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Mechanisms of high-dose citalopram-induced death in a rat model

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ABSTRACT

Citalopram, a selective serotonin reuptake inhibitor, is generally considered to be of low toxicity. However, serotonin syndrome, seizures, electrocardiographic abnormalities as well as respiratory failure and death have been described in patients with citalopram overdose. The mechanisms of severe toxicity remain unclear. Our objective was to study the mechanisms of death following high-dose citalopram administration in Sprague Dawley rats. The median lethal dose (MLD) of intraperitoneal (i.p.) citalopram was measured using Dixon & Bruce's up-and-down method at 102 mg/kg. Dose–effect relationships of citalopram-induced clinical features, alterations in arterial blood gas and plethysmography, and disturbances in blood lactate, plasma and platelet serotonin concentrations were studied. Seizures were significantly increased in rats receiving 80% and 120% of citalopram MLD versus controls ($p < 0.05$ and $p < 0.01$, respectively). A significant decrease in body temperature was observed after 90 min in rats treated with doses >60% MLD in comparison to controls ($p < 0.05$). The occurrence of serotonin behavioural syndrome was comparable in all groups. Citalopram administration did not result in significant hypoxemia, hypercapnia and lactate elevation. However, a significant moderate increase in the inspiratory time ($p < 0.05$) accompanied with an expiratory braking was observed. A significant dose-related linear decrease in platelet serotonin and increase in plasma serotonin concentrations were measured ($p < 0.05$). Pre-treatments of rats receiving 120% of citalopram MLD with diazepam (1.77 mg/kg) and cyproheptadine (17.1 mg/kg) prevented seizures and death, but propranolol pre-treatment was ineffective. Neuroprotection with diazepam and cyproheptadine was not associated with decreased serotonin plasma concentrations. In conclusion, citalopram-induced deaths resulted from seizures in relation to serotonin release, whilst respiratory and metabolic toxicity was mild. Our observations support the role of serotonin-induced neurotoxicity in citalopram overdose and suggest that cyproheptadine and benzodiazepines, but not beta-blockers, may have a role in the management of citalopram toxicity.

1. Introduction

The prescription of selective serotonin reuptake inhibitors (SSRI) is increasing world-wide due to fewer toxic effects in comparison to cyclic antidepressants (Whyte et al., 2003). In 2010, the American Association of Poison Control Centers reported 45,095 SSRI and 12,619 cyclic antidepressant exposures responsible for 97 versus 349 severe poisonings (0.21 and 2.8% of all exposures respectively) and 6 versus 21 deaths (0.01 and 0.17% of all exposures respectively) (Bronstein et al., 2011). SSRI overdoses generally have only mild clinical features, even in large ingestions; common clinical features include sinus tachycardia, diastolic hypertension, headache, drowsiness and minor digestive troubles (Isbister et al., 2004). The most significant complication is serotonin syndrome which is reported in up to 15% of SSRI overdoses, in relation to an excess of extracellular serotonin activating various serotonin receptors including serotonin 2A receptors in the central nervous system (Boyer and Shannon, 2005; Sun-Edelstein et al., 2008). Serotonin syndrome was first defined in 1991, based on non-specific diagnostic criteria, including clonus, agitation, seizures and hyperthermia (Sternbach, 1991). More recently, alternative diagnostic criteria (the Hunter Serotonin Toxicity Criteria) have been proposed (Isbister et al., 2007).

Citalopram is considered the most selective SSRI (Hyttel, 1994). Citalopram overdose can be associated with serotonin syndrome, QT prolongation, and seizures in both adults and children (Isbister

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et al., 2004; Waring et al., 2008; Hayes et al., 2010; Klein-Schwartz et al., 2012). In 30% of citalopram overdoses with ingested doses ≥600 mg, QRS widening may also occur, whilst cardiac failure and arrhythmias are exceptional (Personne et al., 1997). In a few cases, life-threatening respiratory failure has been reported and attributed to citalopram toxicity in the absence of aspiration pneumonia or cardiogenic pulmonary oedema (Chechani, 2002; Kelly et al., 2003; Jimmink et al., 2008). However, fatalities remain rare and usually result from concentrations 20 to 25 times greater than the therapeutic range (Glassman, 1997).

