The Effect of Banol and Paraoxon on the Nadph-Cytochrome c Reductase Activity and Cytochrome P-450 Content in Rats

Nesko K. Neskovic
Department of Pesticides INEP
Baranjska 15, P.O. Box 46
11080 Zemun
Yugoslavia

Slavoljub Lj. Vitorovic
Department of Pesticides
Faculty of Agriculture
University of Belgrade
Nemanjina 6, 11080 Zemun
Yugoslavia

The NADPH-cytochrome c reductase is most probably the limiting factor in the enzyme electron transport (GLAZER and SARTORELLI, 1971), and cytochrome P-450 was identified as the terminal oxidase included in the metabolism of numerous xenobiotics (HRYCAY and O'BRIEN, 1973). It is known that compounds, which are metabolized by the microsomal enzymatic complex, could increase the amount of these enzymes in the microsomal fraction. Phenobarbitone is a well known compound with such effect (ABERNATHY et al., 1971; HRYCAY and O'BRIEN, 1973), as well as some insecticides, like DDT and carbaryl (ABERNATHY et al., 1971; CRESS and STROTHER, 1974).

In this work the effect of banol (2-Chloro-4,5-dimethylphenyl N-methylcarbamate) and paraoxon (0,0-diethyl O-p-nitrophenylphosphate) on NADPH-cytochrome c reductase and content of cytochrome P-450 in rat liver microsomes was investigated.

Materials and Methods

Female rats, weighing between 100 and 150 g, eight animals per group, from our farm were used. Two doses of phenobarbitone (sodium salt), 80 mg/kg, was applied ip 48 and 24 hours before sacrificing. Banol (2 mg/kg) and paraoxon (0.03 mg/kg), were also applied ip two

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hours before sacrificing.

The animals were sacrificed by decapitation, and the liver was taken for the preparation of microsomes. Preparation of microsomes was carried out according to the method described by CINTI et al. (1972).

The activity of NADPH-cytochrome c reductase was estimated by the methods of MASTERS et al. (1967). The reaction mixture in total volume of 3 ml contains: phosphate buffer 50 mM (pH-7.8), microsomal proteins, NADPH, Triton X-100 and cytochrome c.

Cytochrome P-450 was estimated according to the method of OMURA and SATO (1964), and the concentration was calculated from the absorbence difference at 450 and 490 nm using extinction coefficient 91.0 $mM^{-1}cm^{-1}$.

Microsomal proteins were estimated by the colorimetric method of LOWRY et al. (1951).

Analysis of data was by analysis of variance, completely randomized design. The level of significance was chosen as $P<0.05$.

Results and Discussion

As is evident in Table 1, both banol and paraoxon affect the enzymes examined. Banol had an inhibitory effect on both enzymes by reducing the activity of NADPH-cytochrome c reductase and cytochrome P-450 concentration.

The activity of NADPH-cytochrome c reductase was, under the influence of banol, reduced about 27%, and the cytochrome P-450 content about 30% if compared with

TABLE 1

The activity of NADPH-cytochrome c reductase and the concentration of cytochrome P-450 in microsomes isolated from the liver of rats treated with banol and paraoxon[1]

Treatment	No. of animals	Cytochrome P-450[2]	NADPH-cytochrome c reductase[3]
Control	8	0.292 ± 0.057	44.46 ± 2.08
Banol	8	0.204 ± 0.030[4]	32.88 ± 1.90[4]
Paraoxon	8	0.323 ± 0.024	36.39 ± 2.73[4]

[1]Banol (2 mg/kg) and paraoxon (0.03 mg/kg) were applied ip 2 hours before sacrificing. The results present mean value ± SE.

[2]Cytochrome P-450 concentration is expressed in nmol/mg of microsomal proteins.

[3]Activity of NADPH-cytochrome c reductase is expressed in nmoles cytochrome c reduced/min per mg protein.

