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CLINICAL AND EXPERIMENTAL STUDIES ON ACUTE INTOXICATION WITH THE TRIAZOLE FUNGICIDE DINICONAZOLE (SUMI 8 2WP) IN RABBITS. II. BIOCHEMICAL STUDIES

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Summary

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Experiments for evaluation of the toxic effect of the triazole fungicide diniconazole (Sumi 8 2WP) on rabbits were carried out following up the changes in blood biochemical indices: calcium, inorganic phosphate, magnesium, cholesterol, urea, creatinine and blood glucose.

The studies were performed in one control and three experimental groups, treated orally via a gastic tube with increasing doses of diniconazole ($0.1LD_{50}$, $0.5LD_{50}$ and LD_{50} – oral doses for rats).

Blood for analysis was sampled at hours –48, –24 and 0 prior to the treatment and hours 2, 4, 6, 8, 10, 12, 24, 48 and 72 after the treatment from all animals.

It was observed that the triazole fungicide diniconazole could produce a toxic effect manifested by hypercalciaemia, hyperphosphataemia, hypermagnesaemia, hypercholesterolaemia, uremia and hyperglycaemia. Those changes were the most obvious between post treatment hours 2 and 24 and afterwards the analysed parameters restored their initial values.

Key words: biochemical parameters, diniconazole (Sumi 8 2WP), triazole fungicide

INTRODUCTION

Studies upon the blood biochemical parameters under the influence of triazole fungicides (cyproconazole, flusilazole, 1,2,4-triazole and propiconazole) are performed in mice, rats and humans (Machera, 1995). The changes occurring after treatment with triadimenol are reported in cattle (Markelov and Shormanov, 1994) and hens (Binev, 2000d). Those studies evidenced that depending on the amount of the toxic substance, the blood levels of macroelements (calcium, inorganic phosphate, magnesium), urea, creatinine, cholesterol, triglycerides, blood glucose, pyruvate increased.

In previous studies of ours upon the acute intoxication with the triazole fungicide diniconazole in rabbits (Binev, 2001), the changes in the clinical status (hypothermia, tachycardia, polypnea) and some haematological changes (oligochromemia, erythropenia, neutrophilia, lymphocytopenia and slower erythrocyte sedimentation rates) were described. Diniconazole-treated hens showed blood biochemical deviations characterized by hyperglycaemia, lactacidaemia, hyperalbuminaemia, hypoα_1- and γ-globulinaemia, uraemia, hypercholesterolaemia, hypercalciaemia and hyperphosphataemia (Binev, 2000a; 2000b; 2000c). There are no

literature data about the toxic effect of the triazole fungicide diniconazole in rabbits.

Our study aimed to perform complex experimental studies for determination of changes occurring during the acute intoxication in rabbits treated with diniconazole in order to elucidate its pathogenesis and to facilitate its diagnostics, treatment and prophylaxis.

MATERIALS AND METHODS

Animals

The studies were performed with 22 rabbits from both genders, California and White New Zealand breeds, aged 6-8 months and weighing 2.8-3.2 kg. The animals were put in individual cages 30 days prior to the beginning of the experiments under standard hygienic conditions.The rabbits received treatments against endoparasites with piperazine sulfate (Piperatrin, Vetprom, Bulgaria) and against ectoparasites with 0.1% cypermethrin solution (Ectomin, Ciba-Geigy, Switzerland). The daily ration of each animal consisted of 0.1 kg combined concentrate and 0.2 kg alfalfa hay with free access to tap water.

Drugs and treatments

An acute intoxication was provoked with the commercial preparation Sumi 8 2WP containing 2% diniconazole and 98% inert ingredients (including surfactant and tetrahydrofurfuryl alcohol) (Sumitomo Chemical, Japan). It was administered to experimental animals once, internally, using a gastric tube.

Experimental design

The rabbits were divided into 4 groups – one control (n=4) and three experimental groups (n=6 each). The control group was left untreated while the experimental received diniconazole in doses as followed:

- group I: 0.064 g/kg or 3.2 ml of Sumi 8 2WP (= 0.1 oral LD_{50} for rats);
- group II: 0.320 g/kg or 16 ml of Sumi 8 2WP (= 0.5 oral LD_{50} for rats);
- group III: 0.639 g/kg or 32 ml of Sumi 8 2WP (= oral LD_{50} for rats).

