ENZYMATIC ACTIVITY OF THE INTESTINE IN EFFECT OF PESTICIDES OF PYRETHROID GROUP

The purpose of investigation was to study the effect of pesticides from group of pyrethroids (e.g. decis) on the enzymatic function in the homogenate of the mucosal membrane of the proximal and distal segments of the small intestine.

Determination of the degree of the activity of hydrolytic enzymes in the homogenates of some parts of the intestine allowed to show effect of pesticide decis on the gradient of distribution of enzymatic activity along the intestine. For characteristic of the enzymatic activity of the small intestine there was performed study of the activity of dipeptidase, amylase, invertase and alkaline phosphatase in the homogenate of the mucosal membrane from proximal and distal parts in multiple effects of pesticide decis in toxic dose (1/20 LD50) during 4 months.

Changes of the enzymatic activity in acute poisoning were depended on the time of pesticide exposure and site of the bowel. The different digestive enzymes have different response to effect of pesticides of pyrethroid group (decis), there are differences in the reactions of proximal and distal part of the small intestine and there is correlation between changes of activity of the majority hydrolases and administered dose of pesticides.

**Keywords:** Small intestine, enzymes activity, dipeptidase, amylase, invertase, alkaline phosphatase, pesticide Decis

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Disorders of digestion in human and animals, as it is known, may be related both with defects of the membrane hydrolysis and with disorders at the stage of interaction between digestive and transport processes (Dudrikova et al., 1992; Labana et al., 2001a).

In particular, there are data about changes of membrane digestion as a result of various disorders of synthesis and translocation of the enzymes on the mucosal surface under effect of some harmful chemical and physical factors (Gera et al., 2009).

It is also known that enzyme-synthesizing systems of various parts of intestine have different responses to the effect of exogenic and endogenic factors.

The above-presented data about the state of digestive function of the intestine under effect of various factors show that the problem of the effect of toxic substances including pesticide Decis from the group of pyrethroids on the enzymatic fraction of the intestine is very significant (Labana et al., 2001b; Timofeeva et al., 1971).

The purpose of investigation was to study the effect of pesticides from group of pyrethroids (e.g. decis) on the enzymatic function in the homogenate of the mucosal membrane of the proximal and distal segments of the intestine.

In order to give characteristic of the fermentative activity of the intestine the activity of dipeptidase, amylase, invertase and alkaline phosphatase in the homogenate of the intestine mucosa was studied under the effect of pesticide decis.

**Methods**

Determination of the degree of the activity of hydrolytic enzymes in the homogenates of some parts of the intestine allowed showing effect of pesticide decis on the gradient of distribution of enzymatic activity along the intestine.
For understanding of the hydrolytic functions of the intestine there were selected 3 groups of enzymes. Group 1 included enzymes breaking up carbohydrates (invertases and amylases); group 2 comprised of enzymes hydrolyzing peptides (peptidase) and group 3 consisted of alkaline phosphatase related to the group of hydrolysis and taking an active part in the hydrolysis of phosphoric acid.

The experiments were carried out on the white rats-males with body mass 150-190 g. The animals were injected water suspension of pesticide decis intragastric one time (acute experiment) and many times (chronic experiment). The animals of the first group were injected this preparation in single dose 116 mg/kg (3/4 LD50); the animals were killed in 1,2,7 and 15 days after pesticide administration.

The animals of group II underwent the effect of pesticide in dose 1/20 LD50 (7.75 mg/kg) every day for 120 days. The animals of group III were control because they were not on exposure to the pesticide.

Every time of investigations the studied and control animals were killed and there was measured activity of enzymes invertase, amylase, dipeptidase and alkaline phosphatase in the homogenate of the mucosa of the proximal and distal parts of intestine.

Determination of the enzymatic activity, participating in the intestinal digestion was performed with use of the following methods: total amylolytic activity by SmythGRoy in modification of Ugolev et al. (1969); invertasa was measured by increase in gexoz with method of Nelson in modification of Ugolev (Ugolev et al.,1969); glycil-leucine-dipeptidase by Moore and Stein in modification of Ugolev and Timofeeva (1969). Activity of all above-mentioned enzymes was calculated per 1 g of wet mass of the mucosal membrane and was expressed as µmol/hour of hydrolysis products formation.

