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Atropine maintenance dosage in patients with severe organophosphate pesticide poisoning☆

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ABSTRACT

Although the importance of atropine in therapy of organophosphate (OP) poisoning is generally recognized, its dosing is a matter of debate. A retrospective analysis of atropine dosing was undertaken in 34 patients who had been enrolled in a clinical study assessing obidoxime effectiveness in OP-poisoning. All patients were severely intoxicated (suicidal attempts) and required artificial ventilation. Atropine was administered routinely by intensive care physicians for life-threatening muscarinic symptoms, with the recommendation to favor low dosage. The pharmacological active enantiomere *S*-hyoscyamine was determined by a radioreceptor assay.

When RBC-AChE activity ranged between 10% and 30%, *S*-hyoscyamine plasma concentrations of approx. 5 nmol L^{-1} were sufficient. This concentration could be maintained with about 0.005 mg h^{-1} kg^{-1} atropine. Only when RBC-AChE was completely inhibited, therapy of cholinergic crisis required atropine doses up to 0.06 mg h^{-1} kg^{-1}. Elimination half-life of *S*-hyoscyamine was 1.5 h, showing occasionally a second slow elimination phase with $t_{1/2}$ = 12 h. Malignant arrhythmias were observed in some 10% of our cases, which occurred late and often in the absence of relevant glandular cholinergic signs, when the *S*-hyoscyamine concentration was below 2.5 nmol L^{-1}. Arrhythmias mostly resolved on reinstitution of atropine.

1. Introduction

Accidental and suicidal intoxications play the dominant role in poisoning by organophosphorus compounds (OP) (Eddleston et al., 2008; Aardema et al., 2008; Karalliedde and Szinicz, 2001). However, recent incidents like the sarin attacks in Japan (Tu, 2001; Okumura et al., 1996) shifted public attention to another subgroup of OP, the nerve agents. The terrorist attacks of 11th September 2001 and more recently July 2005 in London or September 2006 and November 2008 in Mumbai indicate the changed intention of terrorists, namely to maximize lethality (Sohns, 2000; Miller, 2006). This goal may easily be achieved by using nerve agents. Hence, knowledge about therapy of OP poisoning in more detail is timely and important.

Toxicity and therapeutic strategies of OP poisoning due to pesticides as well as nerve agents follow the same principles (Thiermann et al., 2007). The most important toxic mechanism is inhibition of acetylcholinesterase (AChE), resulting in cholinergic crisis. Antidotal therapy is based on reactivation of inhibited AChE by oximes and competitive displacement of excess acetylcholine by atropine at muscarinic receptors. While the effectiveness of oximes in human OP pesticide poisoning is still debated (Singh et al., 2001; Khan et al., 2001; Balali-Mood and Shariat, 1998; de Silva et al., 1992; Buckley et al., 2011), atropine is regarded as significant cornerstone in treatment of OP poisoning. Recommendations on its dosing, however, vary considerably (Singh S. et al., 1995; Singh G. et al., 2000; Singh et al., 2001; Balali-Mood and Shariat, 1998; Lee and Tai, 2001; Goswamy et al., 1994; Sungur and Guven, 2001; DuToit et al., 1981; Husain et al., 2010). Early suggestions of moderate dosing (Grob, 1956: "4 to 6 mg atropine should be injected intravenously" and "repeated in doses of 2 mg at 3–8 min intervals until bronchial secretions and salivation decrease and convulsions cease", and that "as much as 24–48 mg of atropine may be required the first day") contrast with reports on total doses of 20 g (Golsousidis and Kokkas, 1985) and 30 g (LeBlanc et al., 1986). With such an aggressive atropinization the mark of clearing lungs and improving cardiovascular function (Lee and Tai, 2001; Singh S. et al., 1995; Singh et al., 2001; Balali-Mood and Shariat, 1998; Goswamy et al., 1994; Tafuri

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and Roberts, 1987) is most probably overshot and serious adverse effects may occur (Beards et al., 1994; Kiss and Fazekas, 1983; Finkelstein et al., 1989; Heath and Meredith, 1992; Robenshtok et al., 2002; Arendse and Irusen, 2009). A special concern is the atropine-induced delirium which may become an issue in cases of a terrorist nerve agent attack where central effects of atropine might enhance panic reactions of the extremely terrified population (Tu, 2001; Okumura et al., 1996). In such scenarios, as well as in OP pesticide poisoning it is of interest to know how much atropine may be just sufficient to control cholinergic crisis.

