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EFFECT OF METHYLPARATHION ON GLUTATHIONE S-TRANSFERASE ISOENZYMES IN DIFFERENT RAT TISSUES

In this work we examined the influence of the organophosphorous insecticide (IFO) methylparathion on the activity of glutathione S-transferase in vivo in different male Wistar rat tissues. We examined postmitochondrial, cytosol, and microsomal cell fractions, of the liver, kidney, pancreas, spleen, and brain. The activity of the GST was measured according to each of the two substrates: CDNB and DCNB respectively, what permitted us to differentiate GST isoenzyms. We observed significant increases of the activity of GST which depended on the tissue and cell fraction.

INTRODUCTION

Phosphoro-organic compounds used as insecticides (IFO) considerably disturb cell metabolism in near-lethal doses to which mammals are generally exposed in consequence of using IFO in agriculture and in households.

IFO damage deoxyribonucleic acid and disturb the transfer of genetic information [2–4, 24–26, 28–32]. In vivo they produce mutations and they also can be carcinogenic and teratogenic factors (6). The mechanism which leads to such serious damages is not fully known yet, because the data in investigations in vivo relating to damages to genetic materials are discordant and not univocal.

This paper has been devoted to the investigation of the influence of methylparathion (MP) on glutathione S-transferases of different cell fractions of rat liver, kidney, spleen, pancreas and brain. The metabolism of MP in rat liver has been presented in Fig. 1 [10].

Methylparathion is activated by desulfurization to paraoxon (inhibitor of acetocholinoesterases in microsomal fraction of the liver). Enzymatic

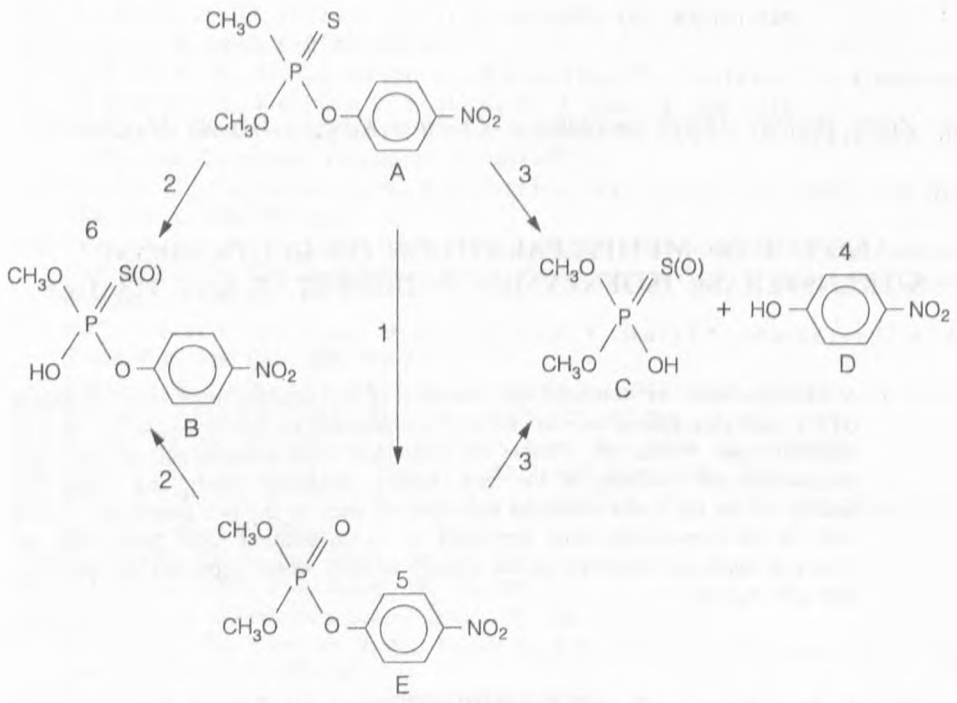

Fig. 1. Metabolic pathway of methyl parathion in rat liver
1 – microsomal mixed-function oxidases, 2, 3 – glutathione S-transferase, 4, 5 – B-esterase, 6 – microsomal mixed-function oxidases inhibition, A – methylparathion, B – 0-methyl-0-para-nitrophenyl phosphorothioate (or 0-methyl-0-para-nitrophenyl phosphate), C – dimethylphosphorothicate, (or dimethyl phosphate acid), D – para-nitrophenol, E – methyl paraoxon

decomposition MP in the rat's organism is catalised by oxidases of mixed effect, hydrolases and glutathion S-transferases.

