Study of mutagenic activity of triazine herbicides in the test of microorganisms / plants

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Abstract. The aim of the study is to explore the mutagenic activity of triazine herbicides in the system bacteria/plant homogenate. Chlorinated triazine herbicides atrazine, propazin, simazine, cyanazine, trietazin that are inactive in direct scan, can be activated into mutagens by the used vegetable microsomal systems. Plant homogenates were prepared from 5-day-old pea germs, wheat, corn, barley, cucumber and spiderwert. In the experiment T more sensitive plasmid strains TA98 and TA100 of S.typhimurium were used. The results showed that propazin, atrazine, simazine, cyanazine and trietazin are plant promutagens and their mutagenic properties appear only in the presence of plant enzymes. The greatest activating effect had homogenates of barley and spiderwert. The relative specificity of their action was revealed: barley homogenate better activates propazin, atrazine and simazine, and homogenate of spiderwert - cyanazine and trietazin.

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Introduction

Relatively new area of the genotoksikology is the explore of the mutagenic activity of the biotransformation products of pesticides in the body of plants [1].

It is revealed that the enzyme systems of plants are capable of modifying the mutagenic effects of chemicals determined in tests with microorganisms that can be expressed in the removal, strengthening or weakening of their activity and even mutagenic transformation into mutagens [2-6].

Methods

In this study were used indicator systems: indicator strains: Salmonella typhimurium: TA-98 his D3052, rfa, uvr B, pKM 101; TA 100 - his G46, rfa uvr B pKM 101, germs of barley, peas, wheat, corn, cucumbers and Spiderwert.

During the study the following methods were used:

1.semiquantitative method of counting for gene mutations in indicator bacteria S. typhimurium. It was used pans Ames test. Mutagenic effect was taken into account by the multiplicity of exceeding the number of revertants induced by drugs over the spontaneous background [7];

2. Preparation of plant activation mixture. Plant germs were grown for 5 days under laboratory conditions with natural photoperiod, and then the plants were harvested, crushed and frozen. The plant material was homogenized in a mortar, adding a double volume of phosphate buffer (pH 7.4) and centrifuged for 15 minutes at 9000g and the temperature 2-4^0 C. The supernatant liquid was collected and used immediately in the experiment;

3.semiquantitative keeping of gene mutations with activated drugs enzyme systems of plants in conditions in vivo. Plant germs were treated with various concentrations of the triazine herbicide for two weeks and then prepared a homogenate from these plants, and then determined mutagenic activity on the indicator bacteria.

The main part

To determine the most effective plant system as a model plant promutagen propazine drug was used. Plant homogenates prepared from 5-day-old pea, wheat, corn, barley, cucumber and Spiderwert germs [8,9]. In the experiment were used a more sensitive plasmid strains TA98 and TA100 S.typhimurium. Experiments' data are shown in Table 1.

It turned out that the number of revertants strain TA98 induced by propazine exceeds the control if using homogenates of pea germs in 6, wheat - 6.4, corn - 6.9, cucumbers - 8.2, Spiderwert - 10.2, barley - 11.4 times, i.e. the greatest activating effect have homogenates of barley, cucumber and Spiderwert. Therefore, we used homogenates of three plants to study the mutagenic activity of the triazine herbicides (Table 2).

Direct mutagens (prometrin, semeron, simetrin, metoprotrin, igran, simeton, noraton, ipaton, primatol) in tests in Salmonella / plants microsome were decontaminated by plants homogenates.

Table 1. Mutagenic activity of propazin in the Ames test with activation of S-9 fractions of various plant

Plants	Concentrationof propazine mg/ml	Amount of colony of revertrants	
		TA98	TA100
Pea	10	138±8,5	280±17,0
	1	125±7,4	225±14,0
	0.1	78±5,3	180±10,0
	control	22±1,2	112±6,6
Wheat	10	140±9,3	272±18,0
	1	120±8,2	256±16,0
	0.1	65±3,8	204±13,0
	control	22±1,3	112±6,4
Con	10	152±8,8	310±20,0
	1	126±7,9	291±18,0
	0.1	63±3,9	264±17,0
	control	22±1,2	112±6,8
Cucumbers	10	182±11,0	341±18,0
	1	138±9,3	290±19,0
	0.1	67±4,5	245±16,0
	control	22±1,2	112±6,2
Spiderwert	10	223±15,0	362±21,0
	1	170±11,0	322±18,0
	0.1	90±5,2	260±17,0
	control	22±1,1	112±7,0
Barley	10	250±17,0	380±23,0
	1	195±12,0	338±22,0
	0.1	132±8,1	280±17,0
	control	22±1,1	112±7,0

However, it appeared that the chlorinated triazine herbicides: atrazine, propazin, simazine, cyanazine, trietazin that are inactive in direct scan, can be activated into mutagens, used by vegetable microsomal systems.

