Original Article

Perspective for using 2,6-dimethylpyridine-N-oxide to reduce the toxic effect of xenobiotics in mammals

Olesia Vasetska[1*], Petro Zhminko[1], Mykola Prodanchuk[1], Anatilyi Galkin[2], Victoria Tsygankova[2]

[1] L. I. Medved Research Center for Preventive Toxicology, Food and Chemical Safety, Ministry of Health, Ukraine (State Enterprise)", Kyiv, Ukraine. [2] V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry, National Academy of Sciences of Ukraine, Kyiv, Ukraine.

Correspondence: Olesia Vasetska, L. I. Medved Research Center for Preventive Toxicology, Food and Chemical Safety, Ministry of Health, Ukraine (State Enterprise)", Kyiv, Ukraine. o.vasetska.medved@gmail.com

ABSTRACT

This work aimed to study the isolated and subsequent effects of 2,6-Dimethylpyridine-N-oxide (Ivin) and 2,4-D-2-EHE on the survival of rats and the population characteristics of the total mRNA that implements molecular genetic processes in rats. The studies on Wistar Han (SPF) rats were performed. It was found that Ivin at doses of 13 and 0.013 mg/kg bw, corresponding to 1/100 and 1/100000 DL50, does not cause clinical signs of intoxication in rats. Ivin increases the level of hybridization in mRNA preparations from both the rat liver and blood cells (to a greater degree at a dose of 13 mg/kg bw than at a dose of 0.013 mg/kg bw). On the contrary, after the action of 2,4-D-2-EHE to rats at a dose of 540 mg/kg bw (corresponding to 1 DL_{50}), pronounced intoxication is observed after 6 hours with the following 40% lethal of the animals. After intraperitoneal administration of Ivin to rats, followed (after 24 hours) by oral administration of 2,4-D-2-EHE at the same doses, the viability of rats is preserved, and the degree of hybridization of cytoplasmic mRNA with [α-^{32}P]-cDNA only slightly decreases at the end of the second day, and the third day, the general condition of the animals' body has returned to normal, there was also an increase in the degree of hybridization between the mRNA molecules isolated from the control rats and [α-^{32}P]-cDNA isolated from the experimental rats. Based on the results obtained, the possible molecular genetic mechanisms of action of the studied compounds are discussed.

Keywords: 2,6-Dimethylpyridine-N-oxide, 2,4-D-2-EHE, Toxicity, Hybridization of mRNA with [α-^{32}P]-cDNA

Introduction

Currently, synthetic chemicals are widely used in almost all spheres of human activity, which threatens the life of ecosystems, including human life. In the scientific literature on many chemicals, the mechanisms of their toxic effects on plants and animals are describes in detail. However, the question of the possibility of preventing their negative impact on both ecosystems and humans remains poorly understood. The search for means of protection against the effects of xenobiotics on living organisms is relevant in this regard. The use of natural or non-toxic synthetic chemicals that are capable to neutralize the effects of toxic substances in the organisms' cells can be one of the current approaches to decide this problem.

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In agriculture, for example, intensive technologies based on the application of physiologically active compounds of natural and synthetic origin to increase crop yields and their resistance to abiotic and biotic stress are widely used.

The polycomponent biostimulants (Radostim, Biolan, Regoplant, Stimpo) created in Ukraine with growth regulation and bioprotective effects for agricultural plants and low toxicity for mammals contain the plant growth regulator of synthetic origin - Ivin, whose active component is 2,6-dimethylpyridine-N-oxide [1]. Researches of the mechanisms of action of plant biostimulants based on 2,6-dimethylpyridine-N-oxide showed their impact on the modification of the plant genome which is an important process in the regulation of plant growth, in particular, a change in the matrix availability of DNA, activation of RNA and protein syntheses, in addition to intensifying nutrient

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transport through membranes, due to a change in lipid composition and an increase in membrane permeability [1].

Molecular genetic studies showed that these plant biostimulants significantly increase plant resistance to various pathogenic organisms due to an enhancing synthesis in plant cells of small regulatory si/miRNAs, which silence (inhibit) the translation of mRNAs of pathogenic organisms by the way of RNA interference [1-4]. Polycomponent plant biostimulants can reduce the phytotoxicity of chemical pesticides and enhance the protective natural immune processes in plants, so they can be used to reduce pesticide consumption without reducing their effectiveness for their intended purpose [2, 4-6]. The use of plant biostimulants makes it possible to reduce the negative effect of toxic to human and animal health pesticides and plant growth regulators, which accumulates in soils and agricultural plants [7-10].

