INDUCTION OF CYTOCHROME P-450 BY TETRAPHENYLPORPHYRIN-Sn^{4+}

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The intensive study of inducers of microsomal mono-oxygenases (MOG) is linked with prospects for creating substances on their basis which will stimulate detoxication processes and protect the internal medium of the organism against xenobiotics of varied nature. Effective inducers of MOG (as well as inhibitors) can also be used to control, at the molecular level, processes of adaptation and compensation of disturbed functions arising during the action of chemicals on the body [6]. This paper gives data obtained in a study of a representative of a new class of MOG inducers, mainly porphyrins and their metallocomplexes.

Natural porphyrins and their metallocomplexes modify activity of hemoproteins and exert their influence on enzymes participating in heme metabolism. Hemin, for instance, which induces hemoxygenase in vivo, lowers the concentration of cytochrome P-450 in the cells [15], whereas a metallocomplex of protoporphyrin IX and tin activates mono-oxygenases through inhibition of hemoxygenase [11]. Synthetic mesosubstitued porphyrins and their metallocomplexes, which are structural analogs of natural porphyrins, can also exert an influence on the activity of heme-containing enzymes and, in particular, on the cytochrome P-450 system. Metallocomplexes of tetraphenylporphyrin (TPP) with various metals of variable valency inhibit cytochrome P-450-dependent systems [4, 8]. The aim of this investigation was therefore to study the effect of TPP-Sn^{4+} on cytochrome P-450 activity and also the intensity of lipid peroxidation (LPO) and hemoxygenase activity.

EXPERIMENTAL METHOD

TPP-Sn^{4+} was synthesized by the method in [10]. Experiments were carried out on noninbred male rats weighinkg 160-200 g. TPP-SN^{4+} was injected once, intraperitoneally, into the animals in a dose of 25 mg/kg body weight. The experiments continued for 30 days (1-30 days). Liver microsomes were isolated by the usual methods [2] and the cytochrome P-450 concentration was determined from the CO-peak of reduced hemoprotein, using a coefficient of molar extinction of 91 $mM^{-1}\cdot cm^{-1}$ [17]. Activity of mono-oxygenases (N-demethylase, aniline hydroxylase, benz(a)pyrene hydroxylase) was determined by the amount of oxidized products formed [7, 16]. The intensity of LPO was judged by accumulation of malonic dialdehyhde (MDA) in the incubation medium. In the case of NADPH and ascorbate-dependent LPO, $5\cdot10^{-4}$ M NADPH, $2\cdot10^{-4}$ M ADP, and $12\cdot10^{-6}$ M $FeSO_4$ were added to the incubation medium in the first case, and $8\cdot10^{-4}$ M ascorbate, $2\cdot10^{-4}$ M ADP, and $12\cdot10^{-6}$ M $FeSO_4$ were added in the second case. Hemoxygenase activity was determined in the postmitochondrial supernatant by the quantity of bilirubin formed, using a molar extinction coefficient of 30,000 $M^{-1}\cdot cm^{-1}$ [13]. The protein concentration was determined by Lowry's method [14].

EXPERIMENTAL RESULTS

Injection of TPP-SN^{4+} into the experimental animals led after 24 h to an increase of 1.4 times in the cytochrome P-450 concentration (Figs. 1 and 2). There was a simultaneous increase in the intensity of N-demethylation of DMA and of p-hydroxylation of aniline by 1.3-1.4 times. The greatest effect was observed in the interval between the 7th and 14th days. For instance, 1 week after injection of the tin complex, the cytochrome P-450 concentration increased by 1.8

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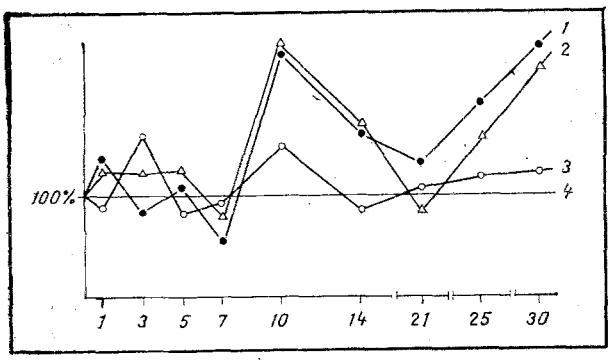

Fig. 1

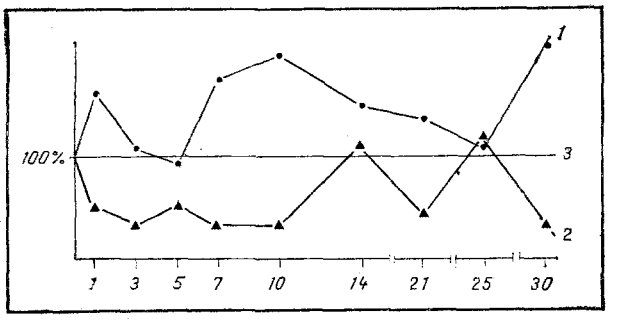

Fig. 2

Fig. 1. Changes in MOG activity in rat liver microsomes after injection of TPP-SN^{4+}. 1) N-demethylase; 2) p-hydroxylase; 3) benz(a)pyrene hydroxylase; 4) control. Abscissa, days; ordinate, %.