Results

The most enzymatic activity was noted in the homogenates of proximal intestinal mucosa in rats of the control group, while enzymatic activity of the homogenates from distal part was less. The administration of single dose of pesticide decis resulted in significant inhibition of invertase activity in the intestinal cells and enzyme translocation on the membrane surface. This is confirmed by the fact that in the animals killed on 1, 2, and 7 day after treatment in the homogenates of the proximal part of the intestine there were found greater changes of enzymatic activity than in distal part.

Amylolytic activity (µmol/g of starch broken) in calculation per 1 g of the intestinal mucosa in control rats fluctuated from 5.14±0.32 to 4.30±0.29 µmol/g.h.

As it may be seen on Fig.1, under the effect of decis in the acute experiment amylase activity in the proximal and distal parts of the intestine reduced during 7 days and in the distal part enzymatic activity was coming to the control on the 15th day.

Dipeptidyl peptidase activity in the homogenates of the mucosa in the both parts in acute poisoning by pesticides in the all terms of investigation reduced. The most activity was found in the homogenates of the proximal part of the intestine and the least activity – in the distal part.

After decis administration to the animals in 1, 2, and 7 days the activity of enzymatic alkaline phosphatase along the intestine reduced, however changes were more marked in the first part of the intestine.

It should be noted that in single intragastric administration of the pesticide decis there was noted inhibition of the activity of invertase, dipeptidase and alkaline phosphatase in the proximal and distal parts of the intestine, particularly marked desactivation was revealed in the proximal part of the intestinal mucosa. Changes of the enzymatic activity in acute poisoning were depended on the time of pesticide exposure and site of the bowel.
Thus, the degree and direction of the changes observed were not similarly in the different time after effect of pesticide decis, as well as in relation to activity of various enzymes in the small intestine.

For characteristic of the enzymatic activity of the small intestine there was performed study of the activity of dipeptidase, amylase, invertase and alkaline phosphatase in the homogenate of the mucosal membrane from proximal and distal parts in multiple effects of pesticide decis in toxic dose (1/20 LD50) during 4 months.

Study of the activity of digestive enzymes along the small intestine in rats from the control group during 120 days showed that activity of the studied enzymes in the both sites of the intestine in all times of investigations remained approximately at the equal level.

Chronic treatment of the animals with decis induced some changes in the studied enzymes (Table 1), because dipeptidase activity of the proximal segment reduced in all periods of investigation. In the distal site of the small intestine in multiple administration of pesticide dipeptidase activity reduced on 30, 60, 90 and 120 day, respectively, to 91.2%; 85.5%; 83.2%; 83%.

| TABLE 1. ACTIVITY OF THE SMALL INTESTINE ENZYMES (M±m) IN MULTIPLE INTRAGASTRIC EFFECT OF PESTICIDE DECIS IN DOSE 7,75 mg/kg (1/20 LD50) |
| Days of examination and groups of animals | Dipeptidase, µ mol/g.h. | Amylase, µ mol/g.h. | Invertase, µ mol/g.h. | Alkaline phosphatase, µ mol/g.h. |
| Parts of the small intestine | Proximal | Distal | Proximal | Distal | Proximal | Distal | Proximal | Distal |
| 30 day: control | 40.94±1.1 | 38.18±0.9 | 5.62±0.4 | 5.82±0.4 | 13.01±0.8 | 11.53±0.6 | 22.11±0.4 | 21.01±0.5 |
| experiment | 34.43±0.7 | 34.81±0.8* | 3.68±0.3* | 3.82±0.3* | 8.93±0.4* | 8.69±0.5* | 18.43±0.5* | 18.12±0.4 |
| 60 day: control | 40.9±1.1 | 39.7±1.2 | 5.76±0.4 | 5.90±0.3 | 13.3±0.7 | 11.37±0.7 | 22.1±0.5 | 20.86±0.6 |
| experiment | 36.66±1.1* | 33.93±0.9* | 4.04±0.37 | 4.05±0.31* | 7.74±0.7* | 7.62±0.8* | 18.04±0.5* | 17.58±0.5 |
| 90 day: control | 42.62±0.95 | 41.12±0.8 | 6.8±0.3* | 6.8±0.4* | 13.45±0.6 | 12.61±0.6 | 21.95±0.7 | 21.31±0.4 |
| experiment | 34.81±0.8 | 34.43±0.8* | 3.89±0.3 | 3.33±0.2* | 7.02±0.5* | 6.9±0.6* | 17.49±0.5* | 17.34±0.4* |
| 120 day: control | 42.9±0.8 | 40.1±1.2 | 5.54±0.3 | 4.87±0.2 | 13.59±0.7 | 12.17±0.7 | 20.78±0.5 | 19.77±0.4 |
| experiment | 33.7±0.9* | 33.5±1.04 | 3.72±0.24* | 3.93±0.2 | 6.56±0.4 | 7.53±0.4* | 17.93±0.4* | 17.62±0.4 |

