Environmental Toxicology and Pharmacology 42 (2016) 63–68

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

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

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

ARTICLE INFO

Article history:
Received 31 October 2015
Received in revised form
22 December 2015
Accepted 26 December 2015
Available online 31 December 2015

Keywords:
Fipronil
Cardiovascular toxicity
Biomarkers
Pesticides

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).

http://dx.doi.org/10.1016/j.etap.2015.12.020
1382-6689/

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

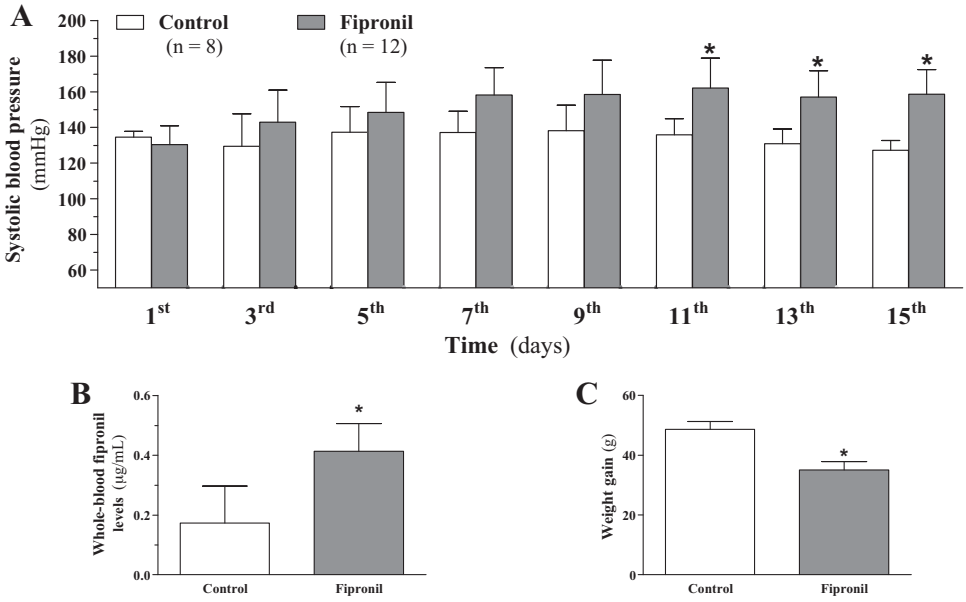

Fig. 1. Systolic blood pressure (mmHg, A), whole-blood fipronil levels (μg/mL, B) and weight gain (g, C) in the non-fipronil-exposed (Control group) or fipronil-exposed (Fipronil group) rats. Data are shown as mean ± S.E.M. Comparisons between groups were assessed by one-way analysis of variance (ANOVA) test for repeated measures. $^{*}p < 0.05$ *versus* Control group.

(Jaffe kinetic method) and urea (urease/glutamate dehydrogenase kinetic method) were assayed on a Dimension EXL 200 Integrated Chemistry System (Siemens, Erlangen, Germany), according to the manufacturer's specifications. Total imprecision, as expressed by the coefficient of variation (CV) was less than 2.5% for all analytes tested, as previously described (Lippi et al., 2006).

2.8. Data analysis and statistics

The results are expressed as means ± SEM. Shapiro–Wilk test was applied to verify normality of data distribution. Comparisons between groups were assessed by one-way analysis of variance (ANOVA) test for repeated measures or Student's paired *t*-test with Bonferroni's correction *post hoc* test, and *t*-distribution and degree of freedom (*df*) values are presented as well. We used Graph Pad Prism® 6.0 for Windows (San Diego, CA, USA) to analyze the results. Differences were considered significant when probability ($^{*}p$) < 0.05.

3. Results

3.1. Effect of fipronil exposure on systolic blood pressure

The effects of fipronil exposure on systolic blood pressure were assessed in conscious rats. As shown in Fig. 1, fipronil caused significant increases in systolic blood pressure (162 ± 16, $t = 4.071$, $df = 18$; 157 ± 14, $t = 4.234$, $df = 17$; and 158 ± 13 mmHg, $t = 5.327$, $df = 16$, in Fipronil group on days 11, 13 and 15 of exposition, respectively (Fipronil group, $^{*}p < 0.05$, Fig. 1A). No significant changes in systolic blood pressure were observed in Control group throughout experimental period (Fig. 1A).