The mechanisms of death resulting from citalopram overdose remain unknown. There have been few animal studies published and these focus on citalopram-induced cardiotoxicity and serotonin syndrome at supra-therapeutic doses (Boeck et al., 1984; Kalueff et al., 2008). The aim of our study was to investigate the mechanisms of high-dose citalopram-induced toxicity and death, in particular to understand the contribution of serotonin syndrome.

2. Methods

All experiments were carried out within the ethical guidelines established by the National Institutes of Health and the French Ministry of Agriculture. The experimental protocols were approved by our institutional ethics committee (No. P2.FB.047.08).

2.1. Animals

Male Sprague-Dawley rats (Janvier, France) weighing between 250 and 350 g at the time of experimentation were used. Animals were housed for 5 days before experimentation in an environment maintained at 21 ± 0.5 °C with controlled humidity and a light–dark cycle (lights on between 8:00 a.m. and 8:00 p.m.). Standard pellet diet and tap water were provided ad libitum. Following each experiment, rats were euthanized using a carbon dioxide chamber.

2.2. Drugs

Citalopram (Lundbeck a/s, Denmark), cyproheptadine (Teofarma, France), diazepam (Francopia, France), and propranolol (Astrazeneca, France) were purchased. Citalopram and propranolol were used at 40 and 5 mg/ml, respectively. Cyproheptadine and diazepam were diluted in saline to obtain solutions at 8 and 2 mg/ml, respectively.

2.3. Catheter implantation for blood sampling

The day before the study, animals were anesthetized with intraperitoneal (i.p.) 70 mg/kg ketamine (Ketalar®) and 10 mg/kg xylazine (Rompum®). The femoral artery was catheterized using 30 cm-silastic tubing with external and internal diameters of 0.94 and 0.51 mm, respectively (Dow Corning Co., Midland, MI). Arterial catheters were subcutaneously tunneled and fixed at the back of the neck. Heparinized saline was injected into the catheter to prevent thrombosis and catheter obstruction. Rats were then returned to their individual cages for a minimum 24 h-recovery period, to allow complete anaesthesia washout.

On the day of experimentation, rats were placed in horizontal Plexiglas cylinders (6.5 cm-internal diameter, up to 20 cm-adjustable length) (Harvard Apparatus, Inc., Holliston, MA, USA), modified by the addition of several holes at the cephalic end to prevent CO_2 rebreathing. Before drug administration, the catheter was exteriorized, flushed, and its patency verified.

2.4. Clinical findings

Body temperature was monitored using rectal probes before (T0), and at 5, 10, 15, 30, 60, 120, 180 and 240 min after i.p. injection of citalopram or its solvent. The serotonin syndrome was quantified using the behaviour serotonin syndrome (BSS) scale (Darmani and Ahmad, 1999). Seizures and death occurrence was noted.

2.5. Measurement of arterial blood gases and lactate concentrations

To analyse arterial blood gases, 100 μl-arterial blood were sampled before (T0), and at 5, 10, 15, 30, 60, 120, 180 and 240 min after i.p. injection of citalopram or its solvent. Samples were immediately analysed using Rapidlab® 248 (Bayer Diagnostics, Germany). Every hour, 200 μl heparinized 0.9% NaCl were administered through the arterial catheter to reduce the risk of catheter clotting and to compensate for volume losses. To measure lactate concentrations, 10 μl-arterial blood were sampled before (T0), and at 20, 60 and 120 min after i.p. administration of citalopram or its solvent and were immediately analysed using Lactate Scout® (EKF Diagnostic, Germany).

2.6. Measurement of plasma and platelet serotonin concentrations

To measure plasma serotonin concentrations, 500 μl-arterial blood were sampled before (T0), and at 30, 60, 90, 120, 150, 180, 210 and 240 min after i.p. injection of citalopram or its solvent. To measure platelet serotonin concentrations, 1000 μl-arterial blood samples were collected before (T0), and at 30, 90, 150 and 240 min after i.p. injection of citalopram or its solvent. Blood samples were collected in tubes containing citrate. Platelet rich plasma (PRP) was prepared by centrifugation of citrated blood at 200 × *g* for 15 min at room temperature. The PRP was collected and centrifuged at 2000 × *g* (4 °C, 15 min). Platelets were counted, and PRP was further centrifuged at 2000 × *g* for 10 min to harvest separately the platelet pellets and the platelet-poor plasma (also referred as plasma). Plasma and platelet aliquots were immediately frozen at −80 °C until measurement (<1 month). Serotonin concentrations were measured using high-performance liquid chromatography as previously described (Kema et al., 1993).