Note: * - statistic c parameter of reliability P<0.05-0.001

From the above-said it was seen that dipeptidase activity under the effect of toxic doses during 120 days reduced, and in the upper sites of the small intestine the inhibition of the activity was more marked in comparison with distal part.

Under the effect of decis in dose 7.75 mg/kg amylase activity in the mucosal membrane of the small intestine reduced in all times (30, 60, and 120) of investigation to 57.2-70.4%. In the distal part there was also found inhibition of the enzymatic activity.

The data presented indicated about that under effect of toxic doses of pesticide amylase activity changed irregularly in the distal part of small intestine, on 90 day of investigation it sharply reduced and increased to 80% on 120 day.

Invertase activity in the proximal part of the small intestine in multiple administration of decis was inhibited on the 30, 60, 90, and 120 day to 68.7%; 58%; 52.2% and 55%, respectively, and in the distal part it reduced to 54.7-75.4%.

As regards with activity of alkaline phosphatase, as it can be seen from the table, in the proximal part of the small intestine in all times of effect it reduced to the equal level (79.7-83.3%). The similar changes of activity were observed in the distal part of the intestine.

The data obtained indicated that under the effect of decis in toxic dose activity of alkaline phosphatase changed equally along the intestine.
Thus, multiple intragastric administrations of pesticide decis in toxic dose promoted to regularly reduction in activity of enzymes dipeptidase, amylase, invertase, alkaline phosphatase in all sites of the small intestine in all times of investigation, and enzyme inhibition was more marked in the proximal part than in the distal part of the small intestine.

Discussion

The pesticides, commonly used in agriculture for achieving better quality products, are toxic substances that have harmful effects on human health. Recent research on pesticides has shown that they are one of the key environmental health issues. Several studied show that pesticides exert effects of the enzymatic activity of the intestine.

Dudrikova et al. (1992) showed that the changed enzymatic activity, particularly alkaline phosphatase, was located in the microvilli of enterocytes of the small intestine. The studies of Gera et al., (2009) showed absence of the activity of such enzymes as maltase, lactase and trehalase in pesticide treated animals, however, Labana et al. (2001b) showed that there was a significant decrease in the activities of sucrase (29%), lactase (20%) and alkaline phosphatase (24%) compares to control animals. These findings suggest that pesticide toxicity may modulate digestive functions in the small intestine.

The data available about changes of membrane digestion as a result of sharp disturbance of the enzyme synthesis and translocation on the mucosa membranes in exposed to some stressor factors indicated that issue about effect of toxic substances including pesticides has been studied insufficiently (Timofeeva, Iezuitov, and Makulina, 1971).

In our work there are presented results of the study of enzymatic activity of invertase, amylase, dipeptidase and alkaline phosphatase in the homogenate if the intestine mucosa in acute and chronic exposure of the pesticide Decis.

Conclusion

The single sublethal (3/4 LD50) and multiple toxic (1/20 LD50) intragastric administration of pesticide decis independently on the duration of experiment resulted in changes of hydrolytic function of the small intestine mucosa in different direction. The different digestive enzymes have different response to effect of pesticides of pyrethroid group (decis), there are differences in the reactions of proximal and distal part of the small intestine and there is correlation between changes of activity of the majority of hydrolases and administered dose of pesticides.

The selected for study the presentation of the group of pyrethroids pesticide Decis induced significant changes in the activity of intestinal enzymes that may result in serious disturbances in the intestinal uptake consumption of the composite part of food. Besides, the overstrain of all sites of the digestive system, related to the maintenance of the high level of hydrolysis and absorption, provokes the occurrence of different pathological conditions and diseases. Thus, the presented above data should be taken into consideration during development of the rations for prophylactic diets for persons working with pesticides of group of pyrethroids.

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