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Joanna Kowalik, Janusz Błasiak

COMPARISON OF THE DNA DAMAGING ACTIVITY OF THE ORGANOPHOSPHORUS INSECTICIDE METHYLPARATHION AND ITS MAIN METABOLITE

The ability of the commonly used organophosphorus insecticide methylparathion and its main metabolite methylparaoxon to induce damage to pUC19 plasmid DNA was investigated. Agarose gel electrophoresis was employed in the study. The changes in optical density of bands corresponding to supercoiled (CCC) and open circular (OC) form of pUC19 were analysed. Methylparathion, in contrary to its metabolite, did not cause any changes as detected by the used system. Methylparaoxon evoked conformational changes of the DNA that were displayed as the increase of the fraction of the OC form. Obtained results indicate that methylparaoxon has potential ability to induce damage to DNA *in vivo*.

1. INTRODUCTION

Organophosphorus insecticides are used in agriculture and food production. They are characterised by high efficiency and low persistence [1]. The organophosphorus insecticides are powerful inhibitors of acetylcholinesterase. This target is responsible for most of the physiological effects, *e.g.* hyperexcitability, convulsions and muscular paralysis, events which precede death in poisoned animals [2–4]. Additionally, other markers of toxicity (memory and visual disturbances, schizophrenia and depression) not related to the inhibition of acetylcholinesterase may chronically develop in poisoned individuals [5–7].

Chronic toxicity in man is related to behavioral, genetic, reproductive, teratogenic and carcinogenic actions [5], but the molecular mechanisms of these effects are far from clear. Therefore, the knowledge of the interactions of insecticide compounds with biomolecules is an imperative task for the design and synthesis of selective compounds.

If the genotoxicity of a compounds is concerned, it is essential to assess the ability of the compounds to induce DNA damage. To do so an *in vitro* system which looks at the effect of the insecticides on purified DNA

directly can be used [8]. This system is organism independent and is free of the masking DNA repair and is also free of any metabolic capacity, *e.g.* in terms of bioactivation and detoxification. Moreover, this system allows to interpret obtained results in relative precise physico-chemical terms.

Plasmid DNA is a useful tool for the investigating of damages to DNA. The plasmid DNA can be in three conformational forms differing in electrophoretic mobility in agarose gel. Native plasmid represents superhelical, covalently closed form (CCC). Single-strand breaks lead to relaxation of plasmid DNA and generation of open circular form (OC). Covalent intercalation between base pairs can cause the same result. Double-strand breaks induce formation of linear form. DNA nicking agents and intercalating substances *in vivo* can lead to genotoxic changes that can be displayed in the electrophoretic pattern of plasmid DNA.

Possibility to induce damage to plasmid DNA by the organophosphorus insecticide methylparathion and its main metabolite methylparaoxon was investigated in this work.

2. MATERIALS AND METHODS

DNA

pUC19 plasmid DNA was obtained from Peterfarm (Sieradz, Poland). The concentration of DNA was estimated spectrophotometrically. Plasmid DNA typically contained about 80% double stranded covalently closed circular supercoiled molecules, 20% open relaxed circular molecules and no linear molecules.

Chemicals

Organophosphorus compounds methylparathion (*O,O*-dimethyl *O*-4 nitrophenyl phosphorothioate) and its main metabolite methylparaoxon (*O,O*-dimethyl *O*-4 nitrophenyl phosphate) at purity of 95–99% were supplied by Instytut Przemysłu Organicznego (Warsaw, Poland) and Dr. Ehrenstorfer GmbH (Augsburg, Germany). Chemical structures of these agents are displayed in Fig. 1.

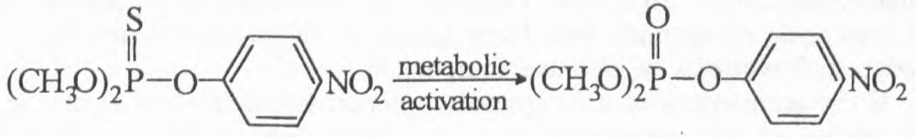

Fig. 1. Chemical structure of methylparathion (a) and methylparaoxon (b)

Chemicals treatment

The insecticide and its metabolite were derived from stock (50 mM) ethanolic solutions to give a final concentration of 150 μM. The DNA and ethanol concentration in all samples were respectively 13,3 μg/ml and 0.384%. The control received, instead of the insecticide, ethanol, the concentration of which did not affect the processes under study. The samples were incubated in the dark for 72 h at 37°C in a buffer comprising 45 mM Tris-borate, 1 mM EDTA, pH 8.0.

