Metabolism of Terbufos in Rat Liver

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Abstract: Metabolism of Terbufos in Rat Liver: Jin-Tong Li, *et al.* Institute of Pharmacology and Toxicology, Academy of Military Medical Sciences, Beijing, China, 100850—Biotransformation of terbufos in rat liver revealed four metabolites in the effluent prepared with a C₁₈ cartridge after the rat liver was perfused for one hour in situ. Analysing the spectrogram of GC-IR and GC-MS, metabolite IV appeared to be an oxidative desulfuration product of terbufos with the formula C₉H₂₁O₃PS₂, the recovery of which in the effluent was 2.13%; metabolite I appeared to be an hydrolysate of metabolite IV with the formula C₅H₁₃O₃PS, the recovery of which was 0.13%; metabolite II appeared to be an hydrolysate of terbufos with the formula C₅H₁₃O₂PS₂, the recovery of which was 2.65%; metabolite III appeared to be a methylate of metabolite II, with the formula C₆H₁₅O₂PS₂, the recovery of which was 1.42%. Relatively the recovery of terbufos was 40.8%. These results were in accord with the regular metabolic pattern in vivo of phosphorothioates with a thioether group.

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Key words: Terbufos, liver metabolism, metabolite, GC-MS, GC-IR

Terbufos is a kind of soil contaminated organophosphorothioate pesticide containing thioether. No reports were seen in literature about its metabolism in rat liver. We studied the biotransformation of terbufos in rat liver perfused in situ, inferred proposed pathways for the mammalian hepatic biotransformation of terbufos by four determined metabolites in the effluent, and determined the recoveries of terbufos and its metabolites in the effluent. We preliminarily discussed the effect of the hepatic metabolic activation and detoxification on the toxicity of terbufos and the toxicity of its metabolites.

Materials and Methods

Chemicals

Terbufos (S-ter-butylthiomethy O,O,-diethyl phosphorodithioate) with a 288.42 molecular weight and 91% purity was purchased from Tian-Jin Pesticides Factory. Bovine serum albumin (BSA) was purchased from Sigma Chemical Co. (electrophoresis grade) and dextran T-40 from Pharmacia LKB (Sweden), methanol and ethyl acetate were HPLC grade, and all other chemicals were reagent grade.

Animals

Male Wistar rats (180–220 g) were obtained from the Laboratory Animal Center of Academy of Military Medical Sciences, and were housed under standard laboratory conditions with free access to water and feed.

Liver Perfusions

Single-pass liver perfusions in situ were performed as described by Sies¹⁾. 100 μ l 0.1 mol/l Terbufos dissolved in methanol was added to the 100 ml perfusate reservoir, the perfusate was a modified Krebs-Ringers NaHCO₃ buffer containing 0.3% BSA, 0.5% dextran T-40 and 0.3% glucose. Flow rates were maintained at 5 ml/liver per min and the length of perfusions was 1 h.

Chemical Analyses

Before terbufos and its metabolites were qualified and quantified by GC-IR and GC-MS, they were separated with a solid phase extraction (SEP) C₁₈ cartridge (Waters Co.). About 50 ml of effluent was passed through an SEP C₁₈ cartridge and eluted from the cartridge with 5 ml of ethyl acetate. Then the eluant was dried under a nitrogen stream and the residue was redissolved in 25 μ l of methanol. The concentrated sample was analyzed by GC-IR and GC-MS. HEWLETT PACKARD 5890 Series II gas chromatography with flame photometric detection and HEWLETT PACKARD VARIO-2 TYPE mass-spectrometer were used, and the infra-red spectrometer was a Bio-Rad FTS-65A. The recoveries of metabolites

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Fig. 1. Gas Chromatogram of Terbufos and its metabolites in rat liver perfusate.

Fig. 2. Mass Spectrum of Terbufos standard.

were expressed as the percentage of the concentration of the parent pesticide entering the liver.

Results

Gas chromatography and GC-IR analyses of terbufos and its metabolites

The concentration sample was well isolated by gas chromatography, compared with that of blank perfusate. Surplus peaks were examined through characteristic absorption groups in an infra-red spectrometer one by one, particularly the group containing P atoms. As a result, as shown in Fig. 1, there were 5 compounds in the gas chromatograph containing P atoms, and the retention times (Rt) of whose peaks were 6.66 min, 7.51 min, 8.35

min, 12.85 min and 13.73 min, respectively.

