BIOCHEMICAL PHARMACOLOGY 75 (2008) 2045–2053

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Reactions of isodimethoate with human red cell acetylcholinesterase

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ARTICLE INFO

Article history:
Received 5 February 2008
Accepted 20 February 2008

Keywords:
Acetylcholinesterase
Dimethoate
Isodimethoate
Obidoxime
Pralidoxime
Pesticides

ABSTRACT

Isodimethoate is a thermal decomposition product that is present in usual pesticide formulations of dimethoate. Owing to its P=O structure the compound is a direct anticholinesterase agent whose properties, to the best of our knowledge, are presented here for the first time. Isodimethoate shows an inhibition rate constant towards human red blood cell acetylcholinesterase (AChE) of $2.3 \times 10^3\ M^{-1}\ min^{-1}$ (pH 7.4, 37 °C), indicating a somewhat higher potency than found with omethoate, the CYP450-mediated active metabolite of pure dimethoate. Isodimethoate-inhibited AChE shows fast spontaneous reactivation and aging kinetics (half-life 2.3 and 25 min, respectively). The inhibited, non-aged enzyme is readily reactivated by obidoxime ($k_r = 9\ min^{-1}$; $K_D = 0.1$ mM) but hardly by pralidoxime at therapeutic concentrations. Interestingly, isodimethoate hydrolyzes readily in buffered solutions at pH 7.4 and 37 °C with liberation of methylmercaptan (half-life 16 min). Liberation of *N*-(methyl)mercaptoacetamide, the expected leaving group, was not observed. These properties make isodimethoate a hit-and-run agent that renders part of AChE non-reactivatable within a short period of time. The clinical consequences of exposure to or intentional ingestion of isodimethoate-containing dimethoate formulations are a partly untractable AChE shortly after incorporation. In fact, aging of AChE in dimethoate-poisoned patients on admission was much more advanced than expected from the reaction with omethoate. Manufacturers, researching scientists and clinical toxicologists should be aware of this problem.

1. Introduction

It has long been known that oximes are of little value in reactivating acetylcholinesterase (AChE) after dimethoate poisoning. Sanderson and Edson were probably the first who showed that there was no advantage of oxime administration in dimethoate-poisoned rats with the tendency that the benefits of atropine were curtailed by inclusion of pralidoxime. In contrast, there was some benefit of oximes (pralidoxime and trimedoxime) in the case of parathion methyl poisoning [1]. The authors reasoned that the difference may be caused by the slower formation of the phosphorylating metabolite in dimethoate poisoning, as opposed to parathion methyl. During the gradual formation of the phosphorylated enzyme in dimethoate poisoning a larger part was already in the aged state when the toxic syndrome becomes evident. In

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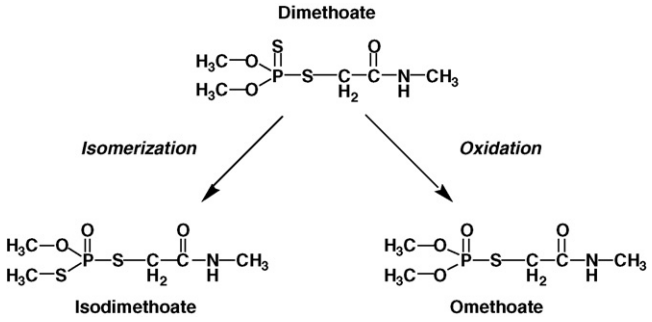
Abbreviations: AChE, acetylcholinesterase (EC 3.1.1.7); ATCh, acetylthiocholine; AU, absorbance units; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid).

0006-2952/$ – see front matter
doi:10.1016/j.bcp.2008.02.021



Scheme 1 – Activation of dimethoate to phosphorylating agents.

contrast, the precipitous inhibition of AChE and the arising cholinergic crisis in parathion methyl poisoning occurred earlier when a smaller proportion of AChE was aged. The resistance of inhibited AChE towards pralidoxime or obidoxime treatment was also shown in dogs poisoned with dimethoate [2], and similar observations were made in man [3]. Inhibited AChE of dimethoate-poisoned patients occasionally showed much faster aging than expected from an *O*,*O*-dimethylphosphoryl enzyme and contrasted with the picture seen, e.g., with oxydemeton-methyl poisoning. This strange behavior suggested either an alternative inhibition reaction or the involvement of dimethoate impurities, or both.