It was established that plant biostimulants based on 2,6-dimethylpyridine-N-oxide are low-toxic substances for laboratory animals. The toxicity of 2,6-dimethylpyridine-N-oxide and other methyl derivatives of pyridine-N-oxide is largely due to the oxygen present in the pyridine molecule near the nitrogen atom, the lower lipophilicity, and value of charge on the nitrogen atom [11-13]. There are toxicity increases with a reduction in the charge on the nitrogen atom in the molecules of methyl derivatives of pyridine-N-oxide. The toxicity of complexes of pyridine-N-oxide with organic acids depends on the structure of proton-donors and can be caused by changes in the state of hydrogen bonds with oxygen $N^+ \rightarrow O$ and hydrogen as a result of their interaction [13].

A study of the combined effect of Ivin with pesticides of various chemical groups showed that Ivin reduces the severity of clinical signs of intoxication and acute toxicity of most of the substances tested on animals, prevented or reduced the lethality of rats observed with separate administration of toxic substances at doses corresponding to ½ and $1DL_{50}$ [6, 14]. Subchronic joint oral exposure of Chlorpyrifos at a dose of 5 mg/kg and Ivin at doses of 13.0 and 0.013 mg/kg in rats showed a decrease in the anticholinesterase effect of Chlorpyrifos in the brain tissue, stabilization of the activity of cytolysis enzymes, glucose levels, and serum cholesterol, which contributed to the relief of intoxication. The authors explain this fact by the hepatoprotective and antioxidant properties of Ivin [14, 15].

It was shown that Ivin stabilizes the mitochondrial membranes of hepatocytes, reduces lipid peroxidation in animal liver tissues, intensifies protein-synthetic processes, increases the content of DNA, RNA, and the mitotic index of rat hepatocytes [14, 16-18], which can also lead to a decrease in the toxicity of the pesticide.

The authors of works [14, 15, 19] have found that Ivin causes hepatoprotective and antioxidant effects. It was shown that Ivin is not a promoter of carcinogenesis [20].

The ability of 2,6-dimethylpyridine-N-oxide to alter cytogenetic effects in mouse bone marrow cells caused by the alkylating mutagen Cyclophosphamide was studied. It was established that Ivin did not have a mutagenic effect. When Ivin was administered to mice CD-1 together with Cyclophosphamide, Ivin at low doses significantly reduced the frequency of metaphases with chromosome aberrations (by 55.7-72.9%), the number of chromosome aberrations of chromatid type, polyploid and multi-aberrant cells, which may be associated with the gene-protective effect of Ivin, due to membrane stabilization and antioxidant action [21]. The antimutagenic effect of Ivin was confirmed by research when it is combined with prooxidant mutagen Dioxidine [22].

Since Ivin has low toxicity, high biological activity, lack of long-term effects of action, and the presence of pharmacological properties inherent in adaptogens (hepato- and genoprotective, membrane-stabilizing, and antioxidant effects), we believe that Ivin is promising for deeper research as a protective agent against the negative effects of xenobiotics.

Based on the above, Ivin was selected for our study as a promising protective agent against the action of xenobiotics. The toxic substance 2-Ethylhexyl-2,4-dichlorophenoxy acetate (2,4-D-2-EHE) was used as a model, which, like 2,4-D, causes free radical oxidation of lipids, inactivates some enzymes, interacts with lipids of biological membranes, and has membranolytic and genotoxic effects, damages the DNA [8, 19].

The presented literature data show that 2,4-D and its Ethylhexyl ether have the opposite effect of Ivin on the state of biological membranes, lipid oxidation, protein-synthetic processes, and also on the main markers of the genome state. It is possible that under their combined action Ivin can change the functioning of the cellular genome and, therefore, change the toxicity of 2,4-D-2-EHE.

Consequently, the important task of toxicology is to study the effect of chemicals on molecular genetic processes in the cells of the body, which will be of both theoretical and practical importance.

This work aimed to study the isolated and subsequent effects of Ivin and 2,4-D-2-EHE on the survival of animals and the population characteristics of the total mRNA that implements molecular genetic processes in the cells of the body.