Identification of Peak V (Rt=13.73 min) in Fig. 1

Examination of standard control by GC-MS revealed that its mass spectrum accorded with the mass spectrum of the peak with a retention time of 13.73 min in Fig. 1 (as shown in Fig. 2 and Fig. 3). Therefore, the peak with the retention time of 13.73 min in Fig. 1 was determined as terbufos (S-ter-butylthiomethy O,O,-diethyl phosphorodithioate) and its molecular structure was (Fig. 12).

Identification of Peak I (Rt=6.66 min) in Fig. 1

Peak I (Rt=6.66 min) showed metabolite I. Its

Fig. 3. Mass Spectrum of Terbufos in rat liver perfusate.

Fig. 4. Mass spectrum of metabolite I in rat liver perfusate.

molecular ion peak and fragment peak (Fig. 4) were completely identified with the mass spectrum of $C_5H_{13}O_3PS$ by looking up its mass spectrum in The "Wiley/NBS Registry of Mass Spectral Data"⁷²⁾. This was confirmed by finding that metabolite I had the characteristic absorption group of $P=O$ which was seen at $1,280\text{ cm}^{-1}$ wave velocity in its infrared spectrum (Fig. 5). Therefore, metabolite I could be determined as $C_5H_{13}O_3PS$ (O,O-diethyl-S-methyl phosphorothioate) and its molecular structure is presented in Fig. 12.

Identification of Peak II ($R_t=7.51\text{ min}$) in Fig. 1

Peak II ($R_t=7.51\text{ min}$) in Fig. 1 showed metabolite II. Its molecular ion peak and fragment peak (Fig. 6) were

Fig. 5. IR spectrum of metabolite I in rat liver perfusate.

Fig. 6. Mass spectrum of metabolite II in rat liver perfusate.

Fig. 7. IR spectrum of metabolite II in rat liver perfusate.

completely identified with the mass spectrum of $C_5H_{13}O_2PS_2$ by looking up its mass spectrum in the abovementioned Data book. This was also confirmed by finding that metabolite II had the characteristic absorption group of $P=S$ which was recognized at $760\text{--}780\text{ cm}^{-1}$ wave velocity in its infra-red spectrum (Fig. 7). Therefore, metabolite II could be determined as $C_5H_{13}O_2PS_2$ (O,O-diethyl-S-methyl phosphorodithioate) and its molecular structure is presented in Fig. 12.

Identification of Peak III ($R_t=8.35\text{ min}$) in Fig. 1

Peak III ($R_t=8.35\text{ min}$) was metabolite III. Its molecular ion peak and fragment peak (Fig. 8) were rightly identified with the mass spectrum of $C_6H_{15}O_2PS_2$ by looking up its mass spectrum in the abovementioned Data book. This was also confirmed by finding that metabolite III had the characteristic absorption group of $P=S$ which was recognized at $760\text{--}780\text{ cm}^{-1}$ wave velocity

in its infra-red spectrum (Fig. 9). Therefore, metabolite III could be determined as $C_6H_{15}O_2PS_2$ (O,O,S-triethyl dithiophosphate) and its molecular structure is presented in Fig. 12.

Identification of Peak IV ($R_t=12.85\text{ min}$) in Fig. 1

Peak IV ($R_t=12.85\text{ min}$) showed metabolite IV, its molecular ion peak was 272 and decreased 16 particles less than compared with the molecular ion of terbufos, suggesting that metabolite IV was an oxidized desulfuration product (oxon derivant) of terbufos. In its mass spectrum, the primary peak was 215 m/e , base peak was 171 m/e , and another main fragment peak was 57 m/e (Fig. 10). Analysis of their formation in the mass spectrum was as follows: first, S-methyl of metabolite IV was β -eliminated as $(C_2H_5O)_2POSCH_2S^+$ ($m/e\ 215$) and C^+ (CH_3)₃ ($m/e\ 57$), then $C_2H_5OPOHCH_2S^+$ ($m/e\ 171$) was formed from $(C_2H_5O)_2POSCH_2S^+$ ($m/e\ 215$) by breaking down a $^+C_2H_4O$ ($m/e\ 44$) and rearranging H^+ . In further analysis of the infra-red spectrum of metabolite IV (Fig. 11), at 1280 cm^{-1} , wave velocity and characteristic absorption group of $P=O$ were recognized. Therefore, metabolite IV could be determined as $C_9H_{21}O_3PS_2$ (S-ter-butylthiomethyl O,O-diethyl phosphorothioate) and its molecular structure is presented in Fig. 12.

Recovery of terbufos and its metabolites in the effluent

As shown in Table 1: In the effluent of rat liver perfused in situ, the recovery of terbufos was 40.8%, and the recovery of metabolites I, II, III and IV was 0.13%, 2.65%, 1.42% and 2.13%, respectively.