Sample analysis

Covalently closed circular DNA (CCC), such as the pUC19 plasmid used in this experiment, is a sensitive probe for detecting strand scission [9]. Such plasmid can be cleaved at any of phosphodiester linkages which permits the DNA strands to unwind, resulting in relaxed form OC DNA [10]. Introduction of another nick adjacent to the first but on the opposite strand gives linear form DNA. Some chemicals may not only cause DNA strand breakage but also unwinding of negatively supercoiled DNA by intercalative covalent modification [11–13]. Both processes lead to a formation of a relaxed circle which agarose gel electrophoretic mobility is about half that of the supercoil. The samples were ran in 0.8% agarose, stained with ethidium bromide, placed in a UV transilluminator and photographed with a Polaroid camera using a black and white Polaroid film type 665. The negatives were scanned with a Desaga densitometer, model CD 60.

3. RESULTS

Result of agarose gel electrophoresis of pUC19 plasmid DNA after 72 h incubation with methylparathion and methylparaoxon at a concentration of 150 μM is displayed in Fig. 2. The control (lane 1) shows three bands corresponding to DNAs of different electrophoretic mobilities. The first one of high optical density, migrating through agarose gel at highest rate, represents native supercoiled plasmid DNA (CCC). The second, slower migrating band, corresponds to relaxed circular form (OC). The third visible band in the electrophoretic pattern is chromosomal DNA contaminating plasmid DNA.

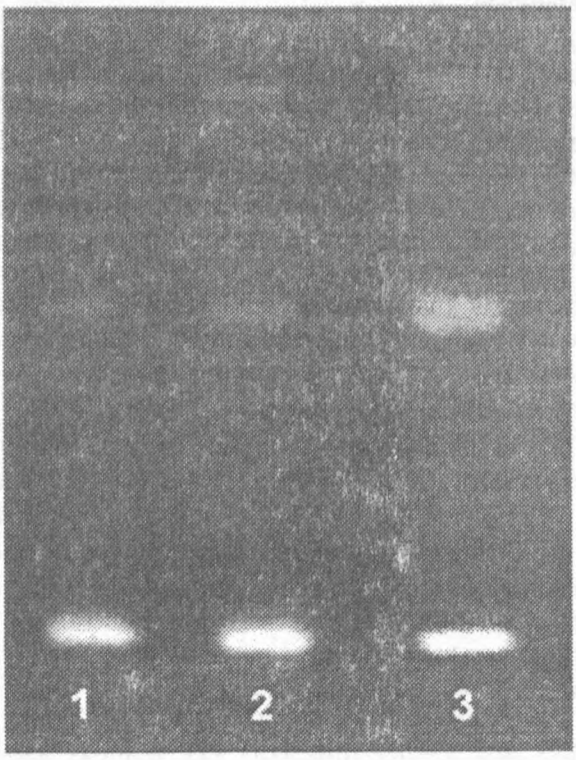

Fig. 2. Electrophoresis of plasmid pUC19 DNA after 72 h incubation at 37° C with methylparathion (lane 2) and methylparaoxon (lane 3) at a concentration of 150 μM. Lane 1 is the control. 100 ng of DNA was loaded on each slot of 0.8% agarose gel and electrophoresis was performed at 5 V/cm for 3 h

Comparing to control sample, no differences were observed in electrophoretic pattern after incubation of plasmid pUC19 DNA with methylparathion (lane 2). Increase of optical density of the band of OC form and decrease of optical density of CCC form band followed after incubation of plasmid DNA with methylparaoxon.

4. DISCUSSION

Comparison of samples treated with methylparaoxon with control shows the differences in optical density of bands corresponding with superhelical (CCC) and open circular (OC) forms of plasmid DNA. Therefore, methylparaoxon has an ability to induce conformational transformation of studied

DNA by disturbing its superhelical structure. This can be a consequence of single-strand breaks or intercalative covalent modification. Performed experiment does not allow to differentiate which of these two mechanisms participate in observed transition of CCC into OC form.