Materials and Methods

We used Ivin (2,6-dimethylpyridine-N-oxide, purity 99.0%) and 2,4-D-2-EHE (2-ethylhexyl-2,4-dichlorophenoxy acetate, purity 95.0%), their chemical structures, names, and molecular weights are shown in **Figure 1.**

Structures	Names	Molecular weights
H_3C N^+ CH_3 O^-	2,6-Dimethyl pyridine N-oxide (**Ivin**)	MW=125.17
Cl O O Cl	2-ethylhexyl-2,4-dichlorophenoxy acetate (**2,4-D-2-EHE**)	MW=333.2

Figure 1. Chemical structures and names of the Ivin and 2,4-D-2-EHE

The experiment was carried out on adult white male Wistar Han rats (180-200 g). Animals were purchased from the SPF nursery of small laboratory animals of the "L. I. Medved Research Center for Preventive Toxicology, Food and Chemical Safety, Ministry of Health, Ukraine (State Enterprise)" and were delivered to the SPF vivarium to maintain animal status.

Acclimatization of animals in the vivarium conditions was carried out within 5 days after acquisition. Throughout the experiment, the rats were kept in the SPF vivarium at a temperature of 21-22°C, air humidity – 40-60%. The lighting was artificial and met the requirements for keeping animals under the conditions of a toxicological experiment. The animals received balanced pelleted food produced by Altromin (Germany) and water (decontaminated, purified, UV-sterilized, deionized) ad libitum. The study was carried out following the principles of bioethics and the requirements of humane treatment of animals (European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, 1986).

Ivin was injected once intraperitoneally in non-toxic doses of 13 mg/kg bw (1/100 DL_{50}) or 0.013 mg/kg bw (1/100000 DL_{50}). 2,4-D-2-EHE was administered orally (as the main route of its entry into the human body with food and water) once at a toxic dose of 540 mg/kg bw (1 DL_{50}). All experimental and control groups included 6 animals each. All animals were examined for clinical symptoms and death. The blood and liver of animals were taken for the study. Blood was collected from the femoral vein of rats after short-term anaesthesia with carbon dioxide into a chemical test tube without anticoagulant. The liver was removed after killing the animals in a CO_2 chamber and frozen at -70°C in Petri dishes.

In the first series of experiments, two animal groups were once intraperitoneally injected with Ivins' physiological saline solution at doses 13 and 0.013 mg/kg bw in a volume of 3 ml per animal. The control animal group was injected once with physiological saline solution in the same volume. Biomaterials (blood and liver) were taken 6, 12, 24, and 72 hours after Ivins' administration.

In the second series of studies, the experimental group of animals was intragastrically injected with a solution of 2,4-D-2-EHE in distilled water with an auxiliary substance OP-10 in a single dose of 540 mg/kg bw in a volume of 1 ml per 100 g of rat body weight. Control animals were similarly injected with a solution of emulsifier OP-10 in distilled water in the same volume. After 6 and 24 hours (respectively, the life periods of short-lived and long-lived mRNAs), biomaterials (blood and liver) were taken in the same way as described above.

In the third series of studies, the experimental rats were once intraperitoneally injected with Ivins' physiological saline solution at non-toxic doses of 13 mg/kg bw or 0.013 mg/kg bw in a volume of 3 ml per animal and after 24 hours, experimental animals were orally administered with 2,4-D-2-EHE at a dose of 540 mg/kg bw in the form of a uniform emulsion with OP-10. The control group of animals was injected intraperitoneally with physiological saline solution in a volume of 3 ml per 1 animal and, after 24 hours, distilled water with OP-10 was orally administered. Biomaterials (blood and liver) were taken 6 and 24 hours after administration, in the same way as described above.

The liver and blood of the experimental animals were selected for the study for the following reasons. The liver plays a multifunctional role in the body. It removes toxic substances that enter the body, synthesizes various structural and functional proteins, and supplies them to the plasma. The blood consisted of different types of cells and many physiologic active substances of endogenous origin, penetrates all tissues of the body, delivering them nutrients, oxygen, and regulatory elements for various purposes to coordinate cell functions and helps to remove catabolic products from the body.

To solve molecular genetic issues, a specific test was used in the work – the synthesis of mRNA, as a kind of highly sensitive indicator that quickly responds to internal and external signals entering the body's cells, in the form of synthesis of certain types of RNA with a lifetime corresponding to the duration of the signal.

