Content of cytochrome P-450 in microsomal fractions of the liver of pregnant rats and their embryos

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Abstract. This article presents a comprehensive examination of the impact of the pesticide "dropp" on the lipid peroxidation (LPO) of microsome membranes and the levels of cytochrome P-450 enzyme within microsomal fractions of pregnant rats and their developing embryos. The study delves into the intricate biochemical responses within these biological systems following exposure to the pesticide. The research uncovers a significant intensification of NADP.N- and ascorbate-dependent lipid peroxidation in the liver microsomes of both pregnant rats and their embryos upon exposure to the "dropp" pesticide. This heightened lipid peroxidation is particularly notable on the 3rd day of pregnancy, accompanied by an elevated concentration of malondialdehyde (MDA), a marker of oxidative stress. Moreover, the study reveals a noteworthy impact of "dropp" on the content of cytochrome P-450 enzyme within the microsomal fraction of the liver in pregnant rats. This enzyme, which plays a vital role in various metabolic processes, experiences a reduction in concentration upon pesticide exposure. The effect is most pronounced on the 3rd and 19th days of pregnancy, highlighting specific time windows of vulnerability. Collectively, these findings provide a nuanced understanding of the biochemical repercussions of "dropp" pesticide exposure on the microsomal membranes and enzyme dynamics within pregnant rats and their developing embryos.
Keywords. Pesticide, rat, pregnancy, embryo, liver, microsome, lipid peroxidation, MDA, NADP.H, enzyme, cytochrome P-450.

1 Introduction

The long-term use of pesticides in various branches of agriculture with their ability to accumulate in biosphere objects and food products has led to environmental pollution. Entering the body in various ways, they accumulate in tissues, biological fluids of humans and animals and have a toxic effect on them [1].

Detoxification processes in the body are carried out by all organs, but mainly by the liver [2]. Under the action of various chemical pollutants, systemic enzyme disorganization and labilization of membranes, the most important intracellular organelles, including the

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endoplasmic reticulum, occurs [3].

It is necessary to note the effect of pesticides on the function of microsome membranes. Studies at the cellular level make it possible to characterize the effect of pesticides on a specific process, the role of a foreign substance in certain structures. This is especially important for determining the interaction of a foreign substance with cell components at the early stages [4].

Most of the metabolic transformations that foreign compounds undergo in the body are provided by enzymes localized in the membranes of the endoplasmic reticulum of liver cells. Microsomal enzymes catalyze the hydroxylation reactions of foreign compounds, as well as important endogenous substrates (saturated and unsaturated fatty acids, phospholipids, etc. [5].

The endoplasmic reticulum (ER) of the liver contains a system of biological oxidation of xenobiotics and endogenous substrates that is physiologically important in the sense of "protecting the internal environment of the body" [6]. Most of the metabolic transformations that foreign compounds undergo in the body are provided by enzymes localized in the membranes of ER liver cells [7].

Microsomal enzymes catalyze hydroxylation reactions of compounds foreign to the body [7, 8], as well as important endogenous substrates (saturated and unsaturated fatty acids, phospholipids, etc.) [9, 10]. Activation of molecular oxygen in ER membranes is carried out by an enzyme complex in which the hemoprotein cytochrome P-450 acts as an enzyme that binds the substrate and activates molecular oxygen. The enzyme system uses NADP as a donor of electrons necessary for oxygen activation [11].

At high concentrations of xenobiotics, i.e., when cytochrome P-450 is fully saturated with substrates, mitochondrial oxidation is inhibited and prerequisites arise for regulatory switching of the flow of reduced equivalents from mitochondria to ER. At the same time, cytochrome P-450 is activated and detoxification of foreign substances occurs faster [12]. Microsome monooxygenase, characterized by wide specificity due to the existence of multiple forms of cytochrome P-450, has the ability to oxidize hundreds of substances of various chemical nature, preventing the accumulation of toxic hydrophobic compounds in the body [12, 13].

The total content of cytochrome P-450 hemoprotein in the liver microsomes of pregnant rats is significantly higher than in non-pregnant rats [14, 15]. In the embryonic liver of rats, ER is represented mainly by a rough subfraction. At first it looks like bubbles, which then turn into tanks [16]. Smooth membranes appear in the postnatal period. There is very little ER in the third phase of pregnancy, its monooxygenase activity is very low. A significant amount of GER appears at the age of one week [17].