The Fipronil group presented higher whole-blood fipronil levels (0.46 ± 0.09 μg/mL) compared with non-fipronil-exposed rats (0.17 ± 0.11 μg/mL in Control group, $t = 1.583$, $df = 13$, $^{*}p < 0.05$, Fig. 1B). In addition, Fipronil group showed lower weight gain (35 ± 3 g) compared with Control group (48 ± 3 g) ($t = 4.176$, $df = 16$, $^{*}p < 0.05$, Fig. 1C).

Table 1
Serum levels of creatinine, creatine kinase (CK), urea, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyltransferase (GGT) in the non-fipronil-exposed (Control group) or fipronil-exposed (Fipronil group) rats. Data are shown as mean ± S.E.M.

Biomarkers of renal and hepatic/biliary functional abilities	Control group	Fipronil group
Creatinine (mg/dL)	0.500 ± 0.036	0.455 ± 0.017
CK (U/L)	797 ± 143	757 ± 78
Urea (mg/dL)	39 ± 2	41 ± 2
ALT (U/L),	62 ± 3	59 ± 3
AST (U/L)	121 ± 8	123 ± 9
ALP (U/L)	27 ± 2	26 ± 1
GGT (U/L)	9 ± 0.9	9 ± 0.1

3.2. Effects of fipronil exposure in circulating biomarkers levels

Fipronil group presented higher concentrations of endothelin-1 (0.152 ± 0.008 pg/mL) compared with Control group (0.197 ± 0.019 pg/mL) ($t = 1.928$, $df = 16$, $^{*}p < 0.05$, Fig. 2A). Also, lower antioxidant capacity (Throlox, 0.075 ± 0.021 *versus* 0.242 ± 0.080 equiv./L, $t = 2.407$, $df = 8$) and levels of circulating MMP-2 (4498 ± 300 *versus* 3260 ± 130 pg/mL, $t = 4.072$, $df = 16$) and NOx (13 ± 1 *versus* 19 ± 1 μmmol/L, $t = 4.617$, $df = 16$) were found in fipronil treated rats compared with Controls (Fipronil *versus* Control group, $^{*}p < 0.05$, Fig. 2B–D, respectively).

No changes were observed in serum levels of creatinine, creatinine kinase, urea, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyltransferase in fipronil treated rats compared with controls (Table 1, respectively).

4. Discussion

The main results of this present study reveal, for the first time, that fipronil exposure resulted in elevation of both systolic blood pressure and levels of endothelin-1. Additionally, rats exposed to fipronil presented lower weight gain and decreases in antioxidant capacity and levels of MMP-2 and NOx in plasma, and no significant changes were observed in serum levels of creatinine, creatinine