Experimental results show that the most effective plant systems are homogenates of barley and Spiderwert. Moreover, the efficiency of plant systems depends on the drug. So, for atrazine, simazine, and propazin activating effect of barley homogenate is higher than of Spiderwert and maximum induction ratio (K_I-multiplicity of exceeding the number of induced reversion compared with control) was 5.9, 7.9 and 11.2, respectively.

Conversely, for cyanazine and trietazin more effective was the homogenate of Spiderwert. The maximum number of induced colonies for revertants in strain TA98 exceeds the control in 5.4 and 6.3 times, whereas in the system of bacteria / barley microsome - only in 4.2 and 5 times.

Table 2. The mutagenic activity of triazines in the Ames test with activation by S-9 fractions of plants in vitro

pesticides	Concentration mg/ml	Amount of colony of revertants					
		S. typhimurium TA98			S. typhimurium TA100		
		barley	cucumbers	spiderwert	barley	cucumbers	spiderwert
atrazine	10	143±8,3	102–6,0	113±7,3	370–25,0	342±21,0	327±21,0
	1	125±11,0	91=5,6	97±6,1	336=19,0	307±20,0	310±22,0
	0,1	98±6,1	74=4,2	83±5,5	278=18,0	275±17,0	260±15,0
simazine	10	190±12,0	158–9,9	175±12,0	402=27,0	398±27,0	360±22,0
	1	166±9,7	130=7,5	98±6,3	367=22,0	359±22,0	336±23,0
	0,1	120±7,6	126..7,8	76±4,0	299..18,0	280±19,0	276±14,0
cyanazine	10	100±6,3	90=5,8	129±7,6	325=19,0	282±19,0	354±24,0
	1	85±5,2	61=3,8	96±5,6	274=18,0	246±16,0	298±19,0
	0,1	51±3,2	42=2,3	63±3,7	219=14,0	192±13,0	231±15,0
trietazin	10	122±7,1	113=7,2	151±10,0	350=23,0	300±17,0	375±25,0
	1	103±7,0	97..6,3	116±7,8	314..18,0	264±14,0	341±20,0
	0,1	59±4,1	43=2,9	68±4,6	232=14,0	210±13,0	254±17,0
propazin	10	250±17,0	182=11,0	223±15,0	390=23,0	141±18,0	375±25,0
	1	195±12,0	138=9,3	170±11,0	338=22,0	290±19,0	341±20,0
	0,1	132±8,1	67=4,5	90±5,2	280=17,0	245±16,0	254±17,0
control		24±1,2	24–1,3	24±1,1	110–6,2	110±6,9	110±6,3

Most sensitive from the used testers to the herbicides strain TA 98. Mutation induction level if activated the strongest drug propazin on the strain TA 98 was 11, and on 3.4 TA- only 100. Therefore, in subsequent experiments, this strain was used.

Results of the experiments on the activation of triazine herbicides in plants in vivo have shown that they are activated to a mutagen and the effect was even higher than in experiments in vitro. It appears that under these conditions it becomes possible to determine the mutagenic activity of all of the metabolites while under conditions in vitro, where the contact with the drug was by the microsomal fractions in the cup where it was considered mutagenic activity only of short-soluble metabolites.

Further experiments were conducted to determine the fate of the mutagenicity of plant metabolites of pesticides in animal activation conditions. Under these conditions, in the reaction mixture along with the indicator bacteria the extract obtained from the pretreated from barley plants triazines and the microsomal fraction of rat liver S9 were added. Adding of the rat liver microsomal fraction has no significant effect on the mutagenic activity of plant metabolites. In the triazine compounds which are able to activate in plants, animal after the activation level of the mutagenic effect is not changed.

This indicates that the mutagenic metabolites of triazine herbicides in plants formed from promutagens, when they ingested in mammals, do not detoxicate. Consequently, their potential genetic risk to the human body increases [10].

Conclusions

From the results of the study the following conclusions were made:

propazin, atrazine, simazine, cyanazine and trietazin are plant promutagens, their mutagenic properties appear only in the presence of plant enzymes;

homogenates of barley and Spiderwert have the greatest activating effect. The relative specificity of

the above-mentioned plants was revealed: barley homogenate better activates propazin, atrazine and simazine, and homogenate of Spiderwert - tsianzin and trietazin.

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