On the 20th day of pregnancy, cytochrome P-450 was not detected spectrophotometrically in the embryonic liver of intact rats. In the following days until the moment of delivery, the concentration of hemoprotein gradually increases. The peak of the differential spectrum of cytochrome P-450 during these periods is stably fixed at a wavelength of 450-450.5 nm [18].

In the rat embryo, the cytochrome P-450 apobel is present just before delivery and remains at approximately the same level for several days after delivery. Since cytochrome P-450 was not detected in the liver, it was assumed that heme does not attach to the protein before birth [19]. Most ER lipids consist of phospholipids, which account for 47-54% of liver homogenate phospholipids [106, 107, 127, 139], including 50-60% phosphatidylcholine, 20% phosphatidylethanolamine and 10% phosphatidylinosite. It should be noted that cytomembranes are identical in lipid composition. Phospholipids play a significant role in lipid-protein interaction and in the molecular organization of membranes [11].

A characteristic feature of the organization of microsome membranes is the high content of polyunsaturated fatty acids (PUFA) in them, capable of undergoing intensive oxidation by the free radical peroxide mechanism [12]. Currently, there is no doubt that lipids of microsome membranes play an important role both in the construction and stabilization of protein-lipid complexes of membranes and in the regulation of enzyme systems. At the same time, an important role belongs to fatty acids - both free and part of phospholipid molecules [3]. Having

a high degree of unsaturation, fatty acid chains of membrane phospholipids are potential substrates for the processes of free radical lipid peroxidation (FR LPO). A large amount of experimental data has been obtained confirming the assumption of B.N.Tarusov about the leading role of LPO processes in the development of various pathological conditions [5-7].

2 Materials and methods

The introduction of lysophosphatidylcholine into rat liver microsomes causes the conversion of cytochrome P-450 to P-420, which is reflected in their differential spectra [7]. Phospholipids are essential components of the monooxygenase system of microsomes that promote cytochrome P-450 binding of metabolized substrates.

At the early stages of ontogenesis in rat liver microsomes, the rate of non-enzymatic and enzymatic LPO increases markedly. The rate of ascorbate-dependent LPO reaches maximum values both in microsomes and in the mitochondria of the liver of 2.5-month-old rats (an increase of almost 5 times compared to the liver of newborns). The maximum rate of non-enzymatic LPO was observed in the liver of 3-day and 2-month-old rats. According to various authors, the rate of non-enzymatic LPO in the membrane structures of the embryonic liver is higher or lower than in adult animals [8, 9].

The rate of enzymatic NADP the H-dependent LPO of microsomal phospholipids of the liver changes extremely during ontogenesis and reaches a maximum level in the liver of 2-month-old rats (an increase of almost 20 times compared to the liver of newborns). A sharp increase in the rate of NADF.H-dependent LPO in the liver at the early stages of rat ontogenesis is probably due to the fact that in the first weeks of their life, the content of the oxidation substrate - polyunsaturated fatty acids (PUFA) increases in the liver microsomes [5].

The intensity of LPO in rat fetal liver homogenates is higher than in adult animals, and after birth until the age of 6-10 days, it gradually decreases.

According to other authors, in the placenta and liver of fetuses of rats, rabbits and guinea pigs, NADPH-H-dependent LPO is insignificant. NADF level the H-dependent LPO in the fetal liver is higher than in adult animals [3].

Among the membrane structures, the most effective processes are POL in ER. Damage in its membranes increases parallel to the development of POL [4, 5]. The processes of peroxidation in microsomes are closely related to the metabolism of xenobiotics. They can compete for reducing equivalents and oxygen. In addition, xenobiotics and their metabolites can have a direct antioxidant or pro-oxidant effect. As a result, ribosome dissociation occurs in microsomes, inhibition of protein synthesis and microsomal oxidation processes.

Activation of POL processes causes degradation of cytochrome P-450 and weakening of microsomal oxidation processes. The metabolic activation of xenobiotics, leading to the formation of free radicals, is carried out in the electron transport chain of the ER. Cytochrome P-450 plays a key role in this [18].

The sequence of ER damage and accumulation of lipoproteins in the liver was shown within 1 hour after administration of CCl_4. Lysosomes and nuclear structures are damaged after 4 hours, mitochondria are damaged after 3-10 hours after inhalation of CCl_4. After the introduction of CCl4, the activity of microsomal enzymes, in particular cytochrome P-450, decreases, and the peroxidation of unsaturated fatty acids of membrane lipids accelerates.