Discussion

Many organophosphorus pesticides such as

Fig. 8. Mass spectrum of metabolite III in rat liver perfusate.

Fig. 9. IR spectrum of metabolite III in rat liver perfusate.

Fig. 11. IR spectrum of metabolite IV in rat liver perfusate.

Fig. 10. EI-MS spectrum of metabolite IV in rat liver perfusate.

Fig. 12. Molecular structures of terbufos and its metabolites.

phosphorothioate undergo metabolic activation to form the corresponding oxons, which are potent inhibitors of cholinesterase, but these oxons can undergo further biotransformation (detoxification) once formed within the liver³. Metabolic activation is mainly oxidation reaction catalyzed by cytochrome P-450 dependent monooxygenases, including oxidized desulfuration of phosphorothioate, formation of sulphoxides and sulphones, formation of amine oxides and *O*-dealkylation^{4,5,6}. Among them, oxidized desulfuration may be the most important activation. Four main routes of hepatic detoxification have potential importance in toxicity levels^{7,8}. The first, cytochrome P-450 mediated dearylation of phosphorothionates; the second, aliesterases should be able to destroy a significant amount of the oxons; the third, the Ca^{++} -dependent A-esterases, are capable of catalytic oxon hydrolysis; and the fourth, glutathione (GSH) conjugation, is a potential route of xenobiotic detoxification. In addition, methylation of phosphorothioate is supposed to be a detoxification pathway⁹.

In mammals, the relative rates of formation and detoxification of oxons are important factors in determining their acute toxicities^{7,10}. In our results, metabolite IV (terbufos oxon) is an oxidized desulfuration product of terbufos which reaction was catalyzed by cytochrome P-450 located in the liver microsome, and its recovery in the effluent is 2.13%. Metabolite I is a hydrolysate of metabolite IV which reaction was catalyzed by A-esterase located in the liver microsome and mitochondrion, and its recovery was 0.13%; Meanwhile, metabolite II is hydrolysate of terbufos which reaction was also catalyzed by A-esterase located in the liver microsome and mitochondrion, and its recovery was 2.65%. But metabolite III was a methylate of metabolite II which reaction was suggested to be a detoxification reaction catalyzed by SAM methyl transferase, and its recovery was 1.42%. Relatively, the recovery of terbufos is 40.8%. Sulphoxides and sulphones were not detected in the effluent. These results are

Table 1. Recovery of terbufos and metabolites in effluent

Preparation*	Effluent perfusate [#]				
	Parent compound (%)	Metabolite I (%)	Metabolite II (%)	Metabolite III (%)	Metabolite IV (%)
1	42.7 ± 0.4	0.10 ± 0.04	2.48 ± 0.35	1.53 ± 0.15	1.30 ± 0.05
2	42.8 ± 0.3	0.20 ± 0.03	3.78 ± 0.29	1.55 ± 0.04	3.03 ± 0.01
3	37.0 ± 1.7	0.10 ± 0.01	2.04 ± 0.21	1.00 ± 0.28	2.07 ± 0.58
Mean ± SD	40.8 ± 3.3	0.13 ± 0.06	2.65 ± 0.98	1.42 ± 0.37	2.13 ± 0.87

*Each preparation was made by three liver perfusions. [#]Values are expressed as the percentages of the concentration of the parent pesticide entering each liver.

accorded with the regular metabolic pattern of phosphorothioate with thioether in mammals⁹⁾.

Nevertheless, it is worth mentioning that metabolites I, II and III are all trialkylphosphorothioates analogues. Although the major of these compounds caused cholinergic signs, in some, second phase toxicity affecting the lung was also seen¹¹⁻¹³⁾. Histopathological studies revealed that the lung lesion is initiated from damage caused by type I pneumocytes and subsequent proliferation of type II pneumocytes which lead to progressive diffuse interstitial thickening in adult rat lung^{14, 15)}. These compounds can also interfere with normal biochemical and physiological maturity of fetal lung through intrauterine exposure^{16, 17)}. Lung injury could precipitate hypercapnia to result in hypothermia¹⁸⁾. Study on the structure and pulmonary toxicity relationship indicated that S-alkyl moieties are residues determining whether a compound mainly induces pulmonary or acute cholinergic symptoms^{12, 13)}. Furthermore, it must be remembered that the hepatic metabolism of terbufos is complex, potentially involving several simultaneous and sequential reactions, so that liver perfusion studies only give partial information regarding specific metabolic steps, and more details of the metabolism of terbufos and the toxicological action of its metabolites await further research.

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