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Exposure to fipronil elevates systolic blood pressure and disturbs related biomarkers in plasma of rats

Joao Leandro Chaguri[a], Antonio Francisco Godinho[a], Daniel França Horta[a], Victor Hugo Gonçalves-Rizzi[b], Jose Sergio Possomato-Vieira[b], Regina Aparecida Nascimento[b], Carlos Alan Dias-Junior[a,b,*]

[a] Center for Toxicological Assistance, Biosciences Institute of Botucatu, Sao Paulo State University – UNESP, Botucatu, Sao Paulo, Brazil
[b] Department of Pharmacology, Biosciences Institute of Botucatu, Sao Paulo State University – UNESP, Botucatu, Sao Paulo, Brazil

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ABSTRACT

Recent reports show that fipronil affects non-target organisms, including environmental species populations and potentially humans. We aimed to examine if fipronil exposure affects the systolic blood pressure and related biomarkers. Thus, fipronil was orally administered to rats (30 mg/kg/day) during 15 days (Fipronil group) or physiological solution (Control group). While fipronil increased significantly the systolic blood pressure (158 ± 13 mmHg), no significant changes were observed in Control group (127 ± 3 mmHg). Significantly, higher levels of fipronil in plasma were observed in Fipronil group (0.46 ± 0.09 μg/mL *versus* 0.17 ± 0.11 μg/mL in Control group). Fipronil group showed lower weight gain compared with Control group. While fipronil resulted in higher concentrations of endothelin-1, reduced antioxidant capacity and lower levels of circulating matrix metalloproteinase 2 (MMP-2) and nitric oxide (NO) metabolites compared to Control group, no alteration was observed in serum biomarkers of renal and hepatic/biliary functional abilities. Therefore, this study suggests that fipronil causes hypertension and endothelin-1 plays a key role. Also, these findings suggest that reductions of both MMP-2 and NO may contribute with the elevation of systolic blood pressure observed with fipronil.

1. Introduction

Fipronil [(±)-5-amino-1-(2,6-dichloro-α,α,α-trifluoro-p-tolyl)-4 trifluoromethylsulfinylpyrazole-3-carbonitrile] is the first member of the phenylpyrazole insecticide class, and possesses a trifluoromethylsulfinyl functional group on the heterocyclic ring. The fipronil has a broad spectrum of action against insects, being used to control fleas, ticks, termites, mole crickets, ants, rootworms, beetles, cockroaches and other insects (Tingle et al., 2003). Fipronil was initially developed to replace pesticides, such as organophosphates, carbamate and pyrethroids insecticides, having action against resistant pest strains (Narahashi et al., 2007), becoming an insecticide widespread used in agriculture. Narahashi et al. (2010) demonstrated that LD_{50} values of fipronil are 0.13 and 41 mg/kg in houseflies and rats, respectively, thus providing highly specificity for fipronil (Narahashi et al., 2010). Jackson et al. (2009) have suggested that the "no observed adverse effect level" (NOAEL) for acute oral dose of fipronil in rats is 2.5 mg/kg.

Importantly, recent evidences have shown that fipronil may be a potent toxicant affecting non-target organisms, including environmental species populations and potentially humans (Le Faouder et al., 2007) and deleterious effects were observed on the early stages of the animal development (Udo et al., 2014; Badgujar et al., 2015; Terçariol and Godinho, 2011). Although the fipronil's actions are blocking the gamma-aminobutyric acid (GABA)-ergic receptors and glutamate (Glu)-gated chloride channels (Narahashi et al., 2007; Zhao et al., 2005), concerningly, evidence has demonstrated that fipronil induces the releasing of reactive oxygen species, resulting in increased oxidative stress (Overmyer et al., 2007; Vidau et al., 2011). Supporting these findings, it has been shown that fipronil induced oxidative stress in kidney and brain of mice (Badgujar et al., 2015). Interestingly, we have received some cases of intoxication with fipronil in our center for assistance and control of intoxications, who has presented symptoms typically associated with the blockade of the GABAergic receptors function (such as nausea, headache and seizures) and surprisingly, also presented elevation of blood pressure. Together, these findings point to the

* Corresponding author at: Department of Pharmacology, Biosciences Institute of Botucatu, Sao Paulo State University (UNESP), Distrito de Rubiao Junior, S/N, 18.618-970 Botucatu, Sao Paulo, Brazil.
E-mail address: carlosjunior@ibb.unesp.br (C.A. Dias-Junior).

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importance of further exploring the mechanisms responsible for fipronil-induced intoxication. However, no previous study had examined the effects of exposure to fipronil on systolic blood pressure, and to our knowledge, this is the first study to elucidate the mechanisms involved in the very early development of fipronil-induced hypertension in rats.

Therefore, we expanded previous reports and we hypothesized that fipronil exposure (during 15 days) increases systolic blood pressure in rats and the fipronil-induced hypertension is associated with significant changes in circulating levels of related biomarkers: endothelin-1, matrix metalloproteinase type 2 (MMP-2), NO and antioxidant capacity.

2. Materials and methods

2.1. Animals and experimental design

All procedures for animal experimentation were approved by the Ethics Committee, Biosciences Institute of Botucatu, Sao Paulo State University (Protocol n° 620/2014), which is complied with international guidelines of the European Community for the use of experimental animals. Twenty male *Wistar* rats (250 ± 20 g) were used in this study. The animals were housed in standard rat cages and maintained at 22 °C on a 12-h light/dark cycle, and were given free access to water and rat chow.