Glutathione S-transferases (GST – E. C. 2.5.1.18) are a multigene enzyme family which perform protective functions against toxic effects of xenobiotics [1, 7, 27]. The study seems to be purposefull, because of the lack of univocal data on the subject of possible processes which lead to a damage of cell genetic material.

MATERIALS AND METHODS

In this experiment have been used male Wistar rats which weigh from 250–300 g. Methylparathion (0,0-dimethyl-0,4-nitrophenylthiophosphate), minimum purity 98% (obtained from the Institute of Organic Chemistry

in Warsaw), was administered once into the tail vein 0,5 LD_{50} (21 mg MP in olive oil per 1 kg of body weight).

Exposure time to the insecticide was up to 150 minutes. The prepared rat tissues: liver, kidney, spleen, pancreas and brain, were kept at the temperature of –70°C. From each of these tissues was made homogenate solution in 0,25 M sucrose in 0,1 M phosphate sodium-potassium buffer (pH 7,4) on the total concentration of 20% (w/v).

Enzyme activity GST was estimated by the method of conjugating GSH (Habig et al. [8]) with 1,2-dichloro-nitrobenzene (DCNB) and 1-chloro-2,4-dinitrobenzene (CDNB). Activity was measured in postmitochondrial, microsomal and cytosol fractions obtained by Jacoby's method [11] using 50 to 300 μg protein for CDNB and DCNB respectively.

Protein concentration was estimated by using Lowry's et al. method [13]. GST activity was expressed in specific activity units 1U/1nmol S-1--conjugation formed within 1 minute in the conditions of enzymatic reaction in terms of mg of protein. The results were analysed by Student's t-test and confidential level approximation of $\alpha = 0{,}05$.

Changes in GST activity have expressed in % according to the formula:

$$\frac{SApoisoned - SAcontrol}{SAcontrol} \times 100\%$$

were SA – Specific Activity.

RESULTS AND DISCUSION

In our work we have observed a statisticaly significant increase in the activity of glutathione S-transferase towards DCNB and CDNB in all examined cell fractions in rats poisoned with MP in the liver, spleen, pancreas, brain and the kidney.

The degree of the increase of the activity of cytosolic GST was different in each of the examined tissues. In the liver, the increase of cytosolic GST towards CDNB as a substrate was 47% in the kidney, 53% in the spleen, 19% in the pancreas, and 69% in the brain – Fig. 2. As regards the increase of cytosolic GSTs towards DCNB as a substrate was 25,8% in the liver, 6,4% in the kidney, 34% in the pancreas, 18% in the spleen, and 50% in the brain – Fig 3.