Many organochlorine pesticides, entering the body, are metabolized mainly by the monooxygenase system of the liver parenchymal cells, inducing significant changes in the functional state of this enzyme system. Acute poisoning with the pesticide lindane at a dose of 18 mg/kg against the background of a balanced diet after 2 hours leads to a significant increase in the content of cytochrome P-450 in preparations of liver microsomes. Induction of the monooxygenase system after 24 hours or more is accompanied by a significant increase in the concentration of microsomal protein [6].

A relationship was established between the inhibitory effect of malabion on microsomal liver metabolism and its ability to damage components of oxidase systems of mixed function and other enzymes of xenobiotic metabolism. Damage to the microsomal liver metabolism of experimental mice by malabion is due to its effect on the content of cytochrome P-450 [6].

Under the influence of hexochlorobenzene, the content of cytochrome P-450 in the liver increases [4, 8]. Thus, pesticides cause significant changes in the structure and metabolism of tissues, cells and subcellular structures. In other words, they are non-specific structural and metabolic poisons.

3 Results and discussion

The objects of research were white female Wistar rats weighing 180-200 g. The animals were seeded with a drop at a dose of 1/10 LD_{50} on the 3rd, 13th and 19th days of pregnancy intragastrically with a special probe for 5 days. For fertilization of rats in the proestrus-estrus stage, they were planted at night with males in a ratio of 3:1. The first day of pregnancy was considered the day of detection of spermatozoa in vaginal smears. The slaughter of animals was carried out on the 20th day of pregnancy, when the embryo reached a significant size, at the end of organogenesis. In the experiments, microsomes of the liver of embryos and the maternal organism were used.

The microsomal fraction from the liver was isolated by differential centrifugation. The activity of ascorbate-dependent and NADPH-dependent lipid peroxidation in microsomes was determined by the content of malondialdehyde (MDA). The MDA content was determined using 2-thiobarbituric acid. At a high pH in an acidic medium, MDA reacts with 2-thiobarbituric acid to form a colored tremitin complex with an absorption maximum at 532 nm. The molar extinction coefficient of this complex is 1.56-10 $cm^{-1} M^{-1}$.

Determination of cytochrome P-450 was carried out [12], based on a change in the amount of absorption of the complex reduced by carbon monoxide. Measurements of cytochrome P-450 content in a suspension of microsomes 1-2 mg/ml of protein in an incubation medium containing 0.1M K-phosphate buffer, pH- 7.4 were carried out on SPECORD UV VIS [FRG]. Microsomes were previously passed through one of the cuvettes with a suspension for 1 min. carbon monoxide, then several crystals of sodium dithionite were added to both cuvettes and the differential spectrum of the reduced form was recorded with carbon monoxide at a maximum absorption of 450 nm and a minimum. The absorption difference between 450 and 490 nm ($A_{450-490}$) served as an indicator of the cytochrome content, which was calculated using a molar extension coefficient equal to 91x10 $cm^{-1} m^{-1}$.

Unsaturated fatty acids of phospholipids of biological membranes are optimal peroxidation substrates by structure. In recent years, the LPO of biological membranes has played a special role in the pathogenesis of various diseases. With a far-reaching process of LPO in phospholipids of biomembranes, the amount of unsaturated fatty acids decreases while increasing the amount of saturated ones.

We studied the effect of drop on the content of MDA, one of the end products of LPO, in the liver of pregnant rats and their embryos (Table. 1 and 2). The effect of drop was studied in the 3rd, 13th and 19th terms of pregnancy and embryonic development. At the same time, it was found that when administered on the 3rd day of pregnancy in rats, an increase in enzymatic and non-enzymatic LPO was observed, respectively, by 60 and 50% in microsomes. Poisoning of rats on the 13th day of pregnancy led to an increase in the content of MDA in microsomes by 50 and 37%, respectively, when pregnant rats were primed on the 19th day of pregnancy, the level of MDA increased in microsomes - by 47 and 45%.

The high content of enzymatic LPO in liver microsomes can be explained by the fact that with NADP.The H-dependent LPO substrate in microsomes are polyunsaturated fatty acids -

phospholipid acyls localized near the components of the electron transport chain, and with ascorbate-dependent - other fatty acids (Table 1).

Table 1. Content of LPO products in the liver microsomes of pregnant rats under the action of drop (mmol/mg protein).