It is known that in cells of living organisms, mRNAs account for only 2 to 4% of the total mass of RNA [23]. The rest of the RNAs (98-96%) are nuclear RNAs, ribosomal RNAs, transport RNAs, and small regulatory si/miRNAs. Although the percentage of mRNAs is small, their diversity is very large. mRNAs are classified as low-component RNAs, while ribosomal RNAs and transport RNAs are polycomponent. Each mRNA molecule is unique in its nucleotide sequence, molecular weight, spatial organization, lifespan (from 1 minute to a day or more).

Taking into account the population differences of mRNA molecules in this work we investigated levels of homology between populations of cytoplasmic mRNA isolated from the liver and blood of experimental and control rats that were determined according to the degree of hybridization (%) between cytoplasmic mRNA isolated from control rats with [α-^{32}P]-cDNA from experimental rats using dot-blot hybridization method [3, 24-26].

Isolation of total RNA from rat liver and blood cells was conducted using the method described in detail in our previously published works [3, 25, 26]. The polymericity of the isolated total RNA preparations was analyzed by electrophoresis in a 1.5% agarose gel in the presence of 7M urea (gels were stained with ethidium bromide solution before photographing RNA fractions under UV light) using the Locker method [3, 25, 26]. As described in detail in our works [3, 25, 26], using total RNA chromatography on the oligo(dT)-cellulose columns, Poly(A)$^+$mRNA (that is, mRNA) separation from Poly(A)$^-$ mRNA molecules was done. The isolated cytoplasmic poly(A)$^+$mRNA purity was analyzed using the Northern-blot method [3, 24-26] according to which electrophoresis fractionation of the poly(A)$^+$RNA preparation in an agarose gel in the formalin presence (5% solution of formaldehyde) was done; electrophoretically pure poly(A)$^+$RNA fractions were transferred on nitrocellulose filters and hybridized with single-stranded [α-^{32}P]-cDNA.

Single-stranded [α-^{32}P]-cDNA was synthesized in vitro transcription reaction on poly(A)$^+$mRNA template using reverse transcriptase and [α-^{32}P]-labeled dCTP as described in detail in our works [3, 25, 26]. The hybridization mixture

electrophoretically pure poly(A)$^+$RNA fractions was transferred on nitrocellulose filters and hybridized with synthesized single-stranded [α-^{32}P]-cDNA contained 50% formamide, 5X Upper buffer (pH=7.0), 5X Denhardt's solution (5g ficoll, 5g polyvinylpyrrolidone, 5 g BSA (Penta, Fraction V), 500 ml H_2O), denaturated calf thymus DNA (100 mg/ml), poly(A)$^+$RNA (1 mg/ml), 0.1% SDS, and [α-^{32}P]-cDNA ($1{,}5 \cdot 10^8$cpm) [3, 25, 26]. After hybridization (throughout the day), the filters were carefully washed free from the exogenous label and exposed for 24 h to X-ray film PM-1 with an intensifying screen (at –70°C). The sizes of the poly(A)$^+$RNA limits were evaluated according to radioautograph distribution of labeled poly(A)$^+$RNA-[α-^{32}P]-cDNA hybrids along the gel lanes (concerning marker polynucleotides and fractions of total RNA) [3, 25, 26].

Dot-blot hybridization [α-^{32}P]-cDNA with mRNA. The mRNA preparations of control rats were subjected to hybridization with [α-^{32}P]-cDNA of experimental rats using dot-blot hybridization method [3, 25, 26]. for the purpose to study levels of homology between populations of mRNA from experimental and control rats.

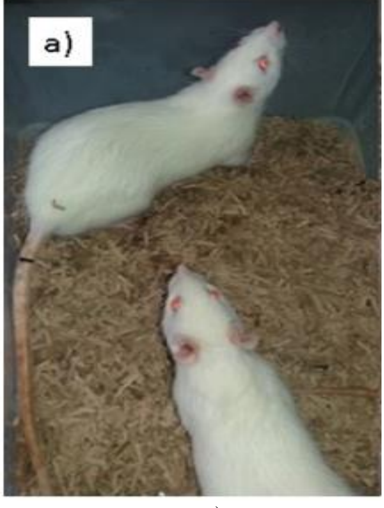
Hybridization experiments were performed on Millipore AP-15 glass fiber filters (Amersham-Pharmacia Biotech, UK) to avoid potential loss of nucleic acids, after which the filters were dried, and using Beckman LS-100C scintillation counter in a toluene-based scintillation fluid containing a fluorescent reagent 2,5-diphenyloxazole (PPO), we measured the radioactivity of hybrid molecules per 20 μg ±SD of mRNA [3, 25, 26].