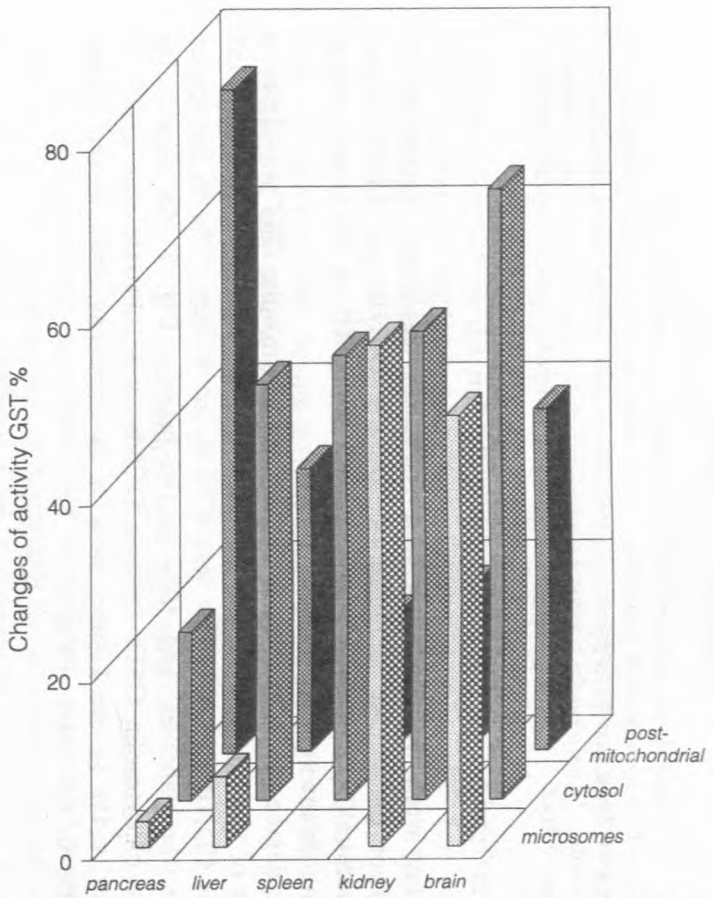

Fig. 2. Effect of MP on GST activity in tissues of the Wistar rat. Enzyme activities were measured by the conjugation of GSH to CDNB

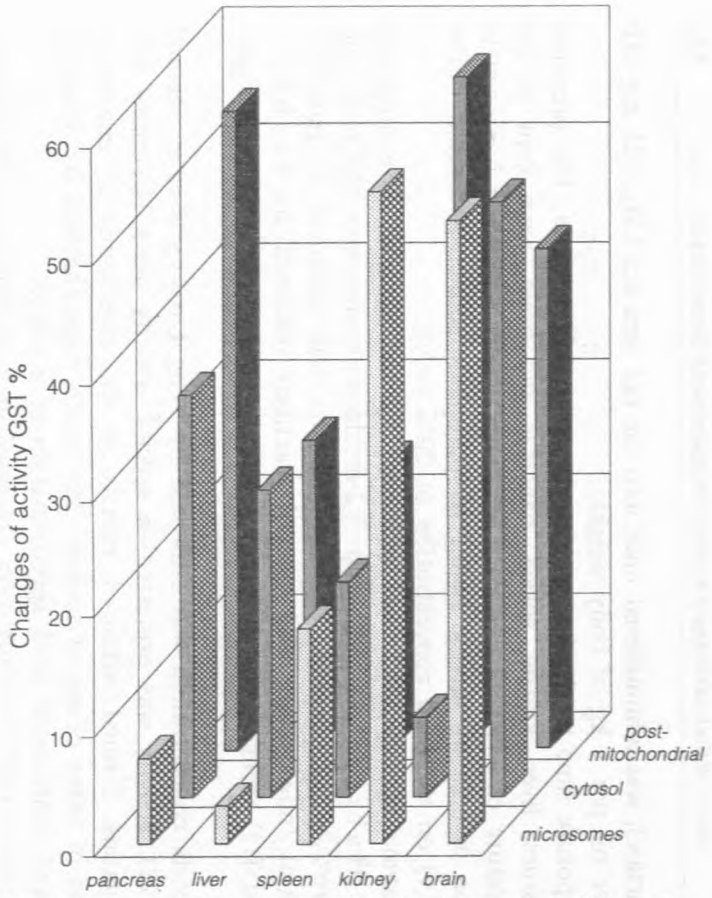

Fig. 3. Effect of MP on GST activity in tissues of the Wistar rat. Enzyme activities were measured by the conjugation of GSH to DCNB

Cytosolic fraction GSTs of rat liver belong to the best known enzymes of this type. Many isoenzymes are present there. They are proteins from 40 to 50 kDa, built of two different or identical subunits from 22 to 27,5 kDa. Till now, at least 10 subunits and 15 molecullar forms of this enzyme have been described in the rat tissues.