Fig. 2. Reciprocal character of changes in cytochrome P-450 concentration (1) and hemoxygenase activity (2) in rat liver after injection of TPP-SN^{4+}; control (3). Abscissa, days; ordinate, %.

times. On the 10th day of the experiment the concentration of the hemoprotein continued to increase, and its level was twice as high as that of cytochrome P-450 in animals of the control group. On the 14th-21st day there was a gradual fall of the microsomal hemoprotein level, but these values exceeded the control level. On the 30th day of the experiment a twofold increase was found in the cytochrome P-450 concentration. In the interval between the 7th and the 14th days, enzymic processes dependent on cytochrome P-450 were considerably activated. For instance, DMA N-demethylase activity was increased by 2.4 times and p-hydroxylase activity by 2.6 times. At the same time oxidation of benz(a)pyrene was increased. This enzymic process is mainly dependent on cytochrome P-448 [16]. Admittedly, whereas activity of MOG dependent on cytochrome P-450 was higher than the control values at virtually all times of the investigation, the increase in benz(a)pyrene hydroxylase activity took place only on the 3rd and 10th days of the experiment. The cytochrome P-450 level 1 month after injection of TPP-Sn^{4+}, as already stated, was increased, and along with it N-demethylation of DMA was increased by 2.5 times and p-hydroxylation of aniline by 1.6 times. This kind of effect is probably mainly connected with the low solubility of the given chemical compound and, as a result, its special character of its absorption from the site of injection. Since TPP-Sn^{4+} is colored, its distribution in the internal cavity can be clearly seen. Thus sufficient quantities of the injected preparation are thus still observed on the 30th day at the site of injection.

Microsomal hemoxygenase not only regulates the heme content in the cell, but its activity is functionally directly opposite to that of the hemoprotein [11, 12]. Our experimental results also confirmed this fact. On the 1st day of the experiment, activity of this enzyme was reduced almost by half. Later during the investigation the hemoxygenase level was significantly lower than the control, and in the interval between the 3rd and 10th days it was only 37% of the level in the intact control. Thus TPP-Sn^{4+} is an inhibitor of hemoxygenase activity in vivo. Regulation of porphyrin metabolism in the body and of activity of heme-containing enzymes is closely connected with the intenstiy of lipid peroxidation (LPO) in the biomembranes for many hemoproteins and, in particular, cytochrome P-450, are membrane-bound enzymes. Intensification of peroxidation in biomembranes converts cytochrome P-450 into its inactive form P-420, and it ceases to be bound to the membrane [3]. Many inducers of the hemoprotein reduce LPO in vivo and largely prevent the degradation of cytochrome P-450 [3]. We therefore decided to investigate the effect of TPP-Sn^{4+} on the intensity of LPO in rat liver microsomes.

In the first 5 days of the experiment (Table 1) no significant changes were found in MDA formation during the study of spontaneous LPO. Only on the 7th day was the intensity of this process reduced, to 40% of the normal value. On the 10th-14th days slight changes took place in MDA formation, and compared with the control, the rate of accumulation of this product amounted to 43-46%. Later, on the 21st, 25th, and 30th days after the beginning of the experiment, the intensity of LPO was increased by 1.5-1.7 times. Closely similar changes in the intensity of this process were obtained during the study of enzymic and nonenzymic LPO. Thus, just as in the case of the spontaneous process, no significant differences were observed in the first 5 days of the experiment. Not until 1 week after the experiment began was the in-

TABLE 1. Effect of TPP-Sn^{4+} on Intensity of LPO in Biomembranes of Rat Liver Microsomes (M ± m; n = 5-7)

Variant / Day	Spontaneous LPO, nmoles/min·mg protein	NADP.H-dependent LPO, nmoles/min.mg protein	Ascorbate-dependent LPO, nmoles/min·mg protein
1-st	0,88±0,1	10,2±3,1	6,7±0,8
3-rd	0,67±0,05	8,5±0,9	5,2±0,5
5-th	0,73±0,06	10,6±2,0	4,7±0,6
7-th	0,3±0,02***	6,0±0,5*	2,5±0,2***
10-th	0,35±0,04***	5,2±0,4*	3,0±0,4***
14-th	0,33±0,04***	5,1±0,4*	2,2±0,3***
21-st	0,95±0,09	13,2±1,2	7,1±0,9
25-th	1,1±0,1*	18,5±3,4	10,7±2,0**
30-th	1,3±0,20*	24,1±4,2**	12,1±1,5***
Control	0,76±0,07	10,4±2,0	5,4±0,6