Judgment of appropriate atropine dosing is hampered by the high diversity of poisoning courses (e.g. due to different types and amounts of OP ingested, time to first treatment, various treatment strategies) (Eddleston et al., 2006, 2008). Furthermore, a clear look on antidotal effects is shadowed by the wide variety of the various mechanisms going on during poisoning and antidotal treatment, e.g. competition of atropine and acetylcholine, ongoing inhibition of AChE by persisting poison, spontaneous and oxime-induced reactivation, aging of the OP-AChE complex; additional therapeutic measurements (e.g. sedation and artificial ventilation) as well as complications (pneumonia, circulatory insufficiency). To consider all these variables, more detailed information, including the reactions at the synaptic site (activity of AChE, presence of active poison) and knowledge on the plasma concentration of atropine would be desirable. A specific study, providing these information has not been published. However, a detailed set of data was available from a clinical trial that was performed on effectiveness of obidoxime in patients with severe OP pesticide poisoning, using red blood cell (RBC)-AChE as surrogate parameter for synaptic AChE (Thiermann et al., 1997, 1999, 2005, 2009; Eyer et al., 2009). Hence, we exploited the data from this trial as background information and performed a retrospective analysis to determine the dose of atropine necessary to cope with the vital muscarinic symptoms and to describe the pharmacokinetics of atropine in the poisoned patients. To this end, we employed a radioreceptor assay which specifically determines the pharmacodynamically active *S*-hyoscyamine enantiomere. In contrast to our previous reports (Thiermann et al., 1996, 2009), we present our data consistently as *S*-hyoscyamine concentrations, since it has been shown that the two enantionmeres behave differently in humans (John et al., 2010). Additionally, we present three patients in whom (a) RBC-AChE activity was substantially inhibited, (b) plasma cholinesterase (Pl-ChE) started to re-increase while (c) cholinergic signs were either minor or completely missing and (d) plasma *S*-hyoscyamine concentration was 2.5 nmol L^{-1} when severe arrhythmia developed. Administration of atropine (*S*-hyoscyamine plasma levels above 5 nmol L^{-1}) led to a recovery of the cardiovascular function.

2. Material and methods

2.1. General procedure

Detailed information on the clinical trial, from which the data were derived for the retrospective analysis, were given previously (Eyer et al., 2009): In short, either life-threatening OP-intoxicated patients (need for artificial ventilation) were transported to our intensive care unit (ICU), or patients where monitored by an investigator from our team in an external hospital. 34 patients severely intoxicated by either diethyl-OP (14), dimethyl-OP (19) or phosphoramidates (1), could be included in the trial. Patient numbers in the different centers and duration of artificial ventilation are given in Table 1. In 33 cases poisoning followed intentional oral ingestion and in one case by subcutaneous injection due to suicidal attempt. All patients were treated with obidoxime (250 mg i.v. bolus, followed by 750 mg/24 h) as long as reactivation could be anticipated (maximally 7 days) (Thiermann et al., 1997, 1999; Worek et al., 1997; Eyer, 2003). We investigated the clinical course as well as the cholinesterase status, i.e. activity of RBC-AChE (RBC-AChE in vivo), reactivatability of RBC-AChE (RBC-AChE in vitro, following incubation with 100 μmol L^{-1} obidoxime) and Pl-ChE activity as well as the plasma concentration of obidoxime and the presence of active poison, and partly the plasma concentration of *S*-hyoscyamine. To combine optimal treatment and reasonable standardization, only the administration of obidoxime and blood sampling schedule necessary for judgment of cholinergic crisis (cholinesterase status) and the effect of obidoxime were regulated. All other necessary measures were at the discretion of the responsible physician at that time, including the administration of atropine, for which moderate dosing was recommended to clear the lungs, keep the heart rate above 80 beats per min and achieve dry axilla. Blood sampling was not scheduled primarily to monitor *S*-hyoscyamine plasma concentrations.

