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Effect of fipronil on energy metabolism in the perfused rat liver

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HIGHLIGHTS

- The effects of fipronil on energy metabolism were examined in perfused rat livers.
- Fipronil inhibits energy-dependent processes such as gluconeogenesis and ureogenesis.
- Mitochondrial respiratory chain was inhibited.
- CYP-derived metabolites of fipronil had an important role in the observed effects.

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ABSTRACT

Fipronil is an insecticide used to control pests in animals and plants that can causes hepatotoxicity in animals and humans, and it is hepatically metabolized to fipronil sulfone by cytochrome P-450. The present study aimed to characterize the effects of fipronil (10–50 μM) on energy metabolism in isolated perfused rat livers. In fed animals, there was increased glucose and lactate release from glycogen catabolism, indicating the stimulation of glycogenolysis and glycolysis. In the livers of fasted animals, fipronil inhibited glucose and urea production from exogenous L-alanine, whereas ammonia and lactate production were increased. In addition, fipronil at 50 μM concentration inhibited the oxygen uptake and increased the cytosolic NADH/NAD^+ ratio under glycolytic conditions. The metabolic alterations were found both in livers from normal or proadifen-pretreated rats revealing that fipronil and its reactive metabolites contributed for the observed activity. The effects on oxygen uptake indicated that the possible mechanism of toxicity of fipronil involves impairment on mitochondrial respiratory activity, and therefore, interference with energy metabolism. The inhibitory effects on oxygen uptake observed at the highest concentration of 50 μM was abolished by pretreatment of the rats with proadifen indicating that the metabolites of fipronil, including fipronil sulfone, acted predominantly as inhibitors of respiratory chain. The hepatoxicity of both the parent compound and its reactive metabolites was corroborated by the increase in the activity of lactate dehydrogenase in the effluent perfusate in livers from normal or proadifen-pretreated rats.

1. Introduction

Fipronil, developed by Rhône-Poulenc Agro in 1987, is a pesticide that belongs to the phenylpyrazole chemical group (Tingle et al., 2003). It is an insecticide with widespread use in the control of many agricultural and domestic pests. Fipronil toxicity is attributed to its ability to act at the GABA receptor as a noncompetitive inhibitor of the GABA-gated chloride channels of neurons in the central nervous system. Impediment of the influx of the chloride ions affects the transmission of nervous impulses, causing insect death by neuronal hyperexcitation and paralysis (Rhône-Poulenc, 1995; Zhao et al., 2004).

Fipronil binding is stronger to the chloride channels of insects than to those of mammals, resulting in an insecticide with selective toxicity. Thus, fipronil has a greater ability to block GABA-gated Cl^- channels of insects than those of vertebrates and is therefore considered safe and is widely used in veterinary medicine (Hainzl and Casida, 1996; Hainzl et al., 1998; Coutinho et al., 2005; Gunasekara and Troung, 2007). However, Zhao et al. (2005) indicate that the metabolite fipronil sulfone, which results

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from the hepatic biotransformation of fipronil via cytochrome P450, is at least 20 times more potent to block mammalian $GABA_A$ receptors.

There are several cases in the literature of animal and human poisoning due to intentional ingestion, accidental exposure or incorrect use of fipronil (Gasmi et al., 2001; Jennings et al., 2002; Chodorowski and Anand, 2004; Mohamed et al., 2004; Lee et al., 2010; Anadon and Gupta, 2012; Gill and Dumka, 2013). Silva (2008) evaluated the effects of prolonged fipronil exposure in rats and observed hepatic cell swelling and increased liver weights in animals treated with a 10 mg/kg dose administered by oral gavage. This dose corresponds to one tenth of the LD50 in the rats established by Hainzl and Casida (1996). The hepatotoxic mechanisms of fipronil are unknown. Vidau et al. (2011) showed that fipronil exerts an uncoupling effect in isolated rat liver mitochondria. In another study, Palma et al. (2013) demonstrated that fipronil exerts an inhibitory effect on the electron transport chain, specifically in complex I. Both studies presented a concentration-dependent effect of fipronil starting at a concentration of 5 μM.

The liver is the central organ in metabolism because it is interposed between the digestive tract and the general circulation. Among the main liver functions is the uptake of amino acids, lipids, carbohydrates and vitamins, with subsequent storage, metabolic conversion and release into the blood, as well as the production of bile. The liver is also capable of accumulating, biotransforming and inactivating many xenobiotics, converting them into water-soluble substances and thus facilitating the removal of these compounds by the organism (Guillouzo, 1998). However, this process has been considered responsible for the toxic effects of many chemicals because the produced metabolites can exert adverse effects on the organism (Ioannides and Lewis, 2004; Mingatto et al., 2008).