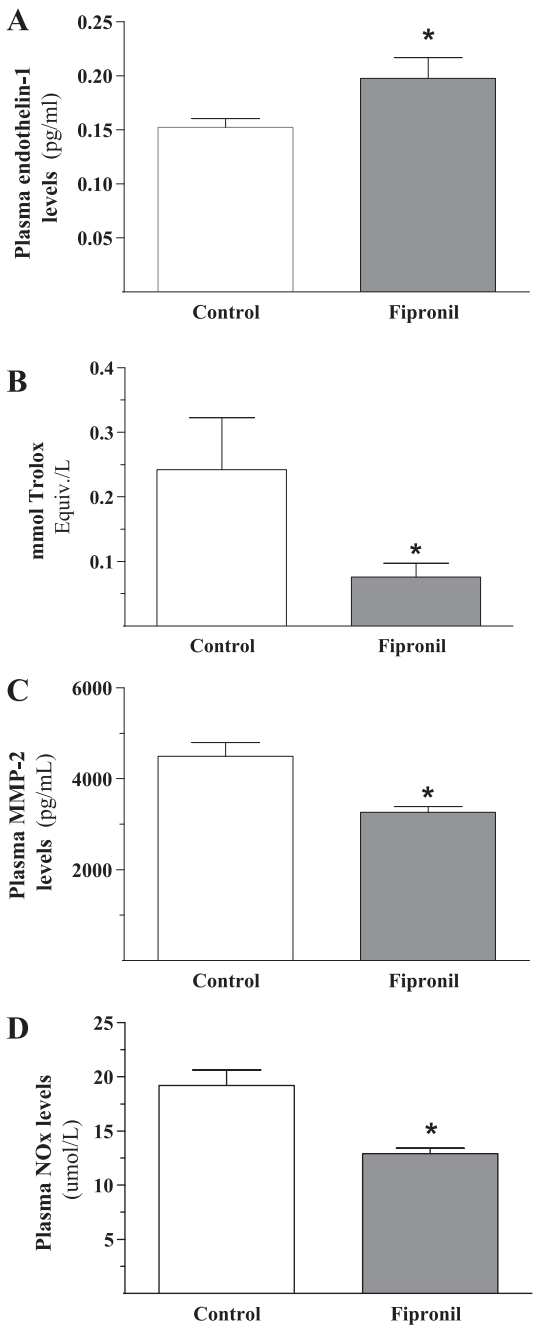

Fig. 2. Plasma endothelin-1 (A), Throlox (B), MMP-2 (C) and NOx (D) in the non fipronil-exposed (Control group) or fipronil-exposed (Fipronil group) rats. Data are shown as mean ± S.E.M. Comparisons between groups were assessed by Student's paired *t*-test with Bonferroni's correction *post hoc* test. $^{*}p < 0.05$ *versus* Control group.

kinase, urea, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyltransferase. Thus, these findings suggest that endothelin-1 may play an important role in fipronil-induced hypertension; also, the reductions of both MMP-2 and NO contributed to the elevation of systolic blood pressure observed with fipronil.

Accidental overexposure to the fipronil by farm works has been reported since its introduction in the market in the 90s (Tingle et al., 2003). The existence of fipronil-containing foodstuffs in many households has raised concerns and the possibility of contamination has been considered by governments (Tingle et al., 2003; Le Faouder et al., 2007; Jennings et al., 2002). Although fipronil's selectivity to the insects has been attributed to the inhibition of chloride channels, which results in uncontrolled neural excitation and, eventually, the death of the insect (Jackson et al., 2009), lower potency has been saw by antagonizes mammalian GABAergic receptors (Ikeda et al., 2004). Accordingly, the human fipronil toxicity results in symptoms and complications typically associated with the blockade of the GABA receptor function, featured by nausea, headache and seizures (Mohamed et al., 2004). However, adverse effects non-related to this fipronil's mode of action has been reported in experimental studies, since low doses of fipronil revealed reproductive toxicity (Moser et al., 2015; Ohi et al., 2004), disturbed maternal behavior (Udo et al., 2014) and disruption of thyroid function in rats (Leghait et al., 2009).

The present study shows that fipronil elevated the systolic blood pressure. Although the mechanisms elucidating how the fipronil exposure resulted in hypertension had never been investigated before, our findings show that fipronil-induced hypertension (Fig. 1) could be explained by increases of endothelin-1 and decreases of NOx levels in plasma (Fig. 2). Supporting our results, it has been suggested that the normal function of the vascular endothelial lining the circulatory system is essential for vascular homeostasis (Murakami and Simons, 2009; Koyama et al., 2013). However, if the endothelial dysfunction is present as in many vascular diseases, including hypertension, there are decreases in endothelium-dependent vasodilators (NO and prostacyclin) and increases in endothelium-dependent vasoconstrictors (endothelin-1 and thromboxane A_2) (Chiu and Chien, 2011). Accordingly, studies in humans have demonstrated that endothelin-1 levels are increased in hypertensive patients (Saito et al., 1990) and endothelin-1 receptor antagonists reduced the blood pressure in resistant hypertension (Weber et al., 2009) and improved the endothelium-dependent vasodilation in essential hypertension (Cardillo et al., 2002). In regard to the NO, it has been demonstrated that lesser NOx plasma concentrations compared with control subjects, confirming biologically that decreases in NO bioavailability during elevation of blood pressure (Panza et al., 1993; Kleinbongard et al., 2006).