Assessment of toxicity

Blood from *v. jugularis* or *v. femoralis* was sampled three consecutive days prior to the treatment (hours -48, -24 and 0) and 2, 4, 6, 8, 10, 12, 24, 48 and 72 hours thereafter for determination of concentrations of calcium (Ca) - by complexometry, magnesium (Mg) – by the method of Basinski (Angelov et al., 1999), of inorganic phsophate (P), cholesterol, blood glucose, urea and creatinine by diagnostic kits (Roche, Germany) using an automated biochemical analyser (Reflotron Manual, Germany).

Statistical analysis

Data are presented as mean ± SEM. The statistical significance of results was determined using the Student t-test at a level of significance of $p<0.05$. Comparisons between control values and the values in experimental groups for each experimental interval were performed. All p-values, mentioned thereafter are referring to those differences.

RESULTS AND DISCUSSION

Blood calcium concentrations in control animals (Fig. 1) during the control period varied between 2.12 ± 0.18 mmol/l and 2.38 ± 0.21 mmol/l and during the experimental period – between 2.16 ± 0.19 and 2.44 ± mmol/l . After the treatment of experimental rabbits from group I and II with diniconazole, statistically significant elevations were reached at post treatment hour 6 (3.08±0.26 mmol/l; $p<0.05$ for the former group) and at post treatment hour 2 (3.10±0.32 mmol/l; $p<0.05$ for the latter

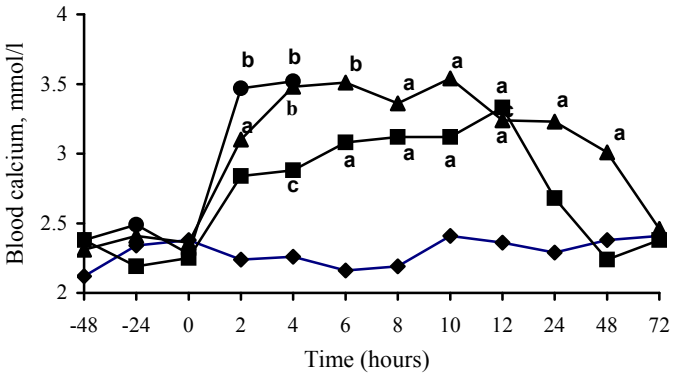

Fig. 1. Changes in blood calcium concentrations in rabbits - untreated (controls) and treated orally with diniconazole at doses of 0.064 g/kg (0.1×LD_{50}, group I); 0.320 g/kg (0.5×LD_{50}, group II) and 0.639 g/kg (LD_{50}, group III). Level of significance vs controls: [a] $p<0.05$; [b] $p<0.01$; [c] $p<0.001$. Legend: ◆ control group; ■ group I; group II; ● group III.

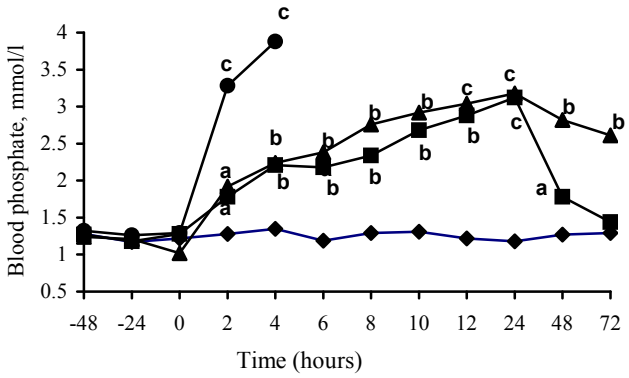

Fig. 2. Changes in blood phosphate concentrations in rabbits - untreated (controls) and treated orally with diniconazole at doses of 0.064 g/kg (0.1×LD_{50}, group I); 0.320 g/kg (0.5×LD_{50}, group II) and 0.639 g/kg (LD_{50}, group III). Level of significance vs controls: [a] $p<0.05$; [b] $p<0.01$; [c] $p<0.001$. Legend: ◆ control group; ■ group I; group II; ● group III.

one). The peak concentrations in group I were measured by the 12th hour (3.33±0.28 mmol/l, $p<0.05$), in group II – by the 10th hour (3.54±0.24 mmol/l, $p<0.05$) and in group III – by the 4th hour, immediately prior to the lethal issue (3.52±0.24 mmol/l, $p<0.01$). Between hour 48 an 72, blood Ca levels in groups I and II became normal.