The animals were randomly distributed into one fipronil-exposed group (Fipronil, $n = 12$) and one Control group (non-fipronil-exposed, Control, $n = 8$) for 15 days, as follows: animals in the fipronil-exposed group were treated by gavage with dose of 30 mg/kg of commercial product containing fipronil (Regent® 800WG, BASF, Agro Brazil, Sao Paulo, Brazil). The animals in the Control group were treated by gavage with physiological solution. The protocol of intoxication using 30 mg of fipronil *via oral* used in this study was chosen with basis in previous studies, which evaluated the dose range to fipronil (Udo et al., 2014; Moser et al., 2015). At the end of the experimental protocol (15th day), we evaluated the weight gain and the rats were anaesthetized with 2% isoflurane (Isoforine, Cristalia, Sao Paulo, Brazil) at a continuous oxygen flow (2 L/min), having confirmed immobility and loss of righting reflex, the rats were submitted to euthanasia.

The whole-blood samples were collected in siliconized vacuum tubes containing no additives (for serum) or lyophilized ethylenediaminetetraacetic acid (EDTA) or heparin (*Vacuntainer* Becton-Dickinson, BD, Oxford, UK) for plasma and fractions were used after serum separation or whole blood centrifugation, and both were stored at −80 °C until use for biochemical analysis.

2.2. Measurement of arterial blood pressure in conscious rats

Indirect systolic blood pressure (mmHg) was measured 1 h before (1st day) and during (1st to 15th) at alternated days (between 8 and 12 am) using tail-cuff plethysmography (catalog # EFF 306, Insight, Ribeirao Preto, Sao Paulo, Brazil). Conscious rats were restrained for 5–10 min in a warm box (Insight, Ribeirao Preto, Sao Paulo, Brazil) in a quiet room and conditioned to numerous cuff inflation-deflation cycles by a trained operator. Systolic blood pressure was measured, and the mean of three measurements was recorded, as previously described (Gonçalves-Rizzi et al., 2015; Nascimento et al., 2015).

2.3. Determination of fipronil concentrations in plasma

Whole-blood levels of fipronil were determined by liquid–liquid extraction and high-performance liquid chromatography with ultraviolet detection (HPLC-UV) system (Prominence Shimadzu®, Kyoto, Japan), according to the method proposed by Cid et al. (2012) and adapted from Xavier et al. (2014). Briefly, blood samples were subjected to extraction by stirring with acetonitrile (Merck, Germany) and the filtered material was evaporated at room temperature and re-suspended with acetonitrile, then, passed with hydrophilic syringe filter with 13 mm diameter and pore 0.22 μm (PTFE membrane, VWR, Atlanta, GA, USA). A volume of 10 μL was injected into HPLC-UV, using a chromatographic column (C18). The whole-blood fipronil levels were expressed in μg/mL.

2.4. Determinations of plasma endothelin-1 and MMP-2 concentrations

For measurements were used commercial enzyme immunoassay (ELISA) kits for endothelin-1 (R&D Systems Inc, Minneapolis, MN, USA, catalog # DET100) and for MMP-2 (Abcam Inc, Cambridge, MA, USA, catalog # AB100730) plasma level assays, according to the manufacturer's instructions. The plasma endothelin-1 and MMP-2 levels were expressed in pg/mL.

2.5. Measurements of total antioxidant capacity

Serum antioxidant activity was determined by the Trolox (a water-soluble vitamin E analog, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma, St. Louis, MO, USA) equivalent antioxidant capacity assay as previously described (Ortner Hadžiabdić et al., 2015). Briefly, the antioxidant capacity is measured as the ability of the test compound (serum) to decrease the color of the reaction mixture by reacting directly with the ABTS (2,20-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical cation. The ABTS solution was mixed with 300 mL of the diluted serum sample and the reaction mixture was allowed to stand in the dark. After 3 min, the inhibition of the initial absorbance was recorded at 734 nm using an ultraviolet-visible spectrophotometer (UV 4-100, ATI Unicam, Cambridge, UK). Antioxidant activity of the sample was determined using a standard curve approach and was expressed as mmol of Trolox equivalent/L of sample.

2.6. Determinations of plasma nitrite/nitrate (NOx) concentrations

The plasma NO metabolites (nitrite/nitrate) – NOx concentrations were determined in duplicate using the Griess reaction as described previously (Gonçalves-Rizzi et al., 2015; Nascimento et al., 2015). Briefly, 40 μL of plasma was incubated with the same volume of nitrate reductase buffer (0.1 M potassium phosphate, pH 7.5, containing 1 mM b nicotinamide adenine dinucleotide phosphate and 2 U of nitrate reductase/mL) in individual wells of a 96-well plate. Samples were allowed to incubate overnight at 37 °C in the dark; 8 μL of freshly prepared Griess reagent (1% sulfanilamide, 0.1% naphthylethylenediamine dihydrochloride in 5% phosphoric acid) was added to each well, and the plate was incubated for an additional 15 min at room temperature. A standard nitrate curve was obtained by incubating sodium nitrate (0.2–200 lM) with the same reductase buffer. The absorbance values were measured at 540 nm using a microplate reader (Spectrophotometer, Synergy 4, BIOTEK, Winooski, VT, USA). The NOx levels in plasma were expressed in μmol/L.

2.7. Evaluations of liver/biliary or kidney functional abilities

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (IFCC method with pyridoxal phosphate activation), alkaline phosphatase (ALP) (IFCC method with aminomethylpropanol buffer), gamma-glutamyltransferase (GGT) (Szasz-Persijn method, using L-g-glutamyl-3-carboxy-4-nitroanilide), creatine kinase (CK) (N-acetylcysteine-activated IFCC method), creatinine