Otherwise, endothelial dysfunction has been often associated with a paradoxical increased oxidative stress, according to reduction in antioxidant capacity (Ortner Hadžiabdić et al., 2015; Chiu and Chien, 2011), thus, oxidative stress induced by fipronil (Badgujar et al., 2015; Bebe and Panemangalore, 2005) may has impaired the bioavailability of NO observed here (Fig. 2). Accordingly, previous reports stated that acute fipronil exposure induced oxidative stress (Badgujar et al., 2015) and reduced antioxidant status (Gill and Dumka, 2013) in animals. In this concern, toxicity mediated by pesticides involves excessive production of reactive oxygen species, which may lead to alterations in the cellular antioxidant defense system resulting in susceptibility of cells to oxidative stress (Badgujar et al., 2015; Bebe and Panemangalore, 2005; Moser et al., 2015). Reduction of cellular antioxidant status makes up the cell to become vulnerable to modify lipids, nucleic acids and proteins leading to cell disruption and increased production of lipid peroxidation induced by oxidative stress (Salminen and Paul, 2014). Thus, it is reasonable to consider that endothelial dysfunction has contributed with the fipronil toxicity in the present study.

In addition, decreases in MMP-2 levels in plasma were also observed with fipronil (Fig. 2), supporting earlier findings in which increases in tissue inhibitor of MMP (TIMP) were showed after fipronil exposure (Moser et al., 2015). Accordingly, both reduced levels of MMP-2 and NO could increase vessel rigidity and decrease its plasticity, contributing to increased vascular resistance and hypertension. These suggestions are consistent with previous reports that MMP-2 (a typical gelatinolytic enzyme) is decreased while the collagen deposition presents increased in arteries from hypertensive patients (Ergul et al., 2004) and from hypertension in pregnant rats (Li et al., 2014).

Importantly, the fipronil's mechanism is blocking the chloride channels of GABA-ergic receptors and glutamate-gated chloride channels (Narahashi et al., 2007; Zhao et al., 2005) and GABA antagonists activate vasopressin release (Isobe and Nishino, 1997; Magnusson and Meyerson, 1993; Sladek and Armstrong, 1987). Furthermore, vasopressin mediates the expression of the endothelin-1 (Emori et al., 1991; Imai et al., 1992; Li et al., 2003), which, in turn, is responsible for control the heart rate and systemic arterial pressure (Faraci, 1989). Therefore, it is possible that fipronil causes the vasopressin release and vasopressin had induced the expression of endothelin-1, elevating the systolic blood pressure as observed in the present study.

Earlier studies have reported that liver, kidney and brain are the target organs for toxic insults of fipronil (Badgujar et al., 2015; Terçariol and Godinho, 2011), owing to the fact that these tissues are extremely perfused organs and receive high blood volumes. In fact, liver toxicity to fipronil has been reported in mice following subchronic and chronic exposures (De Oliveira et al., 2012). On the other hand, this was not observed in our current short-term (15 days) study (Table 1). The present results show that although Fipronil group presented lower weight gain (Fig. 1), suggesting biological toxicity, no significant alterations were observed neither liver/biliary or kidney functional abilities as reflected by no significant changes in alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyltransferase, creatine kinase, creatinine and urea levels in plasma (Table 1). Importantly, a previous study showed that with repeated dosing fipronil accumulated (Moser et al., 2015) and increases in fipronil levels have been related to induction of hepatic metabolizing enzymes (Moser et al., 2015; Das et al., 2006). However, these differences could be explained, at least in part, by physiological and compensatory responses, as well as differing levels of fipronil and its metabolites resulted from different dosage regimen.

Therefore, it should be taken into account that reference levels and acceptable daily intakes for fipronil are set at doses much lower than those used here, which were approached to try to identify biological changes. In addition, although these doses may reflect high peaks in exposures with high contamination or poisonings (Jennings et al., 2002; Mohamed et al., 2004), a logical follow-up study should be driven to assess lower doses and longer exposure periods, to start approaching environmentally relevant exposures.

5. Conclusion

The main conclusions of the present study are that repeated oral exposition of 30 mg/kg/day of fipronil caused hypertension and disturbed related biomarkers that regulate the blood pressure. In addition, increases in endothelin-1 and decreases in antioxidant capacity, MMP-2 and NO were observed after 15 days of fipronil exposure. However, no changes in hepatic/biliary or renal function after 15 days of exposure were seen with fipronil. Therefore, our results suggest that endothelin-1 plays a key role in fipronil-induced hypertension and are in accordance with the notion that reductions of both MMP-2 and NO contribute to the elevation of blood pressure.

