clinical observations on the antioxidant effect of UDCA in patients with alcoholic liver cirrhosis and cholesterol gallstone disease have been published (Grigorescu, Petrica, Grigorescu & Damian, 1998; Von Ritter, Sreejayan, Meyer & Jungst, 1998).

Recently we showed antioxidant effect of UDCA in γ-irradiated rats (Buko, Artsukevich, Lukivskaya, Zavodnik, Ignatenko, Sadovnichy, Sushko & Tauschel, 1998). The whole body γ-irradiation of rats with a single dose of 1 Gy caused an increase of $O_2^{\cdot}$, liver carbonyl products of peroxidation detected as dinitrophenylhydrazones (polar carbonyls, zone III (alkanals, alkenals, ketones), zone II (ozazones) and zone I (hydroxyalkenals) and MDA content, the NADPH-dependent chemiluminescence amplified by luminol or lucigenin and hydrogen peroxide production in liver microsomes and SOD activity in the liver. The UDCA treatment of rats normalized all the above mentioned parameters.

The exposure of rat to γ-rays induced a statistically significant depletion of the microsomal GSH content which amounted to 26% of the control GSH value. The rat liver microsomal GSSG content increased almost two-fold in radiation-treated animals. FAD-stimulated glutathione reductase was reduced after γ-irradiation, whereas the basal enzyme activity did not change. The UDCA treatment normalized the FAD-stimulated glutathione reductase activity, whereas the activities of other glutathione-related enzymes remained unchanged.

The decrease of $O_2^{\cdot}$ content and superoxide dismutase activity under the influence of UDCA confirmed the scavenging effect of this bile acid toward $O_2^{\cdot}$. At the same time the decreased hydrogen peroxide production by UDCA is indicative of $HO^{\cdot}$ interception.

The decrease of either luminol- or lucigenin-attenuated chemiluminescence of microsomes by UDCA is probably connected with an inhibition of not only $O_2^{\cdot}$ generation, but also with a diminution of the formation of other reactive oxygen species. Therefore, the definition of the radical scavenging function of UDCA requires further investigations.

PROSTAGLANDINS

Data on a hepatoprotective effect of prostaglandins E series in alcoholic fatty liver (Buko, 1991) and CCl4 poisoning (Ruwart, Rush, Friedle, Piper & Kolaja, 1985) were obtained previously. Mechanisms of these effects were unclear and partially explained by an improvement in cAMP-dependent signal transduction (Buko & Zavodnik, 1990). Because free radicals play an important role in alcohol- and CCl_4-induced liver damage (Poli, 1993), we tried to evaluate an antioxidant effects of both prostaglandin E1 and prostaglandin E2 (4 mg/kg i.p., last 10 days) in rat experiment with chronic alcohol intoxication (5 g/kg/d; 56 days). The data obtained suggests a significant activation of lipid peroxidation in ethanol-treated rats (Maltsev, Sadovnichy & Buko, 2000). Ethanol increased a formation of end-products of lipid peroxidation in the liver: MDA, diene conjugates and carbonyl-containing products, alkanals, alkenals and ketones (zone III) and hydroxyalkenals (zone I). The administration of prostaglandin E1 and prostaglandin E2 did not effected diene conjugates content, but significantly decreased MDA and liver carbonyls. Ethanol induced Fe-stimulated chemiluminescence of liver homogenates and NADPH-stimulated chemiluminescence attenuated by luminol in liver microsomes whereas the prostaglandins normalized both the above parameters.

Based on our previous studies we proposed that one of the mechanism of the antioxidant effect of prostaglandin E1 is due to decreased radical species generation by cytochrome P-450 in rat liver microsomes (Buko & Sadovnichy, 1996). The chronic alcohol intoxication increased NADPH oxidation, cytochrome P-450 content and NADPH-stimulated chemiluminescense of micro-

Table 1. The effect of PGE1 on cytochrome P-450, related enzymes and parameters characterizing free radical generation in liver microsomes of rats with chronic alcohol intoxication

Parameters	Control	Ethanol	Ethanol + PGE1
Cytochrome P-450, nmol/mg protein	0.62 ± 0.05	0.83 ± 0.03*	0.42 ± 0.05* †
NADPH-cytochrome P-450 reductase, nmol/mg protein/min	234.0 ± 39.3	234.7 ± 34.8	204.0 ± 19.8
NADPH oxidase, nmol/mg protein/min	1.37 ± 0.06	1.91 ± 0.19*	1.57 ± 0.14
MEOS, nmol/mg protein/min	238.4 ± 12.7	286.5 ± 14.6*	134.5 ± 7.9* †
H_2O_2 production, nmol/mg protein/min	0.45 ± 0.02	0.48 ± 0.036	0.46 ± 0.02
Superoxide dismutase, % of blocking	32.8 ± 0.49	40.2 ± 1.25*	35.7 ± 1.65
NADPH-induced chemiluminescence, c.p.m./mg protein/s	619 ± 80	1526 ± 178 *	521 ± 52

*P < 0.05 compared to the control group

†P < 0.05 compared to the ethanol group

somes. Ethanol also raised superoxide dismutase (SOD) activity in microsomes. Prostaglandin E1 decreased cytochrome P-450 content, normalized NADPH oxidation, NADPH-induced chemiluminiscence and SOD activity in the liver of alcohol-treated rats. Prostaglandin E1 exerted a similar effect after the microsomal induction by acetone combined with starvation or phenobarbital, normalizing all the above parameters (Table 1) Therefore, prostaglandin E1 affected both the ethanol-inducible IIE1 and phenobarbital-inducible IIB1 isoforms.