The levels of homology between populations of cytoplasmic mRNA isolated from experimental and control rats were determined according to the degree of hybridization (%) between cytoplasmic mRNA from control rats and [α-^{32}P]-cDNA from experimental rats. The degree of "self-hybridization" between cytoplasmic mRNA from control rats and [α-^{32}P]-cDNA from control rats was taken as 100%.

Statistical analysis. All experiments were performed in three replicates. Using dispersive Student's t-test with the significance level at $P<0.05$, statistical analysis of the data was performed; the values are mean ±SD [27].

Results and Discussion

The control and experimental rats exposed to the isolated injection of 2,4-D-2-EHE and sequential injection of Ivin and 2,4-D-2-EHE are shown in **Figure 2**.



a)

b)

c)

Figure 2. Clinical signs of intoxication of rats during exposure to Ivin and 2,4-D-EHE:
a) – Control (intact rats);
b) – 2,4-D-EHE (dose – 540 mg/kg) after 48 hour;
c) – Ivin (dose 13 mg/kg) + 2,4-D-2-EHE (dose 540 mg/kg) after 24 hour

The single intraperitoneal injection of Ivin at doses of 13 mg/kg bw or 0.013 mg/kg bw during 72 hours showed that there were no clinical signs of intoxication and no death of the animals was

observed. The general condition and behavior of the experimental rats did not differ from the control animals **(Figure 2a)**.

Oral administration to rats with 2,4-D-2-EHE at a dose of 540 mg/kg bw after 6-24 hours resulted in decreased locomotion, salivation, nasal discharge, shortness of breath, hunched back, ataxia, tail rigidity, diarrhea; after 48 hours, the body lateral position, hind limbs paresis and enuresis were also found **(Figure 2b)**. And after 72 hours the death of animals was noted, which amounted to 40%.

The same dose of 2,4-D-2-EHE, but after preliminary administration of Ivin to animals at a dose of 13 mg/kg bw, after 6 hours caused a decrease in activity, tousled fur, impaired coordination of movements, after 24 hours in some rats also revealed enuresis, arching of the back. The state of the animals returned to normal on the second day of the study **(Figure 2c)** and no death of the animals was observed. Against the background of Ivin, which was used at a dose of 0.013 mg/kg bw, 2,4-D-2-EHE for 24 hours caused a decrease in activity and only slightly tousled fur. The death of animals was not found. The state of the animals returned to normal on the second day of the study.

Thus, there were no pronounced clinical signs of intoxication (ataxia, tail rigidity, and hind limbs paresis), when 2,4-D-2-EHE was administered with Ivin at both doses. The lethality of the rats was not found. Animals' state returned to normal earlier than in the animals that received only 2,4-D-2-EHE. The data obtained indicate that Ivin in doses 13 and 0.013 mg/kg bw neutralizes the manifestations of intoxication and prevents 2,4-D-2-EHE-induced lethality in animals.

The results of dot-blot hybridization between cytoplasmic mRNA from control and [α-^{32}P]-cDNA from experimental rat liver and blood cells after once Ivins' intraperitoneal injection are presented in **Figures 3** and **4**.

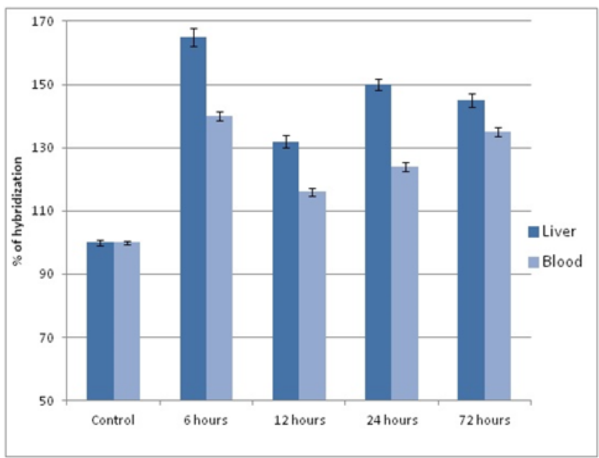


Figure 3. The degree of hybridization (%) between cytoplasmic mRNA isolated from control and [α-^{32}P]-cDNA from experimental rat liver and blood cells after effect of Ivin (dose - 13 mg/kg) as compared to the degree of hybridization between cytoplasmic mRNA and [α-^{32}P]-cDNA isolated from control rat liver and blood cells