Conflict of interest

There is no conflict of interest to declare.

Acknowledgements

This study was funded by Fundacao de Amparo a Pesquisa do Estado de Sao Paulo (FAPESP, Brazil).

References

Badgujar, P.C., Pawar, N.N., Chandratre, G.A., Telang, A.G., Sharma, A.K., 2015. Fipronil induced oxidative stress in kidney and brain of mice: protective effect of vitamin E and vitamin C. Pestic. Biochem. Physiol. 118, 10–18.

Bebe, F.N., Panemangalore, M., 2005. Pesticides and essential minerals modify endogenous antioxidant and cytochrome P450 in tissues of rats. J. Environ. Sci. Health B 40, 769–784.

Cardillo, C., Campia, U., Kilcoyne, C.M., Bryant, M.B., Panza, J.A., 2002. Improved endothelium-dependent vasodilation after blockade of endothelin receptors in patients with essential hypertension. Circulation 105, 452–456.

Chiu, J.J., Chien, S., 2011. Effects of disturbed flow on vascular endothelium: pathophysiological basis and clinical perspectives. Physiol. Rev. 91 (1), 327–387.

Cid, Y.P., Ferreira, T.P., Medeiros, D.M.V.C., Oliveira, R.M., Silva, N.C.C., Scott, F.B., 2012. Determination of fipronil in bovine plasma by solid-phase extraction and liquid chromatography with ultraviolet detection. Quim. Nova 35 (10), 2063–2066.

Das, P.C., Cao, Y., Cherrington, N., Hodgson, E., Rose, R.L., 2006. Fipronil induces CYP isoforms and cytotoxicity in human hepatocytes. Chem. Biol. Interact. 164 (3), 200–214.

De Oliveira, P.R., Bechara, G.H., Denardi, S.E., Oliveira, R.J., Mathias, M.I., 2012. Cytotoxicity of fipronil on mice liver cells. Microsc. Res. Tech. 75 (1), 28–35.

Emori, T., Hirata, Y., Ohta, K., Kanno, K., Eguchi, S., Imai, T., Shichiri, M., Marumo, F., 1991. Cellular mechanism of endothelin-1 release by angiotensin and vasopressin. Hypertension 18 (2), 165–170.

Ergul, A., Portik-Dobos, V., Hutchinson, J., Franco, J., Anstadt, M.P., 2004. Downregulation of vascular matrix metalloproteinase inducer and activator proteins in hypertensive patients. Am. J. Hypertens. 17, 775–782.

Faraci, F.M., 1989. Effects of endothelin and vasopressin on cerebral blood vessels. Am. J. Physiol. 257, H799–H803.

Gill, K.K., Dumka, V.K., 2013. Antioxidant status in oral subchronic toxicity of fipronil and fluoride co-exposure in buffalo calves. Toxicol. Ind. Health 10, 1–9.

Gonçalves-Rizzi, V.H., Nascimento, R.A., Possomato-Vieira, J.S., Dias-Junior, C.A., 2015. Sodium nitrite prevents both reductions in circulating nitric oxide and hypertension in 7-day lead-treated rats. Basic Clin. Pharmacol. Toxicol.

Ikeda, T., Nagata, K., Kono, Y., Yeh, J.Z., Narahashi, T., 2004. Fipronil modulation of GABAA receptor single-channel currents. Pest Manag. Sci. 60, 487–492.

Imai, T., Hirata, Y., Emori, T., Yanagisawa, M., Masaki, T., Marumo, F., 1992. Induction of endothelin-1 gene by angiotensin and vasopressin in endothelial cells. Hypertension 19 (6), 753–757.

Isobe, Y., Nishino, H., 1997. GABAergic control of Arg-vasopressin release from suprachiasmatic nucleus slice culture. Brain Res. 755 (2), 213–220.

Jackson, D., Cornell, C.B., Luukinen, B., Buhl, K., Stone, D., 2009. Fipronil technical fact sheet. National Pesticide Information Center, Oregon State, pp. 1–3.

Jennings, K.A., Canerdy, T.D., Keller, R.J., Atieh, B.H., Doss, R.B., Gupta, R.C., 2002. Human exposure to fipronil from dogs treated with frontline. Vet. Hum. Toxicol. 44, 301–303.