[4]Significantly different from control, $P<0.05$.

the control. Values were significantly different from control at 0.05 level. Paraoxon only reduced the activity of NADPH-cytochrome c reductase somewhat less than 20%; the difference was significant ($P<0.05$). Paraoxon had a stimulative effect on the cytochrome P-450 content; it increased 10.6% compared with the control. The difference, however, was not significant.

As it is known, metabolism of the xenobiotic compounds in the organism takes place mainly in the liver. However,

the compounds, whose transformation includes microsomal enzymes, affect in different ways some components of this complex. CRESS and STROTHER (1974) have thus established that the insecticide carbaryl increases the content of cytochrome P-450 and cytochrome b_5 in liver microsomes. Something similar was found out for DDT by ABERNATHY et al. (1971).

The activity of NADPH-cytochrome c reductase is very often decreased under the influence of various insecticides. Parathion in in vitro experiments reduced the activity of this enzyme 20-30% in relation to the applied dose in comparison with control (NEŠKOVIĆ et al., 1973). A similar tendency was observed with the effect of its active metabolite, paraoxon here.

In phenobarbitone-treated rats (Table 2), both cytochrome P-450 concentration and NADPH-cytochrome c reductase activity were increased. This increase amounted to 19.5% for cytochrome P-450 and 22.6% for NADPH-cytochrome c reductase in comparison with the control. Values were significantly different from control ($P<0.05$).

The same kind of the stimulative effect of phenobarbitone on liver enzymes taking part in the metabolism of xenobiotics, was obtained by other authors (GRAM et al., 1971; HRYCAY and O'BRIEN, 1973; ABERNATHY et al., 1971). When phenobarbitone was applied in combination with paraoxon, the cytochrome P-450 concentration showed a further increase reaching the level of control values. When applied with banol, phenobarbitone increased the cytochrome

TABLE 2

The activity of NADPH-cytochrome c reductase and cytochrome P-450 concentration in microsomes isolated from rats liver, treated with phenobarbitone, and combination of phenobarbitone with banol and paraoxon[1]

Treatment	No. of animals	Cytochrome P-450[2]	NADPH-cytochrome c reductase[3]
Control	8	0.292 ± 0.057	44.46 ± 2.08
Phenobarbitone	8	0.349 ± 0.062[4]	54.52 ± 3.10[4]
Phenobarbitone + Banol	8	0.220 ± 0.040[4]	31.40 ± 2.75[4]
Phenobarbitone + Paraoxon	8	0.360 ± 0.059[4]	42.99 ± 3.22

[1] Phenobarbitone (80 mg/kg), banol (2 mg/kg) and paraoxon (0.03 mg/kg) were applied ip as follows: phenobarbitone 48 and 24 hours before sacrificing, and banol and paraoxon two hours before sacrificing. The results present the mean value ± SE.

[2] Cytochrome P-450 concentration is expressed in nmol/mg microsomal proteins.

[3] The activity of NADPH-cytochrome c reductase is expressed in nmoles cytochrome c reduced/min per mg protein.

[4] Significantly different from control, $P<0.05$.

P-450 concentration about 5%, while the activity of NADPH-cytochrome c reductase was insignificantly lowered in relation to the microsomes isolated from animals previously treated with banol only.

Summary

We investigated the effect of banol and paraoxon on the activity of NADPH-cytochrome c reductase and cytochrome P-450 concentration in microsomes of rats treated *in vivo* with these compounds alone, or in the presence of phenobarbitone.

The results showed that banol reduced the NADPH-cytochrome c reductase activity and the concentration of cytochrome P-450, while paraoxon reduced the activity of NADPH-cytochrome c reductase, but increased the cytochrome P-450 concentration.

Phenobarbitone stimulated an increased NADPH-cytochrome c reductase activity and cytochrome P-450 concentration. When applied with banol and paraoxon, phenobarbitone lowered their inhibitory effect.

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