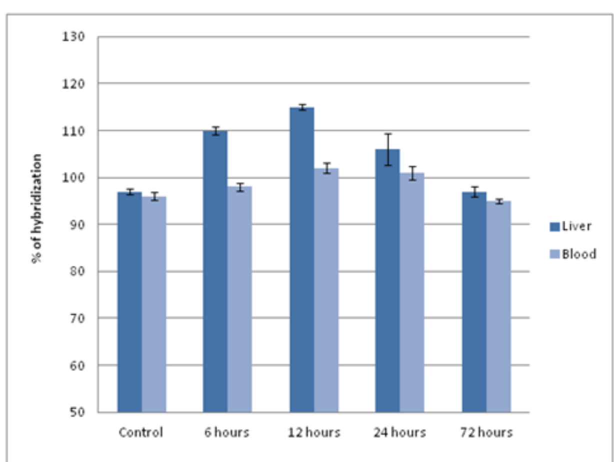


Figure 4. The degree of hybridization (%) between cytoplasmic mRNA isolated from control and [α-^{32}P]-cDNA from experimental rat liver and blood cells after effect of Ivin (dose - 0.013 mg/kg) as compared to the degree of hybridization between cytoplasmic mRNA and [α-^{32}P]-cDNA isolated from control rat liver and blood cells.

Under the isolated action of Ivin at a dose of 13 mg/kg bw during the study period, the significant increase in the degree of hybridization between cytoplasmic mRNA isolated from control rats and [α-^{32}P]-cDNA from experimental rats was observed **(Figure 3)**.

The greatest increase in the degree of hybridization between mRNA and [α-^{32}P]-cDNA isolated from rat liver cells was detected 6 and 24 hours after Ivins' administration, by 69% and 55%, respectively, compared to the control. The degree of hybridization between cytoplasmic mRNA isolated from control and [α-^{32}P]-cDNA from experimental liver cells after 12 and 72 hours after Ivins' administration was lower and amounted to 36% and 48%, respectively, compared to the control.

At the same time, the degree of hybridization between cytoplasmic mRNA isolated from control and [α-^{32}P]-cDNA from blood cells of the experimental rats was less pronounced than in liver cells. The increase of the degree of hybridization between cytoplasmic mRNA isolated from control and [α-^{32}P]-cDNA from experimental rat blood cells was 45%, 21%, 28%, and 39% after 6, 12, 24, and 72 hours, respectively, compared to the control.

When Ivin was injected intraperitoneal at a dose of 0.013 mg/kg bw, similar changes were observed in the population characteristics of expressed mRNA in the liver and the blood **(Figure 4)**.

But, the degree of hybridization between [α-^{32}P]-cDNA from liver and blood cells of the experimental rats and cytoplasmic mRNA isolated from control was less expressed than with a higher dose of Ivin. Population changes in mRNA in liver cells were the more noticeable after 6 and 12 hours after Ivins' administration, the degree of hybridization between [α-^{32}P]-cDNA from the experimental rat liver cells in these time intervals and cytoplasmic mRNA isolated from the control was 14% and 19%, respectively, compared to the control. After 24 hours the degree of hybridization between cytoplasmic mRNA

isolated from the control and [α-^{32}P]-cDNA from the experimental rat liver cells was 9 % higher, and after 72 hours after Ivins' administration it did not differ from the control.

The degree of hybridization between cytoplasmic mRNA isolated from the control and [α-^{32}P]-cDNA from the experimental rat blood cells was insignificant, and significant differences were observed only after 12 and 24 hours after Ivins' administration, and amounted to 6% and 5%, respectively, concerning the control. Thus the identified population changes in mRNA from liver and blood cells evidence that Ivin activates the synthesis of additional short-lived mRNAs, which indicates a protective effect of Ivin at the genetic level.

Population changes in mRNA isolated from liver and blood cells of experimental rats, which were sequentially administrated orally with 2,4-D-2-EHE and Ivin are shown in **Figures 5** and **6**.