Methylparathion is metabolised to methylparaoxon in organism [14] – the phosphorothioate residue is oxidized to phosphate residue (Fig. 2). The properties of natural metabolites should be considered during studies of the influence of a chemical on living organism.

Oxidation is a typical reaction of organophosphorus compounds [5]. It takes place in microsomal fraction of hepatocites and has influence on biological activity of these compounds. In general, oxidative analogues of organophosphorus insecticides are more toxic than parent compounds [5], what was confirmed in our experiment. Double bond between phosphorus and oxygen atoms causes that the former one acquires electrophilic properties. It can cause an attack of insecticide molecule on nucleophilic compounds, such as DNA.

Possible mechanism leading to DNA strand nicking after organophosphorus insecticide treatment is methylation of purines and spontaneous depurinization of alkilated bases. Under physiological conditions (pH 7.4; 37° C) the apurinic sites are unstable and DNA is cleaved by a β-elimination mechanism [15].

Fact that methylparaoxon has an ability to interact directly with DNA generating strand breaks of purified DNA or intercalating between base pairs may not necessarily be a basis of reported genotoxic properties of this compound or inducing identical effects *in vivo*. Further studies are needed to establish possible mechanism of observed changes.

5. REFERENCES

[1] Ware G. W. (1978), *The Pesticide Book*, vol. I, W. H. Freeman and Company, San Francisco, 27–52.
[2] Aldridge A. N., Davison A. N. (1952), Biochem. J., **51**, 61.
[3] Doherty J. D. (1979), Pharmacol. Ther., **7**, 123.
[4] Fukuto R. T. (1990, Environ. Health Perspec., **87**, 245.
[5] Eto M. (1974), *Organophosphorus Pesticides: Organic and Biological Chemistry*, CRC Press, Cleveland, 123–231.
[6] Okhawa H., Oshita H., Miyamoto J. (1980), Biochem. Pharmacol. **29**, 2721.
[7] Herbert G., Peterle T., Gruub T. (1989). Bull. Environ. Contam. Toxicol., **42**, 471.
[8] Griffin D. E., Hill W. E. (1978), Mutat. Res. **52**, 161.
[9] Gamper H. B., Tung A. S. C., Straub K., Bartholomew J. C., Calvin M. (1977), Science, **197**, 671.

[10] Johnson P. H., Grossman L. I. (1977), Biochemistry, **16**, 4217.
[11] Espejo R. T., Lebowitz J. A. (1976), Anal. Biochem., **72**, 95.
[12] Brawn K., Fridovich I. (1981), Arch. Biochem. Biophys., **206**, 414.
[13] Salgo M. G., Stone K., Squadrito G. L., Battista J. R., Pryor W. A. (1995), Biochim. Biophys. Res. Commun., **210**, 1025.
[14] Matsumura F. (1985), in: *Toxicology of Insecticides*, ed. F. Matsumura, vol. 2, Plenum Press, New York and London, 203–289.
[15] Kushida T. (1994), J. Am. Chem. Soc., **116**, 479.

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Department of Molecular
Genetics, University of Łódź
Poland

Joanna Kowalik, Janusz Błasiak

PORÓWNANIE ZDOLNOŚCI DO USZKADZANIA DNA PRZEZ INSEKTYCYD FOSFOROORGANICZNY I JEGO GŁÓWNY METABOLIT

Badano zdolność indukowania uszkodzeń DNA plazmidu pUC19 przez powszechnie stosowany insektycyd fosforoorganiczny metyloparation i jego główny metabolit metyloparaoksonu po 72 h inkubacji z tymi związkami. Stosowano metodę elektroforezy w żelu agarozowym i analizowano zmianę intensywności pasm odpowiadających dwóm formom konformacyjnym plazmidu – kolistej superskręconej (CCC) i kolistej otwartej (OC). Metyloparation, w przeciwieństwie do swego metabolitu, nie wywoływał zmian konformacyjnych plazmidowego DNA. Działanie metyloparaoksonu powodowało przejście konformacyjne DNA z formy CCC do OC. Otrzymane rezultaty wskazują na potencjalną zdolność metyloparaoksonu do wywoływania uszkodzeń DNA *in vivo*.