During the pre-treatment and treatment periods, inorganic phosphate concentrations (Fig. 2) in the control group were between 1.17±0.11 mmol/l – 1.28±0.12 mmol/l and between 1.18±0.11 mmol/l –

1.35 ± 0.14 mmol/l respectively. The earliest significant increase in all experimental groups compared to controls was observed 2 hours following the treatment – 1.78±0.24 mmol/l ($p<0.05$), 1.92±0.28 mmol/l ($p<0.05$) and 3.28±0.30 mmol/l ($p<0.001$) for groups I, II and III respectively. The peak phosphate values in group I occurred by hour 12 (2.88±0.26 mmol/l, $p<0.001$), in group II – by hour 24 (3.18±0.29 mmol/l, $p<0.001$) and in the third group – by hour 4 (3.88±0.31 mmol/l, $p<0.001$). At post treatment hour 72 the inorganic phosphate concentrations in group I decreased to control values while in group II remained elevated (2.48±0.28 mmol/l).

During the pre-treatment and treatment periods, control blood magnesium levels (Fig. 3) in the control group ranged between 1.48±0.13 mmol/l – 1.58±0.14 mmol/l and between 1.51±0.12 mmol/l – 1.60 ± 0.13 mmol/l respectively. Eight hours after the treatment they increased to 2.12±0.21 mmol/l in group I ($p<0.05$) and to 2.18±0.21 mmol/l in group II ($p<0.05$). The highest blood Mg concentrations in both groups were 2.13±0.18 mmol/l at post treatment hour 10 and 2.19±0.24 mmol/l at post treatment hour 8, respectively ($p<0.05$). For the third group the peak Mg levels occurred by hour 4 – 2.24±0.27 mmol/l ($p<0.05$). The concentrations in groups I and II returned to normal by the 12th hour.

Cholesterol levels in controls during the pre-treatment period varied from 0.57±0.02 mmol/l to 0.62±0.03 mmol/l and during the experimental period from 0.58±0.02 to 0.64±0.03 mmol/l. Two hours after the treatment they increased in all experimental groups: 1.01±0.14 mmol/l; 0.89±0.11 mmol/l and 1.08±0.13 mmol/l in groups I, II and III respectively ($p<0.05$). The highest cholesterol concentrations were attained at post treatment hour 10 in groups I and II (3.37±0.21 mmol/l and 3.88±0.27 mmol/l respec-

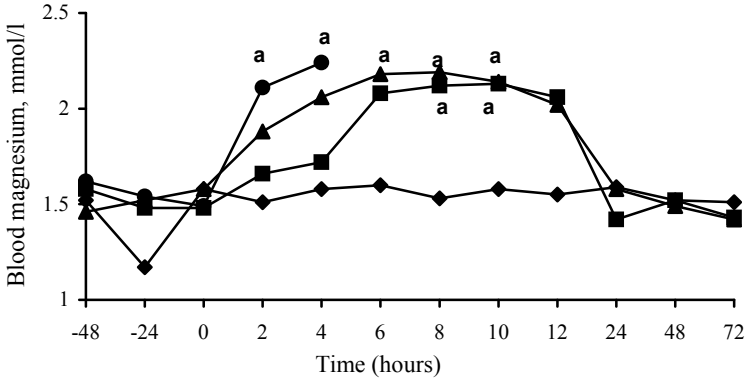

Fig. 3. Changes in blood magnesium concentrations in rabbits - untreated (controls) and treated orally with diniconazole at doses of 0.064 g/kg ($0.1\times LD_{50}$, group I); 0.320 g/kg ($0.5\times LD_{50}$, group II) and 0.639 g/kg (LD_{50}, group III). Level of significance vs controls: [a] $p<0.05$; [b] $p<0.01$; [c] $p<0.001$. Legend: ◆ control group; ■ group I; ▲ group II; ● group III.

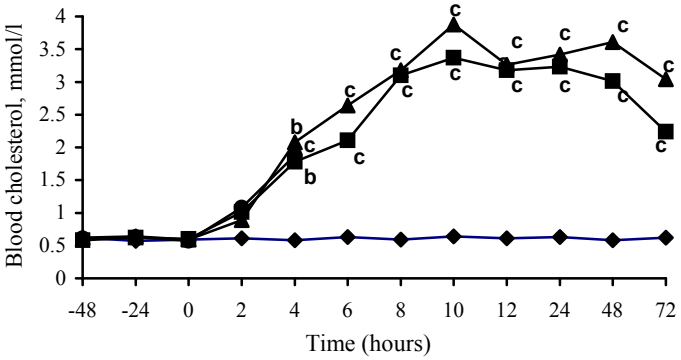

Fig. 4. Changes in blood cholesterol concentrations in rabbits - untreated (controls) and treated orally with diniconazole at doses of 0.064 g/kg ($0.1\times LD_{50}$, group I); 0.320 g/kg ($0.5\times LD_{50}$, group II) and 0.639 g/kg (LD_{50}, group III). Level of significance vs controls: [a] $p<0.05$; [b] $p<0.01$; [c] $p<0.001$. Legend: ◆ control group; ■ group I; group II; ● group III.

