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Acute renal failure enhances the antidotal activity of pralidoxime towards paraoxon-induced respiratory toxicity

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ABSTRACT

We recently showed in a rat model of dichromate-induced acute renal failure (ARF) that the elimination but not the distribution of pralidoxime was altered resulting in sustained plasma pralidoxime concentrations. The aim of this study was to compare the efficiency of pralidoxime in normal and acute renal failure rats against paraoxon-induced respiratory toxicity.

Ventilation at rest was assessed using whole-body plethysmography after subcutaneous administration of either saline or paraoxon (50% of the LD_{50}), in the control and ARF rats. Thirty minutes after administration of paraoxon, either saline or 50 mg/kg of pralidoxime was administered intramuscularly.

ARF had no significant effects on the ventilation at rest. The effects of paraoxon on respiration were not significantly different in the control and ARF group. Paraoxon increased the total time (T_{TOT}), expiratory time (T_E) and tidal volume (V_T), and decreased the respiratory frequency (f).

In paraoxon-poisoned rats with normal renal function, pralidoxime had a significant but transient effect regarding the T_{TOT} and V_T ($p < 0.05$). In the ARF group, the same dose of pralidoxime significantly decreased the T_{TOT}, T_E, and V_T and increased f during 90 min ($p < 0.01$).

In conclusion, pralidoxime had partial and transient effects towards paraoxon-induced respiratory toxicity in control rats; and a complete and sustained correction in ARF rats.

1. Introduction

Acute insecticide poisonings involving organophosphates are a public health concern more especially in developing countries (Eddleston et al., 2008; Pawar et al., 2006). Data from World Health Organisation suggest that each year 3,000,000 people are treated for organophosphates poisoning of whom more than 220,000 die (Satoh, 2006).

The treatment of organophosphate poisoning includes supportive therapy and the combined use of antidotes including atropine and an oxime (Eddleston et al., 2002). Oximes are nucleophilic agents allowing the reactivation of acetylcholinesterase by removing the phosphoryl group (Namba et al., 1971). Since the early fifties, pralidoxime (PRX) has been a worldwide-used oxime in the treatment of organophosphate poisonings (Eddleston et al., 2008; Pawar et al., 2006).

The pharmacokinetics of pralidoxime administered parenterally are characterized by a distribution in most body fluids, the lack of binding to plasma proteins (Eyer, 2003), and renal elimination of unchanged PRX (Garrigue et al., 1990). PRX is rapidly excreted by the kidney, resulting in a short plasma elimination half-life.

The efficiency of PRX in the treatment of organophosphates is still questioned. The results obtained from animal studies (Glickman et al., 1984; Jokanovic and Maksimovic, 1995; Matsubara and Horikoshi, 1983; Serrone et al., 1969; Shlosberg et al., 1976) and clinical studies (De Silva et al., 1992; Pawar et al., 2006; Sungur and Guven, 2001) are controversial. Rahimi et al. (2006) performed a meta-analysis of clinical trials to evaluate the efficacy of oximes in the management of acute human organophosphate poisoning. They concluded that oximes are not effective in the management of organophosphate-poisoned patients. A similar meta-analysis was done by Peter et al. (2006) resulting in the same conclusion. However, these two meta-analyses deserved criticism (Johnston and Rice, 2006). Indeed, at the time of these 2 studies, no adequate clinical trials had been carried out on the effectiveness of the

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oximes. Eddleston et al. (2002) reviewed in 2002 the clinical trials involving PRX. They concluded that the trials used low doses of PRX and/or that the methodology of the trials was unclear. In addition, there has been a trend towards increasing doses to treat human organophosphate poisonings with PRX. Recently, Pawar et al. (2006) performed a prospective, randomized, control study in acute human organophosphate poisonings and showed that high but not low dosage regimen resulted in a significant improvement of principal as well as secondary endpoints including needs for intubation, duration of mechanical ventilation, and outcome. Furthermore, high but not low dosage regimen was associated with the use of significantly lower cumulative doses of atropine.

