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Aqueous extract of *Ilex paraguariensis* decreases nucleotide hydrolysis in rat blood serum

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Abstract

Mate is a xanthine-containing beverage, which is prepared as an infusion of the dried and ground leaves of *Ilex paraguariensis* St. Hil. (Aquifoliacea). Previous reports have shown that *Ilex paraguariensis* has the highest levels of caffeine and theobromine when compared to other *Ilex* species. Furthermore, mate is able to interfere in the circulatory system, acting as a diuretic and hypotensive agent. Many processes of vascular injury result in the release of adenine nucleotides, which exert a variety of effects. Nucleoside 5′ tri- and diphosphates may be hydrolyzed by members of the ecto-nucleoside triphosphate diphosphohydrolase (E-NTPDase) family. The synchronic action of a NTPDase and a 5′-nucleotidase promotes the catabolism of ATP to adenosine, which is able to control the extracellular nucleotides/nucleosides ratio. The chronic ingestion of aqueous extract of *Ilex paraguariensis* by rats during 15 days significantly decreased ATP (55%), ADP (50%) and AMP (40%) hydrolysis in blood serum. These results suggest changes in the balance of purine levels induced by *Ilex paraguariensis* ingestion. Considering the potential effects of *Ilex paraguariensis* in the circulatory system, these results may be relevant since NTPDases are a novel drug target for the treatment of cardiovascular diseases.

Keywords: *Ilex paraguariensis*; NTPDase; Nucleotides; Blood serum; Aquifoliacea

1. Introduction

Mate is a beverage traditionally taken in several Latin American countries, including Southern Brazil, Argentina, Uruguay and Paraguay. Consume of mate in Brazil is approximately 1.2 kg/person/year (reviewed by Fredholm et al., 1999). Mate is prepared as an infusion of the dried and ground leaves of *Ilex paraguariensis* St. Hil (Aquifoliacea) (Schinella et al., 2000). The infusion is drunk for its claimed diuretic, anti-inflammatory and stimulant properties (Cruz, 1982; Mazzafera, 1994). Previous reports have demonstrated that *Ilex paraguariensis* has the highest levels of caffeine and theobromine when compared to other *Ilex* species (Filip et al., 1998). The ingestion of *Ilex paraguariensis* could contribute to the increase of antioxidant defense against the action of free radicals (Schinella et al., 2000). Intake of aqueous extracts of *Ilex paraguariensis* inhibits copper-induced autoxidation of LDL in whole human plasma (Gugliucci, 1996). Furthermore, oral ingestion of *Ilex paraguariensis* infusions was reported to interfere in the circulatory system, acting as a diuretic and hypotensive agent (Mazzafera, 1994).

Circulating nucleotides are released as signaling substances or during pathological events (Zimmermann, 1996; Bodin and Burnstock, 2001a). Many processes of vascular

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injury result in the release of adenine nucleotides which exert a variety of effects (Luthje, 1989; Bodin and Burnstock, 2001b). ATP acts as vasoconstrictor and may be a cytotoxic structure (Opie, 1992), while it could be a potent vasodilator in most vascular beds (Aso et al., 1986; Agteresch et al., 1999). ATP also stimulates cellular production of prostacyclin and nitric oxide, two vasodilators and inhibitors of platelet aggregation (Motte et al., 1995). In contrast, studies have shown that ATP inhibits platelet aggregation acting as a competitive antagonist, whereas ADP is able to promote platelet aggregation (Opie, 1992). There are two distinct families of receptors for purine and pyrimidine nucleotides: P2X and P2Y, which contain eight and seven members, respectively (Ralevic and Burnstock, 1998; Hollopeter et al., 2001; Zhang et al., 2002). These subtypes are expressed with some selectivity on different types of cells (reviewed by Ralevic and Burnstock, 2003), and ATP and ADP may regulate hemostasis through activation of platelet P2Y or P2X receptors (Fredholm, 1994; Jin and Kunapuli, 1998).