Kleinbongard, P., Dejam, A., Lauer, T., Jax, T., Kerber, S., Gharini, P., Balzer, J., Zotz, R.B., Scharf, R.E., Willers, R., Schechter, A.N., Feelisch, M., Kelm, M., 2006. Plasma nitrite concentrations reflect the degree of endothelial dysfunction in humans. Free Radic. Biol. Med. 40, 295–302.

Koyama, T., Ochoa-Callejero, L., Sakurai, T., Kamiyoshi, A., Ichikawa-Shindo, Y., Iinuma, N., Arai, T., Yoshizawa, T., Iesato, Y., Lei, Y., Uetake, R., Okimura, A., Yamauchi, A., Tanaka, M., Igarashi, K., Toriyama, Y., Kawate, H., Adams, R.H., Kawakami, H., Mochizuki, N., Martínez, A., Shindo, T., 2013. Vascular endothelial adrenomedullin-RAMP2 system is essential for vascular integrity and organ homeostasis. Circulation 127 (7), 842–853.

Le Faouder, J., Bichon, E., Brunschwig, P., Landelle, R., Andre, F., Le Bizec, B., 2007. Transfer assessment of fipronil residues from feed to cow milk. Talanta 73 (4), 710–717.

Leghait, J., Gayrard, V., Picard-Hagen, N., Camp, M., Perdu, E., Toutain, P.L., Viguié, C., 2009. Fipronil-induced disruption of thyroid function in rats is mediated by increased total and free thyroxine clearances concomitantly to increased activity of hepatic enzymes. Toxicology 255 (1–2), 38–44.

Li, L., Galligan, J.J., Fink, G.D., Chen, A.F., 2003. Vasopressin induces vascular superoxide via endothelin-1 in mineralocorticoid hypertension. Hypertension 41, 663–668.

Li, W., Mata, K.M., Mazzuca, M.Q., Khalil, R.A., 2014. Altered matrix metalloproteinase-2 and -9 expression/activity links placental ischemia and anti-angiogenic sFlt-1 to uteroplacental and vascular remodeling and collagen deposition in hypertensive pregnancy. Biochem. Pharmacol. 89 (3), 370–385.

Lippi, G., Salvagno, G.L., Montagnana, M., Brocco, G., Guidi, G.C., 2006. Influence of hemolysis on routine clinical chemistry testing. Clin. Chem. Lab. Med. 44 (3), 311–316.

Magnusson, A.M., Meyerson, B.J., 1993. GABA-A agonist muscimol inhibits stimulated vasopressin release in the posterior pituitary of Sprague-Dawley, Wistar. Wistar-Kyoto and spontaneously hypertensive rats. Neuroendocrinology 58 (5), 519–524.

Mohamed, F., Senarathna, L., Percy, A., Abeyewardene, M., Eaglesham, G., Cheng, R., Azher, S., Hittarage, A., Dissanayake, W., Sheriff, M.H., Davies, W., Buckley, N.A., Eddleston, M., 2004. Acute human self-poisoning with the N-phenylpyrazole insecticide fipronil – a $GABA_A$-gated chloride channel blocker. J. Toxicol. Clin. Toxicol. 42, 955–963.

Moser, V.C., Stewart, N., Freeborn, D.L., Crooks, J., MacMillan, D.K., Hedge, J.M., Wood, C.E., McMahen, R.L., Strynar, M.J., Herr, D.W., 2015. Assessment of serum