The occurance of isoenzymes of overlapping substrate specific qualities results from the existance of a number of double combinations of subunits.

Varied terminology is used to classify GSTs. In our paper a division into 3 classes α, μ, π has been adopted according to Mannervic [14]. Specific activity towards the used substrates allows, to a certain degree to draw conclusions that the classes α and μ, react mainly with the substrate CDNB, while the isoenzymes 3–3 and 3–4 of the class μ with DCNB [5]. It is evident that the increase of the activity in the liver concerned both, the isoenzymes of the class α and the class μ. Seven isoenzymes have been found in the pancreas [21]. The increase of GST activity in the pancreas related also to isoenzymes of the classes α and μ.

In the spleen, the increase of GST activity towards CDNB was 50% and towards DCNB 18%. On the basis of division on Sepharose 2, two main forms of isoenzymes 2–2 (class α) and 7–7 (class π) have been characterized in the spleen, so the increase of the activity related to the classes α and π [23].

In the kidney, the increase of cytosolic GST towards CDNB as a substrate was 53%, and towards DCNB 6,4%. According to the investigations [22], in kidneys there are 8 isoenzymes GST belonging to the classes α, μ and π. Our investigations show a particular increase of the enzymes of the α class. Enzymes of the μ class also show a certain increase of activity.

An exceptionally high increase of GST activity has been observed in the cytosolic fraction of the rat's brain. It was 69% in relation to CDNB and 50% in relation to DCNB. It may be concluded that this relates to all classes of cytosolic enzymes. According to Johnson's et al. paper [12], enzymes of all cytosolic GST classes appear in the rat's brain.

Microsomal form of GST has a different structure than cytosolic forms. It has been examined in human and rat livers [15]. It is built of three identical subunits about 17,3 kDa. In our investigations, we have found inconsiderable 10% increase of microsomal GST activity in the pancreas and liver, and about 18% increase in the spleen, while in the kidney, liver, and brain we have observed about 50% increase of the GST activity in relation to to both examined substrates.

There is very little literature data concerning microsomal GST of the brain, so there is some difficulty in the interpretation of this result. Postmitochondrial fraction contains both, the enzymes from microsomal and cytosolic fractions. The results for this fraction seem to confirm,

generally our observations concerning the increase of GST activity in the examined fractions.

The obtained data clearly prove that the effect of methylparathion is an increase in the activity of one of the main enzymes of the xenobiotic metabolism system.

On the, so far available, literature we haven't found data related to the influence of MP on GSTs of all the fractions and tissues of the rat.

Several new papers on GSTs are given some data on the of microsomal GST in rat tissues (also of the brain) [16], the distribution of GSH and GST in mitochondria and cytosol of the nervous system [9, 17], the GSH and GSTs activity in the organs and blood of rats following chronic irradiation at low doses [18], but none of them uses MP as an inductor of GST activity.

There is also a paper about the purpose of using cryopreserved tissue to study the metabolism of pesticides in food-producing animals and rats [20] concluding that there is no qualitative differances in the results but a quantity degrease appears.

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WPŁYW METYLOPARIATIONU NA IZOENZYMY TRANSFERAZY S-GLUTATIONOWEJ W RÓŻNYCH TKANKACH SZCZURA

W niniejszej pracy zbadano wpływ insektycydu fosforoorganicznego – metyloparationu na aktywność transferaz S-glutationowych w tkankach szczura rasy Wistar. Zbadano frakcję postmitochondrialną, cytozolową i mikrosomalną wątroby, nerki, trzustki, śledziony i mózgu. Aktywność oznaczano z dwoma substratami: CDNB i DCNB, co pozwoliło wyróżnić izoenzymy GST. Stwierdzono zmiany aktywności zależne od tkanki i frakcji komórkowej.