Presumably, all tissues have the capacity to metabolize extracellular nucleotides by surface-located enzymes or also by soluble enzymes in the interstitial medium or within body fluids (Zimmermann, 1996, 2001). Nucleoside 5′ tri- and diphosphates may be hydrolyzed by the members of the ecto-nucleoside triphosphate diphosphohydrolase (E-NTPDase) family (Zimmermann, 2001). Recently, studies from our laboratory have demonstrated a possible soluble NTPDase in rat blood serum (Rücker et al., 2003; Oses et al., 2004). The synchronic action of NTPDase and 5′-nucleotidase promotes the catabolism of ATP to adenosine, which is able to control the extracellular nucleotides/nucleosides ratio. Then, adenosine produced by nucleotide catabolism in the circulation can act as a vasodilator and as an inhibitor of platelet aggregation (Olson and Pearson, 1990; Opie, 1992). Adenosine can exert its functions through four types of P1 receptors: A_1, A_{2A}, A_{2B} and A_3 (Ralevic and Burnstock, 1998). Some of these receptors are expressed in endothelial cells (Bodin and Burnstock, 1995), smooth muscle cells (Giaroni et al., 2002), and sympathetic and sensory nerves (Burnstock, 1996). The action of adenosine in A_1 and A_2 receptors can be blocked by caffeine, an unspecific antagonist of adenosine receptors (Fredholm et al., 2001; da Silva et al., 2003).

The fact that *Ilex paraguariensis* has a hypotensive role and nucleotides are signaling molecules able to modulate circulatory system, prompted us to examine the effects of acute and chronic ingestion of aqueous extract of *Ilex paraguariensis* in nucleotides hydrolysis in rat blood serum.

2. Materials and methods

2.1. Preparation of Ilex paraguariensis extract

Samples of mate were obtained from commercial products purchased from local supermarkets in Lajeado, Rio Grande do Sul, Brazil. The plant is produced by the manufacturer in Venâncio Aires, Rio Grande do Sul, Brazil.

The extract was prepared as infusions. Herbal commercial samples (5 g) were weighted and put into 100 mL of boiling distilled water and after left to cool down (Schinella et al., 2000). This extract was filtered using filter paper. The solid contents of the aqueous extract was 0.05 g/mL^{-1}. The yield of the extract was 21.54%. The extracts were prepared daily (chronic treatment) and at the time of infusion (acute treatment).

2.2. Animals

Male Wistar rats (weight, 220–260 g; age, 60–90 days) from our breeding colony were used. The animals were housed five to a cage with food ad libitum and maintained on a 12-h light/dark cycle at temperature of 23 ± 1 °C. In all experiments, the “Principles of laboratory animal care” (NIH publication No. 85–23, revised 1996) were strictly followed.

2.3. Chronic treatment

The animals were allotted into two groups, containing 10 rats each. The first group received *Ilex paraguariensis* infusion and a second group tap water during 15 days ad libitum. The infusion of *Ilex paraguariensis* was administered chronically by giving the animals free access to bottles containing this infusion. The proportion of extract and water was given according to Schinella et al. (2000). The intake of *Ilex paraguariensis* was monitored throughout the experiment. Daily fluid intake (mL/day) was estimated once every day based on the fluid consumption by the subjects over a 24-h period and its body weight. Daily fluid intake in control groups with free access to tap water was monitored for comparison. There was no significant weight difference between groups (data not shown).

2.4. Acute treatment

In the acute treatment, two groups of animals ($n = 10$ for each group) were submitted to an oral administration by gavage (0.5–0.6 mL) of water (control group) or *Ilex paraguariensis* infusion (treated group). After 1 hour, blood samples were taken and used for the enzyme assays.

2.5. Measurement of ATP, ADP and AMP hydrolysis

ATP, ADP and AMP hydrolysis were determined using a modification of the method described by Yegutkin (1997). The reaction mixture containing ADP or ATP as a substrate (at the concentrations indicated), 112.5 mM Tris–HCl, pH 8.0, was incubated with approximately 1.0 mg of serum protein at 37 °C for 40 min in a final volume of 0.2 mL. The reaction was stopped by the addition of 0.2 mL 10% TCA. The samples were chilled on ice and the amount of inorganic phosphate (Pi) released was measured by the method of Chan