Variants	NADPH-dependent LPO			Ascorbate-dependent LPO		
	Pregnancy days					
	3	13	19	3	13	19
	Microsomes					
Control	0.205+0.028	0.184+0.032	0.200+0.020	0.188+0.025	0.178+0.025	0.209+0.021
Dropp	0.302+0.037	0.280+0.038	0.295+0.022	0.286+0.031	0.253+0.030	0.306+0.025

Similar changes in the level of oxidation products were observed in the liver microsomes of embryos poisoned by drop on the 3rd, 13th and 19th days of development.

On the 3rd day of development, the level of enzymatic and non-enzymatic POL in the liver microsomes of embryos increased by 60 and 50%. On the 13th day, the level of enzymatic and non-enzymatic POL increased by 44 and 37% in microsomes. On the 19th day, the enzymatic LPO in microsomes increased by 47%, respectively, and ascorbate-dependent - by 33%. At the same time, the content of enzymatic POL and ascorbate-dependent POL in microsomes was significantly higher than in the control group.

When poisoning with the pesticide drop, there is an intensification of NADP.N- and ascorbate-dependent LPO in liver microsomes of pregnant rats and their embryos. The MDA level is higher in case of drop poisoning on the 3rd day of pregnancy (Table 2).

Table 2. Content of POL products in the liver microsomes of embryos under the action of drop (mmol/mg protein).

Variants	NADPH-dependent LPO			Ascorbate-dependent LPO		
	Development days					
	3	13	19	3	13	19
	Microsomes					
Control	0.308±0.032	0.325±0.027	0.348±0.035	0.328±0.038	0.313±0.031	0.359±0.032
Dropp	0.426±0.052	0.460±0.052	0.508±0.041	0.438±0.048	0.390±0.040	0.479±0.038

Thus, the oxidation products of dropp stimulate peroxidation reactions in the membranes of the microsomes of the liver of the maternal organism and the embryo, which leads to their damage. Apparently, under the action of these pesticides, significant violations of the structural arrangement of microsome membranes occur, which create conditions for intensive oxidation of membrane lipids. The increase in the same NADF.H-dependent liver LPO in the early stages of embryogenesis is probably due to the fact that during these periods the content of the oxidation substrate - PUFA increases in the liver microsomes of embryos.

The enzyme system of microsomal oxygenases plays an important role in the metabolism of chemical carcinogens. The terminal site of this system is the cytochrome P-450 enzyme, which plays a major role in the biotransformation of foreign substances [17]. The changes we found in the lipid matrix of microsomal membranes under the action of pesticides suggest changes in the properties of the cytochrome P-450 enzyme complex.

In the following experiment, the effect of dropp on the content of cytochrome P-450 in the microsomal fraction of the liver of pregnant rats was investigated (Table 3). When dropa was introduced into the body of pregnant rats on the 3rd, 13th and 19th days of pregnancy, the content of cytochrome P-450 in the microsomal fractions of the liver decreased. When rats were poisoned with dropp, the content of cytochrome P-450 decreased by 24% on the 3rd day

of pregnancy, by 13% on the 13th day, and by 23% on the 19th (Table 3).

Table 3. Effect of drop on the content of cytochrome P-450 in microsomal fractions of the liver of pregnant rats, nmol/mg of protein.

Variants	Terms of pregnancy, days		
	3-day	13- day	19- day
Control	0.930±0.084	0.910±0.072	0.920±0.065
Drop	0.710±0.057	0.800±0.065	0.650±0.064

4 Conclusions

As can be seen from the above data, activation of POL processes causes degradation of cytochrome P-450 and weakening of microsomal oxidation processes. The metabolic activation of xenobiotics, leading to the formation of free radicals, is carried out in the electron transport chain of the endoplasmic reticulum. Cytochrome P-450 plays a key role in this. With the introduction of droppa, the content of cytochrome P-450 in the microsomal fractions of the liver of pregnant rats decreases. A more pronounced decrease in the content of this enzyme is observed with drop poisoning on the 3rd and 19th days of pregnancy. In this regard, our data are consistent with the results of earlier studies, according to which the processes of microsomal oxidation and LPO are alternative: activation of one of them leads to suppression of the other. Inactivation of cytochrome P-450 confirms the violation of the processes of LPO in the membranes of microsomes and, in our opinion, plays a leading role in the mechanism of pathology development.

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