We recently showed in a rat model of dichromate-induced acute renal failure that the elimination but not the distribution of PRX was altered resulting in sustained plasma PRX concentrations (Kayouka et al., 2009). We hypothesized that the increased plasma PRX concentrations in the renal failure model.

Respiratory failure is considered the primary cause of death in acute organophosphate poisonings (Durham and Hayes, 1962; Lerman and Gutman, 1988; Pawar et al., 2006; Yamashita et al., 1997). The mechanism of organophosphate-induced respiratory failure remains unclear. In previous studies, paraoxon administered subcutaneously at a dose equal to 50% of the LD_{50} significantly altered the ventilation at rest assessed by whole-body plethysmography in awake unrestrained rats (Villa et al., 2007). Paraoxon at doses equal to 50% (Houze et al., 2008; Villa et al., 2007) and 75% (Villa et al., 2007) of the LD_{50} consistently induced a sustained decrease in respiratory rate resulting from an increase in expiratory time (Houze et al., 2008; Villa et al., 2007) which was completely reversed by atropine (Houze et al., 2008; Villa et al., 2007) but not by methylatropine (Houze et al., 2008).

In the present study, the antidotal effect of PRX toward paraoxon-induced respiratory toxicity was tested using whole-body plethysmography in awaken rats without and with acute renal failure caused by the administration of a single dose of potassium dichromate (Biber et al., 1968; Fatima et al., 2005; Perez et al., 2004).

2. Methods

All animal procedures used in this study were in strict accordance with the European Community Council Directive of 24 November 1986 (86-609/EEC) (protection of animals used for experimental and other scientific purposes) and Decree of 20 October 1987 (87-848/EEC).

2.1. Animals

Male Sprague–Dawley rats (280–300 g) were purchased from Janvier (Le Genest-St-Isle, France).

All the animals were housed in a room with controlled environment (22 ± 3 °C, 55 ± 10% relative humidity), and maintained under a 12-h light/dark cycle. Animals had free access to food and tap water *ad libitum*.

2.2. Chemicals and drugs

Xylazine was obtained from Bayer (Puteaux, France) and ketamine was supplied by Panpharma (Fougères, France). Paraoxon (diethyl p-nitrophenyl phosphate), dimethyl sulfoxide, and sodium carbonate were purchased from Sigma–Aldrich (St Quentin Fallavier, France). Pralidoxime methylsulfate (Contrathion®) was kindly provided by SERB Laboratories (Paris, France).

Paraoxon (PO) was diluted in DMSO to obtain a stock solution of 35 mg/ml. Several dilutions of paraoxon were then prepared in saline solution in order to inject doses equal to 50% of the LD_{50}. The stock and the dilutions of paraoxon were preserved from light and stored at 4 °C during a maximum of 1 month.

2.3. Safety precaution

Due to the toxic properties of paraoxon, special measures have to be taken during its manipulation. The preparation of the solutions should be done under a fume hood. The wearing of nitrile gloves, suitable protective clothes and eye protection is mandatory. All the contaminated objects have to be cleaned by a solution of sodium carbonate 1 M.

2.4. Whole-body plethysmography

Whole-body plethysmography was used to measure ventilation at rest in awaken unrestrained rats, as the barometric method described by Bartlett and Tenney (1970) which was adapted by the use of infra-red telemetry to measure the ambient and core temperatures (Houze et al., 2008). This method allowed us to measure the following parameters: the tidal volume (V_T), the inspiratory time (T_I), the expiratory time (T_E), the total respiratory time ($T_{TOT} = T_I + T_E$), the respiratory frequency (f), and the minute ventilation ($V_E = V_T \times f$).

