Toxicology 250 (2008) 32–38

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

An animal model to study health effects during continuous low-dose exposure to the nerve agent VX

David Rocksén[a,b], Daniel Elfsmark[a], Victoria Heldestad[c], Karin Wallgren[a], Gudrun Cassel[a], Ann Göransson Nyberg[a,*]

[a] FOI CBRN Defence and Security, Swedish Defence Research Agency, Umeå, Sweden
[b] Experimental Traumatology Unit, Department of Neuroscience, Karolinska Institute at Karolinska University Hospital, Stockholm, Sweden
[c] Department of Pharmacology and Clinical Neurosciences, Division of Clinical Neurophysiology, Umeå University, Sweden

ARTICLE INFO

Article history:
Received 17 March 2008
Received in revised form 7 May 2008
Accepted 25 May 2008
Available online 9 July 2008

Keywords:
Telemetry
Osmotic pump
Low-dose
VX
Acetylcholinesterase

ABSTRACT

In the present study, we have developed an animal model to study long-term health effects of continuous exposure of toxic chemical agents, in awake, freely moving rats. The aim was to evaluate the effect of low-dose exposure of the nerve agent VX, and to find specific biomarkers for intoxication. To exclude the influence of stress, we used an implanted radio-telemetric device for online registration of physiological parameters, and an osmotic pump, implanted subcutaneously, for continuous exposure of the toxic agent.

Our results showed that the lowest observable effect dose of VX in Wistar rats was 5 μg/kg/24 h, after continuous exposure by the osmotic pump. Although we observed significant inhibition of acetylcholinesterase (AChE) in blood and a significant decrease in body weight gain at this dose, no change in blood pressure, heart rate or respiratory rate was registrated. However, a significant decrease in the thyroid hormone, free T_4, was measured in blood after 8 weeks, indicating that low doses of VX might affect the thyroid function. Rats given repeated daily injections were more sensitive to VX and needed only 1/10 of the concentration to reach a similar level of AChE inhibition, compared to animals exposed by the osmotic pump. Moreover, the results showed that exposure of VX in our experimental design, does not induce an increase in corticosterone blood levels. Thus, the model used in this investigation renders minimal stress and will not cause unnecessary pain to the animals, indicating that this model could be a useful tool to study long-term effects of various toxic substances in freely moving rats.

1. Introduction

Acute and long-term effects of exposure to organophosphorus (OP) acetylcholinesterase (AChE) inhibitors have been studied extensively (Sidell, 1974; Holstege et al., 1997; Chambers, 1992; Göransson-Nyberg et al., 1998). However, knowledge regarding harmful effects of long-term low-dose exposure to these agents is less defined. Possible low-dose exposure of nerve gases has occurred at Khamisiyah, Iraq in 1991 (Weese, 2001), and in Matsumoto, Japan in 1994 (Nakajima et al., 1999). It has been suggested that the exposure occurring in Iraq was the source of the so-called Gulf War Syndrome. These subjects suffered from a wide variety of symptoms, including weakness, fatigue, headache, memory loss and increased susceptibility to infections (Conn et al., 2002).

Knowledge regarding exposure to non-symptomatic levels of AChE inhibitors might also be of value when investigating health effects in humans exposed to OP insecticides (McCauley et al., 2001; Bazylewicz-Walczak et al., 1999).

Animal experiments studying non-symptomatic exposure of AChE inhibitors has shown physiological effects such as increased arterial blood pressure (Buccafusco, 1996), decreased body temperature (Russell et al., 1986), and effect on behaviour and nociception (Scremin et al., 2003; Russell et al., 1986). Persistent EEG-changes in monkeys repeatedly exposed to low levels of sarin have also been reported (Burchfield and Duffy, 1982). However, other studies investigating OP AChE inhibitors have found no increased incidence of mental, neurological, hepatic or reproductive pathology in humans (Panel on Anticholinesterase Chemicals, 1982; Coordinating Subcommittee, 1985; Moore, 1998). These contradictory conclusions may be the result of different route of exposure, substance, doses or number of repeated exposures.

The nerve agent VX is an especially likely candidate for low-dose exposure, since it remains stable for a long period in the environment (Crenshaw et al., 2001; Genovese et al., 2007), thus posing a threat for low-dose exposure when humans are in contact with contaminated surroundings, clothing or equipment.

* Corresponding author. Tel.: +46 90106736.
E-mail address: ann.goransson@foi.se (A. Göransson Nyberg).