Prior to arrival at the ICU 65 ± 90 mg of atropine (0–424) were administered, including 36 ± 45 mg of atropine by the emergency physician. During the obidoxime infusion the patients received 80 ± 91 mg of atropine (0–353). After stopping the obidoxime infusion, 76 ± 166 mg atropine (0.5–854) was administered in 24 patients.

Lethality, which amounted to 21% (parathion 5; phoxim 1; oxydemeton-methyl 1), was caused by complications of poisoning (e.g. septic multiorgan failure, ARDS after aspiration pneumonia) and occurred about 20 days (8–38) after poisoning.

The trial was approved by the responsible Independent Ethics Committees (9 involved).

2.2. Determination of atropine

The radioreceptor assay developed by Aaltonen (Aaltonen et al., 1984) was used with slight modifications (Thiermann et al., 1996). Receptor material was from rat brain and the displacement of the ligand [N-methyl-^{3}H] scopolamine methyl chloride by atropine was determined (Thiermann et al., 1996).

Atropine doses refer consistently to racemic atropine sulfate monohydrate (MW 694.8 g). The assay was suitable to determine specifically *S*-hyoscyamine concentrations between 2.5 and 25 nmol L^{-1} in 50 μL plasma. Racemic atropine standards were analyzed regularly along with the plasma samples. There was no change in *S*-hyoscyamine content of spiked control plasma (2.5, 5, and 25 nmol L^{-1}) over 4 months (2.5 ± 0.3; 4.9 ± 0.4 and 22.6 ± 1 nmol L^{-1}) stored at −60 °C. Dilution of plasma with phosphate buffer did not disturb the assay, thus allowing quantification of concentrations higher than 25 nmol L^{-1}.

2.3. Determination of the cholinesterase status

Blood was immediately diluted bed-side 1:100 (v/v) with ice-cold phosphate buffer (0.1 mol L^{-1}, pH 7.4; 0.03% Triton X-100) and frozen at −20 °C until analysis. AChE activity was determined according to a modified Ellman method (Worek et al., 1999). Reactivatability of RBC-AChE, Pl-ChE and inhibitory material in plasma were assessed as already described (Thiermann et al., 1997).

2.4. Selection criteria for data to assess the correlation between the area under the curve (AUC) of the plasma S-hyoscyamine concentration vs. time and the atropine amount administered

Inclusion criteria: Availability of a clear and complete record of atropine dosing along with frequent blood sampling allowed 12 patients to be included.

Exclusion criteria: 1. Frequent changes of atropine dosing that was not accompanied by adequate blood sampling (sampling was restricted according to assessment of cholinesterase status by order of the Independent Ethics Committees) (19 patients). 2. Administration of drugs (e.g. amitriptyline) with anticholinergic effects that may have disturbed the assay (1 patient). 3. Clear contrast between the doses that were recorded and the clinical response as well as the atropine plasma levels (2 patients); the reason for this finding remains unclear but may be attributed to recording errors.

Generally, due to restricted sampling, determination of *S*-hyoscyamine-AUC was limited to the observation period of obidoxime treatment, i.e. 70 ± 44 h (4–185).

2.5. Calculations/statistics

AUCs were calculated by the trapezoidal rule up to the limit of quantification. Clearance was estimated by the ratio of infusion rate/plasma concentration at steady state (Css). Css was determined, when a constant dose was administered for a longer period (42 ± 45 h; >5 times $t_{1/2\ atropine}$) during which sampling was appropriate (n = 9). For calculation of elimination parameters one or two phase exponential curves were fitted to the data, as appropriate.

The results are presented as arithmetic means ± SD. For calculations the computer program GraphPadPrism 4.00 (SanDiego, Cal, USA) and MS-Excel were used.

3. Results

3.1. Pharmacokinetics of S-hyoscyamine

The sensitivity of our radioreceptor assay was comparable to those used by other groups (Aaltonen et al., 1984; Ensing et al., 1987; Kentala et al., 1989) and a quantification limit of 2.5 nmol L^{-1} was sufficient for our purpose. Occasionally, large atropine bolus doses were administered during emergency care (up to 200 mg) resulting in initial *S*-hyoscyamine plasma levels