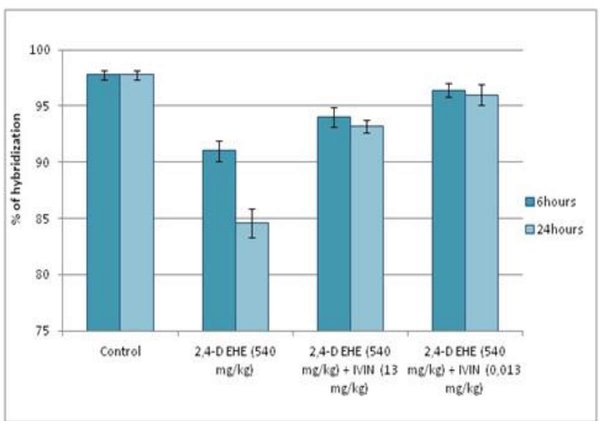


Figure 5. The degree of hybridization (%) between cytoplasmic mRNA isolated from control and [α-^{32}P]-cDNA from experimental rat liver cells after effect of 2,4-D-2-EHE (dose - 540 mg/kg), 2,4-D-2-EHE (dose - 540 mg/kg) + Ivin (dose - 13 or 0.013 mg/kg) as compared to the degree of hybridization between cytoplasmic mRNA and [α-^{32}P]-cDNA isolated from control rat liver cells.

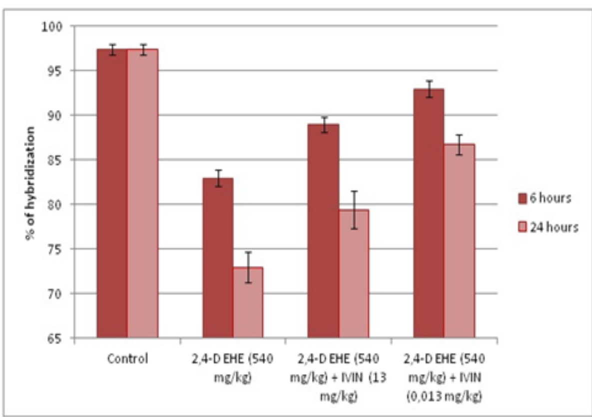


Figure 6. The degree of hybridization (%) between cytoplasmic mRNA isolated from control and [α-^{32}P]-cDNA from experimental rat blood cells after effect of 2,4-D-2-EHE (dose - 540 mg/kg), 2,4-D-2-EHE (dose - 540 mg/kg) + Ivin (dose - 13 or 0.013 mg/kg) as compared to the degree of hybridization between cytoplasmic mRNA and [α-^{32}P]-cDNA isolated from control rat blood cells.

It was shown that under the isolated administration of 2,4-D-2-EHE at a dose 540 mg/kg bw, in contrast to Ivin, the mRNA synthesis is suppressed, as evidenced by a significant decrease in the degree of hybridization between cytoplasmic mRNA isolated from control and [α-^{32}P]-cDNA from experimental rat liver and blood cells.

The decrease in the degree of hybridization between [α-^{32}P]-cDNA from experimental rat liver cells after 6 and 24 hours and cytoplasmic mRNA isolated from control was 7% and 14%, respectively, compared to the control **(Figure 5)**. The degree of hybridization between [α-^{32}P]-cDNA from experimental rat blood cells and cytoplasmic mRNA isolated from control also decreased after 6 and 24 hours by 15% and 25%, compared to the control **(Figure 6)**.

When rats were administered with 2,4-D-2-EHE at doses of 540 mg/kg bw in combination with Ivin at doses of 13 mg/kg bw and 0.013 mg/kg bw **(Figures 5 and 6)**, the degree of hybridization between cytoplasmic mRNA isolated from control and [α-^{32}P]-cDNA from experimental rat liver and blood cells was reduced also, but to a lesser degree than after isolated administration of 2,4-D-2-EHE, compared to the control.

Thus, following the combined action of 2,4-D-2-EHE with Ivin on rats at a dose of 13 mg/kg bw after 6 and 24 hours, the degree of hybridization between cytoplasmic mRNA isolated from control and [α-^{32}P]-cDNA from experimental rat liver cells significantly decreased by 4% and 5%, respectively **(Figure 5)**, and the degree of hybridization between cytoplasmic mRNA isolated from control and [α-^{32}P]-cDNA from experimental rat blood cells significantly decreased by 9% and 19%, respectively **(Figure 6)**, compared to the control.

When rats were administrated with 2,4-D-2-EHE 540 mg/kg bw together with Ivin at a dose of 0.013 mg/kg bw after 6 and 24 hours, the degree of hybridization between cytoplasmic mRNA isolated from control and [α-^{32}P]-cDNA from experimental rat liver cells decreased significantly by 1.4% and 2%, respectively, compared to the control. The degree of hybridization between cytoplasmic mRNA isolated from control and [α-^{32}P]-cDNA from experimental rat blood cells decreased less significantly by 4.5% (after 6 hours) and 11% (after 24 hours), respectively, compared to the control.