2.7. Whole-body plethysmography

Four days before the study, temperature transmitters (DSI, Chatillon, France) were implanted in the peritoneal cavity. Rats were then returned to their individual cages for a minimum 72 h recovery period. Ventilatory parameters were recorded in a whole-body plethysmograph by the barometric method previously described (Bartlett and Tenney, 1970) and validated (Villa et al., 2007). The day of experimentation, rats were placed in a rectangular Plexiglas chamber with a 3 l volume connected to a reference chamber of the same size by a high-resistance leak to minimize the effect of pressure changes in the experimental room. The animal chamber was flushed continuously with humidified air at a 5 l/min rate. During the recording periods, the inlet and outlet tubes were temporarily clamped and pressure changes associated with each breath were recorded using a differential pressure transducer (Validyne MP, 45 cm ± 3 cm H_2O, Northridge, CA), connected to the animal and reference chambers. During each measurement, calibration was performed by three injections of 1 ml air into the chamber. Ambient temperature was then noted. The spirogram was recorded and stored on a computer with an acquisition data card (PCI-DAS 1000, Dipsi, Chatillon, France) using respiratory acquisition software (Acquis1 Software, CNRS, Gif-sur-Yvette, France) for analysis off-line.

This technique was validated daily with a series of leak tests (leak was signalled when reduction in the signal amplitude exceeded 33% in 5 s) (Bonora et al., 2004). The quantification threshold corresponded to a minimum air volume injection of 30 μl. Within the range of tested volumes (0.1–3 ml), measurements were linear. The mean coefficient of intra-day variability (four series of 5 measurements carried out the same day) was 1.32 ± 0.18%. The mean coefficient of inter-day variability (25 measurements carried out on 3 different days) was 1.69 ± 0.11%. We verified that the mean CO_2 measured using an Ohmeda 5250 RGM capnograph (rebreathing test) during clamping periods did not exceed 0.6% of the air contained in the chamber.

During the experiment, the first measurement was performed after a 30–60 min period of accommodation, when the animal was quiet but not in deep or rapid eye movement sleep which can be grossly estimated from observing their behaviour, response to noise, and pattern of breathing. The animal was then gently removed from the chamber for i.p. injection, and replaced in the chamber for the remaining measurements. Ventilatory parameters were recorded at 5, 10, 15, 30, 60, 90, 120, 180 and 240 min, each recording lasting about 60 s. The following parameters were measured: the tidal volume (V_T), the inspiratory time (T_I), the expiratory time (T_E) and the total respiratory time (T_{TOT}). Additional parameters were calculated: the respiratory frequency (f) and the minute ventilation ($V_E = V_T * f$). After sacrifice, rat lungs flotation on water surface was tested to check for the absence of pulmonary oedema.

2.8. Study designs

2.8.1. Study 1 – determination of citalopram median lethal dose (MLD)

We used the up-and-down method of Dixon–Bruce (Bruce, 1985) to limit the number of required animals, as follows: The first rat receives 157 mg/kg citalopram i.p., the estimated 50% lethal dose in rat as kindly provided by the manufacturer, Lundbeck A/S (unpublished data). If the rat survives, the next animal receives a dose 1.3-fold more than this previous dose and so on until the trend changes (i.e. an animal dies). Then, the next animal receives a dose 1.3-fold less than the dose that was lethal for its predecessor. This method progressively brackets the MLD, permitting use of fewer animals than classic MLD designs. Three series starting with 157 mg/kg, 120 mg/kg (1.3-fold less) and 204 mg/kg (1.3-fold more) were used. Animals were observed at 1, 2, 3, 4 and 24 h after citalopram injection and then daily during one week. The total number of animals required depends on the accuracy of the initial estimation of the MLD. The MLD is then determined by the final dose, the fixed percentage of dose change chosen, and the pattern of animal outcome.

2.8.2. Study 2 – description of citalopram toxicity

Temperature, incidence of seizures, BSS and death were determined in four groups of randomized rats receiving saline and 60%, 80%, and 120% of citalopram MLD (N = 8/group). Time between citalopram injection and occurrence of seizures and death was measured. Ventilation parameters were measured using whole-body plethysmography in two groups of randomized rats receiving saline and 80% of