0300-483X/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.tox.2008.05.015

In the present study, we have developed an animal model to study long-term health effects of continuous exposure of toxic chemical agents, in awake, freely moving rats. The aim was to evaluate the effect of low-dose exposure of the nerve agent VX. To exclude the influence of stress, we used an implanted radio-telemetric device for online registration of physiological parameters, and an osmotic pump implanted under the skin for continuous exposure of the toxic agent. Concomitant with the online registration, blood samples were collected to detect changes in different hormones and AChE activity.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (Scanbur, Sweden) weighing 225–275 g at the beginning of the experiments were housed individually in plastic cages during 12 h light–dark cycles (lights on 6 a.m.). Room temperature was maintained at 21 ± 2 °C and relative humidity at 55 ± 5%. Standard rodent pellet food and tap water were available *ad libitum*. The rats were acclimatized to these conditions for a minimum of 10 days prior to surgery. The animal experiments were approved by the Regional Research Ethical Committee in accordance with Swedish laws (SFS 1988:539, LSFS 1989:41).

2.2. Chemicals

The nerve agent VX (*O*-ethyl-*S*-[2(di-isopropylamino)ethyl] methyl phosphonothioate), >95% pure) was synthesized at the Department of Chemistry, FOI CBRN Defence and Security, Sweden, and diluted to its final concentration with the vehicle PPG (polypropylene glycol) 400 (Sigma–Aldrich, Sweden) on the day of the experiment.

2.3. Implantation of telemetry probes

Telemetry transmitters (TL11-M2-C50-PXT, Data Sciences International, St. Paul, MN, USA) were implanted according to the instructions of the manufacturer. Before surgery, the probes were pre-warmed to body temperature in 0.9% NaCl.

The animals were anaesthetized with 4–5% isoflurane (inhalant, Baxter Medical AB, Sweden) in an anaesthesia box and were thereafter placed on an inhalation mask, using a lower concentration (1–2% isoflurane). Hypothermia was avoided using a heating pad, regulated by a rectal temperature probe. Hair was removed from the abdomen and the incision area was disinfected with alcohol.

An abdominal incision was made, and the intestines were retracted using gauze moisturized with saline. The abdominal aorta was exposed by removing tissue and fat with sterile cotton applicators. Thereafter, the aorta was gently separated from the vena cava and a proximal ligature was placed around the aorta to occlude blood flow temporarily. Puncture of the aorta was made with a needle bent in a 45° angle. This needle was also used as an introducer when inserting the telemetry probe catheter into the vessel. The surgical area was thereafter dried with sterile cotton applicators before securing the catheter with tissue glue (Vetbond TM, 3M, Animal Care Products, St. Paul). For further stability, a small cellulose patch was also secured over the catheter. The gauze was withdrawn and the abdominal cavity was rinsed with saline at body temperature. The intestines were thereafter gently massaged back into place.

Bio-potential leads were used to monitor heart rate (HR), and these leads were tunnelled subcutaneously using a trochar (tunnelling device). One electrode was placed in the upper right quadrant of the chest and the other electrode was secured at the left upper abdominal muscle. Finally, the telemetry probe was secured to the abdominal muscles by silk sutures and the skin was closed with additional sutures. These skin sutures were removed 7–10 days after the incision healed.

After surgery, all animals were given prophylactic antibiotic by an intramuscular injection of 0.1 ml Baytril® vet. (25 mg/ml Enrofloxacin, Bayer, Germany) and pain relief by a subcutaneous (s.c.) injection of 0.05 ml Temgesic® (Buprenorphinum, 0.3 mg/ml, Schering-Plough Europe, Belgium). Afterwards, the animals were treated with 0.05 ml Temgesic twice daily for 2 days and with 0.1 ml Baytril once daily for 1 week.

The rats were allowed to recover from surgery using individual housing for at least 1 week, before initiation of data acquisition.

2.4. Data acquisition

The individual rat cages were placed on top of telemetry receiver plates. The transmitted radio frequency was received by the plate and directed to an online acquisition system (DataQuest A.R.T 3.1, Data Science International). Physiological parameters such as heart rate, respiratory rate (RR), body core temperature and mean arterial pressure (MAP) were recorded 15 s every 15 min, during 4 weeks. Daily 24 h-values for each physiological parameter was taken as the average of 24 data-points, each obtained from the mean of four recorded 15-min intervals.