Summarizing the obtained research results, we can conclude that Ivin increases and 2,4-D-2-EHE decrease the degree of hybridization between cytoplasmic mRNA isolated from control and [α-^{32}P]-cDNA from experimental rat liver and blood cells. When rats were sequentially administrated with Ivin and 2,4-D-2-EHE, the degree of hybridization between cytoplasmic mRNA isolated from control and [α-^{32}P]-cDNA from experimental rat liver and blood cells was similar to the degree of hybridization between cytoplasmic mRNA and [α-^{32}P]-cDNA isolated from control rat liver and blood cells. As a result, the toxic effect of 2,4-D-2-EHE was neutralized.

Based on the results of studies and literature data, we can assume the mechanisms of Ivin's protective action against toxic damage to the body by chemical compounds.

The increase in the activity of Ivins' antioxidant system, its hepatoprotective and membrane-stabilizing effect, proven in several experimental studies [14, 15, 17-19] may be one of Ivins' protective mechanisms.

Concerning molecular genetic mechanisms, it is possible to propose that the direct or indirect action of the studied xenobiotics may change the functioning of the genome, which determines and controls life processes in cells, as well as the inheritance of fundamental and acquired in the process of life traits specific to each species. It is known that these processes involve the realization of information encoded in genes throughout mRNA into the final products of gene expression - proteins. Nevertheless, the question of the mechanisms of regulation of gene expression, especially in higher organisms, remains poorly understood, due to the complex structure of their genomes, the presence of intergenic, intergenomic, and intercellular interactions, and interactions of a higher order between organs in the whole organism.

There is also the possibility of an indirect nonspecific action of synthetic compounds through the processes of "methylation-demethylation" of DNA, which are related to the main processes in the epigenetic regulation of gene expression in eukaryotic cells [28, 29].

Indeed, such processes are the basis for the response of genomes to the negative or positive effects of chemical xenobiotics. Based on these prerequisites, it can be assumed that the action of 2,4-D-2-EHE can lead (as a protective reaction of the organism) to an increase in the methylation level of functionally active DNA regions, as well the competitive effect of Ivin on the DNA demethylation.

This assumption is confirmed by the absence of the effect of 2,4-D-2-EHE on the studied parameters (viability of animals, population characteristics of mRNA) after preliminary administration of Ivin.

There are other possible mechanisms for leveling (neutralizing) the toxic effects of one chemical compound with another. Competitive mechanisms of Ivin's interaction with target proteins can play an important role in this process due to the more pronounced affinity of its stereochemical configuration. In addition, an increase in gene expression could enhance the synthesis of protein-enzymes that play a key role in the biotransformation and detoxification of xenobiotics in mammals [30].

Conclusion

Summarizing the results obtained, we can distinguish between clinical signs of intoxication caused by 2,4-D-2-EHE and population changes in mRNA from liver and blood cells of rats under the isolated and combined action of 2,4-D-2-EHE and Ivin. The following conclusions were made:

1. It was found that at the separated administration of rat males by Ivin and 2,4-D-2-EHE, Ivin increases, and 2,4-D-2-EHE decreases the degree of hybridization between [α-^{32}P]-cDNA isolated from experimental rat liver and blood cells and cytoplasmic mRNA isolated from control, which indicates their contrasting effects on transcription processes, and, consequently, on the genome.
2. Ivin neutralizes the toxic effect of 2,4-D-2-EHE when administrated together, therefore, the clinical signs of intoxication and the lethality of rats caused by 2,4-D-2-EHE are reduced. This may be due to Ivin's influence on the genome, as evidenced by the tendency towards normalization of the degree of hybridization between [α-^{32}P]-cDNA isolated from experimental rat liver and blood cells and cytoplasmic mRNA isolated from control, which is similar to the degree of hybridization between cytoplasmic mRNA and [α-^{32}P]-cDNA isolated from control rat liver and blood cells.
3. The effects of chemical compounds at the genetic level may include a variety of mechanisms. The proposed mechanisms in the presented work are direct or indirect effects on the genome, the interaction of chemicals with target proteins due to the pronounced affinity of their stereochemical configuration, the effect of chemicals on DNA methylation-demethylation processes, increased expression of genes encoding the synthesis of proteins-enzymes which play a key role in biotransformation and detoxification of xenobiotic in mammals, require experimental confirmation and may be the subject of further research.

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