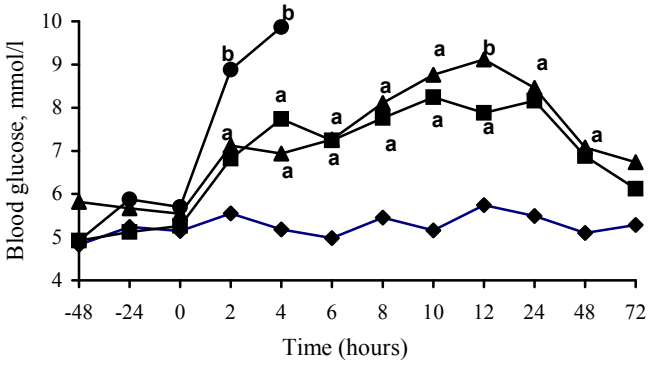

Fig. 5. Changes in blood glucose concentrations in rabbits - untreated (controls) and treated orally with diniconazole at doses of 0.064 g/kg ($0.1\times LD_{50}$, group I); 0.320 g/kg ($0.5\times LD_{50}$, group II) and 0.639 g/kg (LD_{50}, group III). Level of significance vs controls: [a] $p<0.05$; [b] $p<0.01$; [c] $p<0.001$. Legend: ◆ control group; ■ group I; group II; ● group III.

tively, $p<0.001$). Four hours after the treatement, the mean cholesterol levels in group III were 1.88±0.13 mmol/l at $p<0.001$. In the former groups, cholesterol concentrations persisted significantly elevated up to the end of the experiment.

During the pre-treatment and treatment periods, control blood glucose levels (Fig. 3) were between 4.82±0.38 mmol/l – 5.24±0.48 mmol/l and between 4.98±0.41 mmol/l – 5.74±0.43 mmol/l respectively. In group I (Fig. 5) the first significant increase in blood glucose levels occurred at the 4th hour – 7.74±0.76 mmol/l ($p<0.05$) and the peak values were

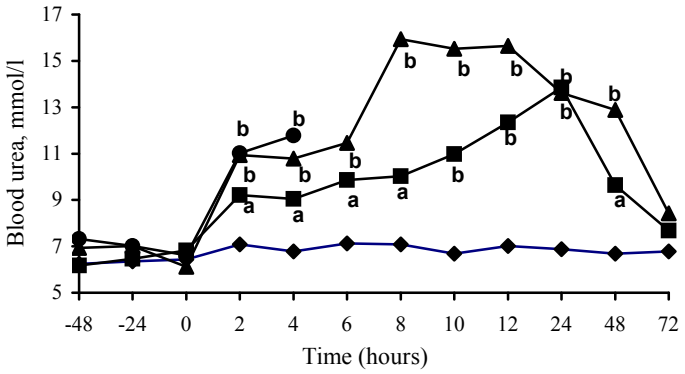

Fig. 6. Changes in blood urea concentrations in rabbits - untreated (controls) and treated orally with diniconazole at doses of 0.064 g/kg (0.1×LD_{50}, group I); 0.320 g/kg (0.5×LD_{50}, group II) and 0.639 g/kg (LD_{50}, group III). Level of significance vs controls: [a] $p<0.05$; [b] $p<0.01$; [c] $p<0.001$. Legend: ◆ control group; ■ group I; group II; ● group III.

reached by hour 10 – 8.24±0.76 mmol/l ($p<0.05$). In the second group, those changes occurred earlier at the second post treatment hour – 7.12±0.58 mmol/l ($p<0.05$) and hour 12 – 9.12±0.88 mmol/l ($p<0.01$) respectively. By hour 4, in the third group, blood glucose concentrations were 9.87±1.02 mmol/l ($p<0.01$). The concentrations were normalized by hour 24 for group I and by hour 72 for group II.