It is generally assumed that P=S organophosphorus esters have to be metabolically activated by oxidative desulfuration, mainly by the CYP450 superfamily, leading to the corresponding oxons that render the phosphorus atom more electrophilic (Scheme 1). The corresponding dimethoate metabolite is omethoate [4,5]. Given that liberation of *N*-methyl-mercaptoacetamide as the expected leaving group of omethoate forms *O*,*O*-dimethylphosphoryl–AChE, the inhibited enzyme should be indistinguishable from the enzyme blocked by other *O*,*O*-dimethyl organophosphates [6]. If, however, an *O*-methyl was the leaving group, a completely different enzyme would result that might be much more prone to aging and spontaneous reactivation as seen with other phosphorothiolates [7]. Such a pathway for dimethoate/omethoate has, however, never been detected and seems quite unlikely for chemical reasons.

Early work with P=S organophosphorus esters was carried out without realizing that some of these agents may change spontaneously into compounds with properties very different from their parent forms [8]. Besides of oxidation of the P=S or mercapto sulfur in the leaving group, thiono–thiolo isomerisation (RO–P=S into RS–P=O) readily occurs, particularly at elevated temperatures. The toxicological impact of post-factory chemical alterations was recognized in 1976 when 2800 spraymen using malathion during a malaria eradication program in Pakistan became poisoned with characteristic cholinergic signs and symptoms, which were usually not seen with this comparably safe pesticide. It turned out that isomalathion was one of the contaminants formed upon storage that markedly increased the toxicity of the malathion formulation [9].

Also dimethoate is prone to thermal and photochemical isomerisation with formation of isodimethoate [10,11], an *O*,*S*-dimethyl oxon that is a directly acting inhibitor of AChE. (A revised FAO specification allowed up to 7% of isodimethoate after storage tests [11].) By this isomerisation, a new center of asymmetry at phosphorus is formed. If *N*-methyl-mercaptoacetamide is the leaving group two enantiomeric *O*,*S*-dimethylphosphoryl–AChE molecules may be formed in analogy to the reaction of isoparathion methyl with AChE [12]. The rate of inhibition by isoparathion methyl of AChE from various sources was generally higher with the S_P-isomer compared to the R_P-isomer. There was, however, a marked species difference in both inhibition rate constants and their ratios, being least with human erythrocyte AChE [13]. There also exist remarkable differences between both inhibited enzyme species with regard to post-inhibitory reactions. Thus spontaneous as well as oxime-induced reactivation was generally higher in case of the S_P-isomer, while non-reactivatability proceeded two-times faster with the R_P-isomer [14].

The importance of the shelf-age of dimethoate formulations on its toxicity was suspected in 1964 when Meleney et al. reported the different dose dependence for toxic effects of dimethoate in sheep [15] and by Gaines for rats [16]. The involvement of dimethoate impurities on the enhanced toxicity in the presence of oximes has already been supposed in 1966 when it turned out that oximes were capable of enhancing AChE inhibition only in the case of impure dimethoate samples and it suggested to the authors that omethoate or isodimethoate may react with the oximes to give more potent inhibitors such as phosphoryloximes [17,18]. At that time omethoate and isodimethoate were not available to allow more in-depth studies. As to our knowledge, no further investigations on this topic were carried out subsequently by the above authors.

With regard to the strange observations in dimethoate-poisoned patients [3] and the availability of isodimethoate we undertook an *in vitro* study to elucidate the kinetics of inhibition, spontaneous and oxime-induced reactivation as well as formation of a non-reactivatable enzyme of human erythrocytes. During these experiments we realized that isodimethoate was surprisingly unstable in phosphate buffer, pH 7.4, at 37 °C which prompted an additional study.

2. Materials and methods

Isodimethoate, 93.4% certified purity, (*O*,*S*-dimethyl-*S*-(*N*-methylcarbamoylmethyl) phosphorodithioate; CAS 3344-11-4) was a generous gift from Dr. K. Lystbaek, Cheminova A/S Lemvig, Denmark. Stock solutions (100 and 10 mM) were prepared in acetonitrile and kept at −20 °C. Omethoate (*O*,*O*-dimethyl-*S*-(*N*-methylcarbamoylmethyl) phosphorothioate; CAS 113-02-6), 95.5% certified puritiy, was obtained from Dr. Ehrenstorfer, Augsburg, Germany. Stock solutions (10 mM) were prepared in acetonitrile and stored at −20 °C. The acetonitrile content in all enzyme assays was ≤1% (v/v). *N*-(Methyl)mercaptoacetamide, 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), acetylthiocholine iodide (ATCh) and pralidoxime chloride were obtained from Sigma, Taufkirchen, Germany, obidoxime chloride monohydrate from Duphar, Amsterdam,