2.5. Telemetry applied to plethysmography

Infra-red telemetry was used in order to repeatedly measure the ambient temperature as well as the central temperature of the rat (Houze et al., 2008). The rats underwent an intraperitoneal surgical implantation of the telemetry transmitters (TA10TA-F20, Data Sciences International, USA) while under xylazine/ketamine (10/70 mg/kg, intraperitoneally) anaesthesia. A small incision exposed the abdomen to allow the insertion of the transmitter. The skin was then closed with sutures (Ethicon, Belgium). Rats were allowed to recover from surgery for at least 72 h prior to the experiment. The transmitter signals were detected by a receiver placed beneath the plethysmograph enclosure. The receiver was related to a computer program which converts the signals and allows the recording of the temperatures every minute.

2.6. Study design

2.6.1. Study 1 design: effects of acute renal failure on ventilation at rest

A rat model of acute renal failure (ARF) induced by potassium dichromate administration at 15 mg/kg subcutaneously was used (Biber et al., 1968; Fatima et al., 2005; Kayouka et al., 2009; Perez et al., 2004).

The animals were randomly divided in two groups (8 animals in each group). Two days before the experiment, the ARF group received potassium dichromate (15 mg/kg, sc); the control group received saline solution. On the day of the experiment, the rat was placed in the plethysmograph enclosure for about 30–60 min to acclimate before the first measurement. The animals of the two groups were then gently removed from its container for a saline subcutaneous injection (the solvent of paraoxon) and replaced in the chamber. A measurement was performed each 15 min for 30 min and immediately followed by the intramuscular injection of saline (the solvent of PRX). The animals were again replaced in the chamber for the rest of the measurements. Thereafter, measurements were recorded at 35, 50, 60, 75, 90, 120, 180 and 210 min after the injection of paraoxon. At the end of the experiment, a blood sample was collected for the measurement of plasma creatinine concentrations. Blood samples were centrifuged and plasma was taken. Plasma specimens were frozen at −20 °C until analyzed.

2.6.2. Study 2 design: effects of acute renal failure on ventilation at rest in paraoxon-poisoned rats

Two days before the experiment, the animals were randomly divided in three groups (8 animals in each group). The ARF and the ARF + PO groups received potassium dichromate 15 mg/kg administered subcutaneously and the control + PO group received only saline instead of dichromate. On the day of the experiment, the same protocol described in study 1 was followed with only one difference; the saline subcutaneous injection was replaced by a subcutaneous injection of a dose paraoxon equal to 50% of the LD_{50} (LD_{50} = 0.430 mg/kg) for the ARF + PO and the control + PO groups.

2.6.3. Study 3 design: effects of PRX on ventilation at rest in paraoxon-poisoned rats with acute renal failure in comparison with rats with normal renal function

Animals were randomly divided in four groups (8 animals in each group), ARF group received saline and saline, ARF + PO group received paraoxon and saline, ARF + PO + PRX group received paraoxon and PRX and control + PO + PRX group received paraoxon and PRX. Two days before the experiment, ARF, ARF + PO and ARF + PO + PRX groups received potassium dichromate 15 mg/kg administered subcutaneously; control + PO + PRX group received saline solution. On the day of the experiment, the same protocol described in study 1 was realized with respective injections for each group.

At the end of each experiment, animals were sacrificed in a closed container with a flow of CO_2.

2.6.4. Study 4 design: pharmacokinetic–pharmacodynamic correlation of paraoxon-induced increase in T_E and plasma PRX concentrations

In a previous study using the same single dose administered by the same route, we assessed the effects of dichromate-induced ARF on the pharmacokinetics of PRX (Kayouka et al., 2009). In the present study, ventilatory parameters were measured at the same time as those used to measure plasma PRX concentrations. Therefore, we assessed the pharmacokinetic–pharmacodynamic correlation between paraoxon-induced increases in T_E (percentage of the basal value observed at the time of pralidoxime injection) and the corresponding plasma PRX concentrations in the control and ARF groups.