For this reason, based on the importance of the liver to the animal organism and the evidence that fipronil exerts action on isolated mitochondria, the present study was planned to investigate the effects of fipronil in the perfused rat liver, a methodology in which the structural and functional integrity of the organ is preserved. Fipronil was infused into the livers at concentrations ranging from 10 to 50 μM, and several parameters related to the energy metabolism were measured in the perfused effluent, including glycogenolysis, glycolysis and oxygen uptake in the livers of fed rats, as well as gluconeogenesis and ureagenesis in the livers of fasted rats. In order to investigate whether fipronil metabolites are implicated in the metabolic alterations, rats were previously treated with proadifen, a well-known cytochrome P-450 inhibitor.

2. Materials and methods

2.1. Chemicals

The liver perfusion apparatus was built in the workshops of the University of Maringá. Enzymes and coenzymes used in the enzymatic assays were purchased from Sigma–Aldrich (St. Louis, MO, USA). Fipronil was a gift from Ourofino Agribusiness, containing 96.6% purity (Cravinhos, SP, Brazil). All other reagents were of the highest commercially available grade.

2.2. Animals

Male albino rats (Wistar) weighing 180–220 g were housed in plastic cages at a constant temperature (23 ± 3 °C) and relative humidity (55 ± 15%) under a regular light/dark cycle (12 h:12 h). They were fed *ad libitum* with a standard laboratory diet (Nuvilab®, Colombo, Brazil). The experimental protocols were approved by the Ethical Committee for the Use of Laboratory Animals of the UNESP – Univ Estadual Paulista, Campus of Dracena, SP, Brazil.

All experiments were started between 7:00 AM and 8:00 AM. Rats used for studies of gluconeogenesis were fasted 24 h before the experiments to deplete the livers of glycogen. In some experiments rats were pretreated with proadifen (25 mg kg body $weight^{-1}$), a cytochrome P450 inhibitor, intraperitoneally for 3 consecutive days (Somchit et al., 2009).

2.3. Liver perfusion

For the surgical procedure, fed or 24 h fasted rats were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg kg body $weight^{-1}$). Hemoglobin-free, non-recirculating perfusion was performed. The surgical technique was the same as that described by Scholz and Bucher (1965). After cannulation of the portal and cava veins, the liver was positioned in a Plexiglas chamber. The perfusion fluid was Krebs/Henseleit-bicarbonate buffer (pH 7.4), saturated with a mixture of oxygen and carbon dioxide (95:5) using a membrane oxygenator with simultaneous temperature adjustment at 37 °C. The flow, provided by a peristaltic pump, was between 30 and 35 ml min^{-1}, depending on the liver weight. Samples of the effluent perfusion fluid were collected according to the experimental protocol and analyzed for their metabolite contents. The oxygen concentration in the outflowing perfusate was monitored continuously using a Teflon-shielded platinum electrode adequately positioned in a Plexiglas chamber at the point where the perfusate exits (Scholz and Bucher, 1965). Fipronil (10, 15, 25 and 50 μM) or L-alanine (2.5 mM) was dissolved in the perfusion fluid. The doses of fipronil were selected on the basis of the previous results using liver cells (Das et al., 2006) and isolated rat liver mitochondria (Vidau et al., 2011; Palma et al., 2013). Control experiments without fipronil addition were previously performed and showed that concentrations of metabolites in the perfusate and oxygen uptake did not change during the experimental time period, implying that the perfused liver was metabolically stable for the duration of the perfusion used in this study.

2.4. Analytical

The following compounds were assayed using standard enzymatic procedures: glucose (Bergmeyer and Bernt, 1974), L-lactate (Gutmann and Wahlefeld, 1974), pyruvate (Czok and Lamprecht, 1974), urea (Bergmeyer, 1974) and ammonium (Kun and Kearney, 1974). Metabolic rates were calculated from input–output differences and the total flow rates, normalized to the wet weight of the liver, and expressed as μmol min^{-1} (gram liver wet $weight)^{-1}$. The activity of the enzyme lactate dehydrogenase (LDH) was measured in the perfusate using an Assay Kit (Bioclin, Quibasa, Brazil) according to the manufacturer's instructions.

2.5. Treatment of the data

The data in the figures are expressed as mean ± standard error of the mean (S.E.M.) of 3–5 liver perfusion experiments. The statistical significance of the differences between parameters among the experimental groups was evaluated using two-way analysis of variance, and differences in the same experimental groups were tested by repeated-measures one-way analysis of variance (ANOVA). Significant differences among means were identified by Bonferroni or Newman–Keuls testing, respectively. The results are given in the text as probability values (P). $P \leq 0.05$ was adopted as the criterion of significance. Statistical analysis was performed using Statistica™ or GraphPAD Software programs.