2.5. Implantation of osmotic mini-pumps

Alzet® osmotic mini-pumps, with a constant delivery rate of 0.55 μl/hr (Model 2002, Alza Corp., Palo Alto, USA), were used to deliver either vehicle (PPG 400) or VX (solved in PPG 400). The osmotic pumps were implanted subcutaneously in a midscapular pocket during isofluran anaesthesia (inhalant).

Control of the delivery rate of the osmotic pump was performed according to the manufacturer's specification. The pumps where filled with a solution coloured with comassie blue (Sigma–Aldrich, Sweden) and primed in physiological NaCl solution at 37 °C for 40 h. The absorbance of a given volume of the physiological solution was then measured continuously for 4 weeks to estimate the delivery rate. The results confirmed that the osmotic pump was functioning as declared by the company.

Stability of VX in the vehicle PPG 400 was analyzed using a solution of 1000 ppm (volume/volume) of VX dissolved in PPG 400. These samples was kept in suitable containers in water bath at 37 °C and analyzed by GC/NPD once a week. The remaining quantity of VX was 80% after 4 weeks.

2.6. Experimental design

In all experiments, the rats were weighed before the experiment, and then once a week. Since the animals gain weight during the acclimatization period, the VX concentrations used throughout the experimental period were based on the estimated weight of the animals 1 week after implantation, based on the normal growth curve for rats in our laboratory. Normal weight gain after 2 weeks was approximately 30%.

Blood samples were withdrawn from the rat tail before the osmotic pump was implanted, and analyzed for baseline AChE activity at the start of all experiments. All blood samples were withdrawn between 9.00 and 11.00 a.m. For visible signs of symptoms, all animals were examined daily during the experiment.

2.6.1. Dose-response after continuous low-dose exposure of VX

Osmotic mini-pumps containing 0/0.5/1/2.5/5/10/15/20/30 or 40 μg/kg/24 h of VX ($n = 4$ for each dose), were implanted. Body weight and AChE activity in blood was measured once a week for 2 weeks. By analyzing the percent change in body weight from the baseline value, the change in body weight gain was achieved. This was performed to estimate an optimal dose of VX, which would significantly inhibit AChE activity in blood without the animal showing any clinical signs of symptoms.

2.6.2. Comparison between repeated injections and exposure through the osmotic pump

In one series of experiments, osmotic mini-pumps containing 0/0.5/1/2.5/5/10 or 15 μg/kg/24 h of VX were implanted as described above ($n = 4$ animals for each dose). To analyze AChE activity, blood samples were withdrawn once a week for 2 weeks.

In a second series of experiments, the animals received a daily subcutaneous injection, 5 days a week, of 0.1/0.25/0.5 or 1 μg/kg of VX ($n = 4$ animals for each dose). As for the group implanted with osmotic pumps, blood samples were withdrawn once a week for 2 weeks and analyzed for AChE activity. The VX concentrations mentioned above were chosen after initial pilot studies.

2.6.3. Telemetric registration during continuous low-dose administration of VX

Based on the results obtained in the dose-response experiment, the dose 5.0 μg of VX/kg/24 h in the osmotic pump (Fig. 1) was chosen for long-term telemetric recording of physiological parameters. Eight animals received surgically implanted radio-telemetry devices and were allowed to acclimatize for 1 week. After an additional week of baseline measurements the rats were implanted with osmotic pumps,

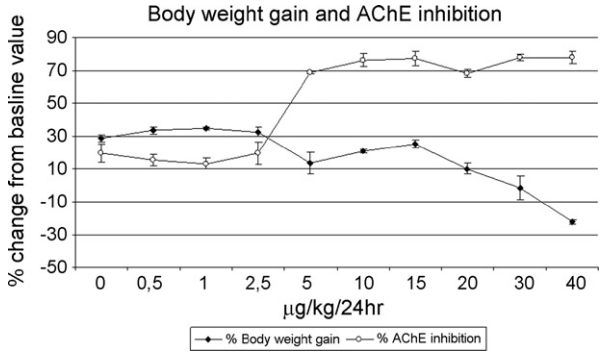

Fig. 1. Percent change from baseline values for body weight gain and inhibition of AChE activity in blood is depicted. VX-doses ranging from 0 to 40 μg/kg/24 h were used, administered by an osmotic pump. The experiment continued for 2 weeks, except in the groups 20–40 μg/kg/day, where we stopped the experiment after 1 week, due to ethical reasons. Each point represents the mean ± S.E.M., $n = 4$ in each group. (■) Body weight gain. (◊) AChE activity in blood.