Thus, prostaglandin E1 decreased or completely normalized all the investigated parameters of cytochrome P-450 system increased under the influence of ethanol. The antiradical activity of prostaglandin E1 was clearly shown in the experimental group, too. We proposed that this effect of prostaglandin E1 could be connected to the decrease of cytochrome P-450 content and, probably, NADPH-cytochrome P-450 reductase activity, which are very important sources of free oxygen radicals in the liver.

POLYUNSATURATED PHOSPHATIDYLCHOLINE

Polyunsaturated phosphatidylcholine (PPC) extracted from soybean and highly purified is widely used for a correction of metabolic disorders in the liver caused by ethanol, different toxins, drugs and radiation. Main hepatoprotective properties of PPC are related to its membranous effect, in particular to the restoration of membrane structures and the normalization of membrane-linked enzyme activities. Consequently, PPC regulates multiple metabolic processes in the liver, including cAMP-dependent signal transduction (Buko, Artsukevich, Maltsev, Nikitin, Ignatenko, Gundermann & Schumacher, 1994) and prostaglandin biosynthesis (Kuntz, 1991).

PPC contains two residues of linoleic acid which is a precursor (via arachidonate formation) of prostaglandin synthesis. However, linoleate as unsaturated fatty acid is an effective substrate for lipid peroxidation. Studying the hepatoprotective effect of PPC in alcoholic fatty liver, we unexpectedly found that PPC decreased the liver content of MDA enhanced by alcohol intoxication (Buko, Nemkevich, Maltsev, Nikitin, Malyshenko, Ignatenko, Gundermann & Schumacher, 1992). We explained this phenomenon by an admixture of vitamin E in the PPC preparation. Later the antioxidant effect of PPC was confirmed in rats with liver fibrosis induced by carbon tetrachloride (Aleynik, Leo, Ma, Aleynik & Lieber, 1997). PPC decreased lipid peroxidation in carbon tetrachloride-treated rats as evaluated by measurements of 4-hydroxynonenal and F2-isoprostanes. However, other authors (Nanji & French, 1989) showed an increase of lipid peroxidation in rats with chronic alcohol intoxication upon supplementation of dietary linoleic acid as triglycerides.

Antioxidant properties of soybean phospholipids are broadly used in the industry to stabilize oils preventing the autooxidation of unsaturated fatty acids (Shahidi, 1996). The antioxidant activity of α-tocopherol was significantly increased by addition of 1% phosphatidylcholine (Linow & Mieth, 1976). Several studies have suggested that phospholipids chelate metals and, consequently, acts as secondary antioxidants (Shukla, Wanasundara & Shahidi, 1996). Additionally, phospholipids can release protons and lead to rapid decomposition of hydroperoxides without the formation of free radicals (Pokorny, Luan, Svobodova & Janicek, 1976). Thus, PPC may realize the antioxidant effect act-

ing as an inactivator of prooxidant metals, synergist of α-tocopherol and a free-radical terminator.

CONCLUDING REMARKS

Despite numerous studies on the role of antioxidants in living organisms, the list of endogenic and dietary antioxidants is not completed yet. Studies on the role of known antioxidants, such as vitamin E, glutathione, antioxidant defense enzymes, etc. point to the existence of new endogenic antioxidants and antioxidant systems and dietary antioxidants and synthesis of novel antioxidants. For instance, antioxidant and radical scavenging properties of melatonin (Tan, Chen, Poeggeler, Manchester & Reiter, 1993), α-ketoacids (Salahudeen, Clark & Nath, 1991), hypotaurine and taurine (Tadolini, Pintus, Pinna, Bennardini & Franconi, 1995) were discovered during the last decade.

We assume that some dietary lipids and endogenous non-structural lipids and lipid derivatives having antioxidant and radical-scavenging properties can be considered as a component of antioxidant defense system protecting cellular homeostasis from oxidative disruption by reactive oxygen species and other reactive metabolites generated from oxygen metabolism.

Concluding our observations described in this paper, we believe that lipids are not only oxidizable substrates, but antioxidants as well.

The biological significance of non-structural lipids and their derivatives as antioxidant defense system is the subject of further studies. Special consideration in this regard should be given to the problem of whether lipids act as either prooxidants or antioxidants under any circumstances. Separate studies on antioxidant effects of endogenous, synthetic and dietary lipids are required. Furthermore, putative mechanisms of antioxidative and radical scavenging actions of these lipids should be explored.

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