Control urea concentrations (Fig. 6) prior to the treatment ranged from 6.24±0.48 to 6.44+0.52 mmol/l and during the treatment period – between 6.78±0.46 and 7.12±0.58 mmol/l. Following the treatment, they increased to a maximum of 13.85±1.46 mmol/l (group I; post treatment hour 24), 15.93±1.68 mmol/l (group II; post treatment hour 8) and 11.78±0.98 mmol/l (group III, post treatment hour 4) at $p<0.01$. By the 72nd hour, blood urea levels in groups I and II decreased to control determinations.

Creatinine deviations in all experimental groups were statistically insignificant compared to controls.

DISCUSSION

The results of our studies showed that the experimental intoxication with the triazole fungicide diniconazole in rabbits was manifested by changes in some of the studied blood biochemical parameters. The elevation in macroelements – calcium, inorganic phosphate and magnesium under the influence of increasing toxic doses could be explained by their lowered excretion with urine (Ronis et al., 1998), caused most probably by the dystrophic changes in renal parenchyma similarly to those observed in dialkylimidazole–treated mice and rats (Machera, 1995), mice (Sivertsen and Muller, 1999) clotrimazole–treated cattle (Markelov and Shormanov, 1994) and guinea-pigs (Thomas et al., 1999) and propiconazole–treated cats (Kurumbaev et al., 1996) and rats (Patel et al., 1999). Another possible cause for observed changes could be the hypothyreosis, observed in mice and rats under the influence of 3-

amino-1,2,4-trizole (Mayberry, 1968); in rats, mice and humans after challenge with cyproconazole, fluzilazole and propiconazole (Machera, 1995) and in cattle treated with triadimenol (Markelov and Shormanov, 1994). Hypothyreosis could impair the excretion of macro- and microelements with urine and result in elevation in their blood concentrations, i.e. in hypercalciaemia, hyperphosphataemia and hypermagnesaemia. It could be suggested that it was the cause for the present hypercholesterolaemia observed also in diniconazole-treated mice and rats (Machera, 1995) and hens (Binev, 2000b) that is accompanied by decrease in fat degradation and their accumulation in internal organs leading to parenchymatous fat dystrophy (Markelov and Shormanov, 1994; Kurumbaev et al., 1996; Patel et al., 1999). The early appearance of hyperglycaemia (by hour 2) could be explained by the increased blood adrenaline (Crofton, 1996; Filipov and Lawrence, 2001) resulting from the neurotoxic effect of triazole fungicides (Allen and McRhail, 1993; Crofton, 1996) that are reported to provoke intoxication with strong signs of excitation (seizures, convulsions, tremor, clonic muscle spasms) (Allen and Mac Rhail, 1993; Crofton, 1996). Such signs have been observed in mice and rats (Allen and Mac Rhail, 1993; Crofton, 1996), hens (Binev, 2000a; 2000c) and rabbits (Binev, 2001) folowing diniconazole treatment as well as in hens after triadimenol treatment (Binev, 2000d). The increase in blood urea concentrations similarly to that observed in triadimenol-treated cattle (Markelov and Shormanov, 1994) and diniconazole-treated hens (Binev, 2000b) were most likely due to renal parenchymal dystrophy, evidenced also in propiconazole-treated cattle (Markelov and Shormanov, 1994) and cats (Kurumbaev et al., 1996) as well as to the decreased excretion of urine reported in Bobwhite quails (Ronis et al., 1998).

The comparison of our data with those in hens treated with the same toxic substance in similar doses ($0.1LD_{50}$, $0.5LD_{50}$ and LD_{50} - oral doses for rats) revealed some species-related features (Binev, 2000a; 2000b; 2000c). In birds there were no changes in studied parameters after challenge with $0.1LD_{50}$, while after the treatment with LD_{50}, the intoxication was surmounted. The interspecies variability in studied parameters' deviations showed that rabbits were more susceptible to diniconazole than hens.

In conclusion it could be summarized that the intoxication with toxic doses ($0.1LD_{50}$, $0.5LD_{50}$ and LD_{50} - oral doses for rats) of the triazole fungicide diniconazole (Sumi 8 2WP) in rabbits provoked changes in some biochemical blood parameters – hypercalciaemia, hyperphosphataemia, hypermagnesaemia, hypercholesterolaemia, uremia and hyperglycaemia. The application of doses equal to the oral LD_{50} for rats resulted in a lethal issue for all experimental animals 4-6 hours after the treatment.

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