biomarkers in rats after exposure to pesticides of different chemical classes. Toxicol. Appl. Pharmacol. 282 (2), 161–174.
Murakami, M., Simons, M., 2009. Regulation of vascular integrity. Mol. Med. (Berl.) 87 (6), 571–582.
Narahashi, T., Zhao, X., Ikeda, T., Nagata, K., Yeh, J.Z., 2007. Differential actions of insecticides on target sites: basis for selective toxicity. Hum. Exp. Toxicol. 4, 361–366.
Narahashi, T., Zhao, X., Ikeda, T., Salgado, V.L., Yeh, J.Z., 2010. Glutamate-activated chloride channels: unique fipronil targets present in insects but not in mammals. Pestic. Biochem. Physiol. 97 (2), 149–152.
Nascimento, R.A., Mendes, G., Possomato-Vieira, J.S., Gonçalves-Rizzi, V.H., Kushima, H., Delella, F.K., Dias-Junior, C.A., 2015. Metalloproteinase inhibition protects against reductions in circulating adrenomedullin during lead-induced acute hypertension. Basic Clin. Pharmacol. Toxicol. 116 (6), 508–515.
Ohi, M., Dalsenter, P.R., Andrade, A.J., Nascimento, A.J., 2004. Reproductive adverse effects of fipronil in Wistar rats. Toxicol. Lett. 146 (2), 121–127.
Ortner Hadžiabdić, M., Vitali Čepo, D., Rahelić, D., Božikov, V., 2015. The effect of the mediterranean diet on serum total antioxidant capacity in obese patients: a randomized controlled trial. J. Am. Coll. Nutr. 5, 1–12.
Overmyer, J.P., Rouse, D.R., Avants, J.K., Garrison, A.W., Delorenzo, M.E., Chung, K.W., Key, P.B., Wilson, W.A., Black, M.C., 2007. Toxicity of fipronil and its enantiomers to marine and freshwater non-targets. J. Environ. Sci. Health B 42, 471–479.
Panza, J.A., Casino, P.R., Kilcoyne, C.M., Quyyumi, A.A., 1993. Role of endothelium-derived nitric oxide in the abnormal endothelium-dependent vascular relaxation of patients with essential hypertension. Circulation 87, 1468–1474.
Saito, Y., Nakao, K., Mukoyama, M., Imura, H., 1990. Increased plasma endothelin level in patients with essential hypertension. N. Engl. J. Med. 322 (3), 205.
Salminen, L.E., Paul, R.H., 2014. Oxidative stress and genetic markers of suboptimal antioxidant defense in the aging brain: a theoretical review. Rev. Neurosci. 25 (6), 805–819.
Sladek, C.D., Armstrong, W.E., 1987. γ-Aminobutyric acid antagonists stimulate vasopressin release from organ-cultured hypothalamo-neurohypophyseal explants. Endocrinology 120, 1576–1580.
Terçariol, P.R., Godinho, A.F., 2011. Behavioral effects of acute exposure to the insecticide fipronil. Pestic. Biochem. Physiol. 99, 221–225.
Tingle, C.C., Rother, J.A., Dewhurst, C.F., Lauer, S., King, W.J., 2003. Fipronil: environmental fate, ecotoxicology, and human health concerns. Rev. Environ. Contam. Toxicol. 176, 1–66.
Udo, M.S., Sandini, T.M., Reis, T.M., Bernardi, M.M., Spinosa, H.S., 2014. Prenatal exposure to a low fipronil dose disturbs maternal behavior and reflex development in rats. Neurotoxicol. Teratol. 45, 27–33.
Vidau, C., Gonzalez-Polo, R.A., Niso-Santano, M., Gomez-Sanchez, R., Bravo-San Pedro, J.M., Pizarro-Estrella, E., Blasco, R., Brunet, J.L., Belzunces, L.P., Fuentes, J.M., 2011. Fipronil is a powerful uncoupler of oxidative phosphorylation that triggers apoptosis in human neuronal cell line SHSY5Y. Neurotoxicology 32, 935–943.
Weber, M.A., Black, H., Bakris, G., Krum, H., Linas, S., Weiss, R., Linseman, J.V., Wiens, B.L., Warren, M.S., Lindholm, L.H., 2009. A selective endothelin-receptor antagonist to reduce blood pressure in patients with treatment-resistant hypertension: a randomised, double-blind, placebo-controlled trial. Lancet 374, 1423–1431.
Xavier, G., Chandran, M., George, T., Beevi, S.N., Mathew, T.B., Paul, A., Arimboor, R., Vijayasree, V., Pradeepkumar, G.T., Rajith, R., 2014. Persistence and effect of processing on reduction of fipronil and its metabolites in chilli pepper (*Capsicum annum* L.) fruits. Environ. Monit. Assess. 186 (9), 5429–5437.
Zhao, X., Yeh, J.Z., Salgado, V.L., Narahashi, T., 2005. Sulfone metabolite of fipronil blocks gamma-aminobutyric acid- and glutamate-activated chloride channels in mammalian and insect neurons. J. Pharmacol. Exp. Ther. 314 (1), 363–373.