Legend. *p < 0.05, **p < 0.01, ***p < 0.003.

tensity of NADPH- and ascorbate-dependent LPO blocked, at 46-58% of the control. Later, on the 10th-14th day, there was further significant inhibition of LPO. Thus on the 10th day the rate of MDA formation was depressed by 44-50%, but 2 days after a single injection of TPP-Sn^{4+} it was reduced by 51-59%. Later there was a gradual increase in the intensity of LPO and the 25th-30th days the velocities of the enzymic and nonenzymic processes were 2-2.3 times higher than the control values. Clear correlation was thus obtained of the dependence of the cytochrome P-450 concentration on the intensity of LPO in the microsomal membrane. For instance, the greatest rise of cytochrome P-450 and activation of the reactions dependent on it took place in the interval between the 7th and 14th days. At the same time the intensity of LPO in the microsomal membranes was blocked. Experimental confirmation also was obtained of correlation between hemoxygenase activity, the intensity of LPO, and the cytochrome P-450 concentration.

It must be pointed out that the metallocomplex of tin and porphyrin differs considerably from other metallocomplexes in its action on cytochrome P-450 and its dependent enzymes. Moreover, whereas the tin salt and its organic complexes of nontetrapyrrole structure are inducers of hemoxygenase and lower the cytochrome P-450 concentration in the liver [9, 11], introduction of tin into a porphyrin (whether natural or synthetic), reverses the direction of its action. This phenomenon can probably be explained as follows: first, tin in the porphyrin is tetravalent, and in this metallocomplex there are two extra ligands, which may considerably modify its physicochemical properties. Most probably, therefore, this compound binds with hemoxygenase and blocks this enzyme; second, some workers suggest [11] that such complexes may have a stimulating action on de novo synthesis of apocytochrome P-450, but no direct proof of this has yet been obtained. So far as its effect on LPO is concerned, we know that all metals of variable valency and their metallocomplexes activate to a greater or lesser degree the intensity of peroxide processes in biomembranes in vitro [3]. Metallocomplexes of tin and porphyrin in vivo probably activate other systems in the cell, and thereby exhibit antioxidative properties indirectly and not directly.

The investigations showed that TPP-Sn^{4+}, given by a single intraperitoneal injection to rats, induces after 24 h a marked increase in the concentration of cytochrome P-450 in the liver, which lasts 30 days. Conjecturally the mechanism of induction is connected with depression of activity of hemoxygenase, an enzyme controlling cytochrome catabolism. An important feature distinguishing this complex was its ability to inhibit lipid peroxidation. Induction of cytochrome P-450 is accompanied by generation of active forms of oxygen and by intensification of lipid peroxidation, the products of which, converting cytochrome P-450 into the inactive form, weaken or completely abolish the induction effect [1, 5]. During the action of TPP-Sn^{4+}, induction of MOG was combined with inhibition of LPO, which evidently determined the lasting effect of action of the preparation.

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MODIFICATION OF PLASMA LIPOPROTEINS BY HEXANAL, A LIPID PEROXIDATION PRODUCT

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Damage to low-density lipoproteins (LDL) by malonic dialdehyde leads to loss of their ability to interact with the B,E-receptor of liver cells, fibroblasts, and smooth-muscle cells, but it enhances their uptake by macrophages, inducing conversion of the latter into lipid-loaded cells [6]. Meanwhile the principle products of lipid peroxidation (LPO) in a composition of peroxidized LDL are not dialdehydes, but monoaldehydes, including hexanal and 4-hydroxynonenal [5].

The aim of this investigation was accordingly to study the action of hexanal on the functional properties of lipoproteins: the rate of elimination of LDL from the bloodstream and the rate of cholesterol acceptance by high-density lipoproteins (HDL).

EXPERIMENTAL METHOD

Hexanal (caproic aldehyde) was obtained by interaction between allylmagnesium iodide and triethylorthoformate, and subsequent hydrolysis of the sulfuric acid acetal thus formed. The physical constants of the substance obtained agreed with data in the literature [3]. LDL and HDL were isolated from blood plasma from a random donor by ultracentrifugation within a density gradient of 1.02-1.05 and 1.063-1.21 g/ml, respectively (Beckman L2-65B ultracentrifuge, 50 Ti rotor, 42,000 rpm, 15°C, 22 h. After dialysis against 0.15 M NaCl containing 0.05 M sodium-phosphate buffer, pH 7.4, the LDL were labeled with ^{125}I by the chloramine method. To remove unbound radioactive iodine the samples were dialyzed against 0.15 M NaCl. Acetylation of labeled LDL was carried out with acetic anhydride. Protein was determined by Lowry's method, using bovine serum albumin as the standard.

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