Cardioprotective effects of *Ilex paraguariensis* extract: evidence for a nitric oxide-dependent mechanism

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**Summary**

**Aim:** To examine the effects of an *Ilex paraguariensis* (Ip) extract on postischemic alterations derived from 20 min of global ischemia and 30 min of reperfusion.  

**Methods:** Isolated rat hearts were treated 10 min before ischemia and the first 10 min of reperfusion with Ip 30 \(\mu\)g/ml. In other hearts, chelerythrine (1 \(\mu\)M), a protein kinase C blocker, or \(\epsilon\)-nitro \(L\)-arginine methyl ester (\(L\)-NAME), a nitric oxide synthase inhibitor, were administered prior to Ip infusion. Left ventricular developed pressure (LVDP), \(+dP/dt\)\(_{\text{max}}\), \(-dP/dt\)\(_{\text{max}}\), and left ventricular end diastolic pressure (LVEDP) were used to assess myocardial function. Thiobarbituric acid reactive substances (TBARS) were measured.

**Results:** Ip treatment produced an improvement of postischemic recovery (LVDP = 96 ± 8%; \(+dP/dt\)\(_{\text{max}}\) = 95 ± 10%; \(-dP/dt\)\(_{\text{max}}\) = 90 ± 12% vs. 57 ± 6%, 53 ± 6% and 57 ± 8%, respectively, in untreated hearts) and an attenuation of the increase of LVEDP and TBARS content. Chelerythrine did not modify and \(L\)-NAME abolished the protection induced by Ip.

**Conclusions:** These data are the first demonstration that Ip extract attenuates the myocardial dysfunction provoked by ischemia and reperfusion and that this cardioprotection involves a diminution of oxidative damage through a nitric oxide-dependent mechanism.

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**Keywords**  
Ischemia; Reperfusion; Ilex paraguariensis; Protein kinase C; Nitric oxide

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Introduction

Herbal mate tea or simply “mate” is a xanthine-containing nutrient beverage traditionally drunk in various countries of South America. Mate is prepared as an infusion of the dried and minced leaves of *Ilex paraguariensis* (Ip) St. Hilaire (Aquifoliaceae). A choleric effect and enhanced intestinal transit,\(^1\) a prevention of methylglyoxal-induced inhibition of plasminogen and antithrombin III,\(^2\) an endothelium-dependent vasorelaxing activity on mesenteric arterial bed\(^3\) and antioxidant effect\(^4\)–\(^7\) are some of the described beneficial actions of Ip extract. These effects have been attributed to a high content of flavonoids and caffeoyl derivatives detected in this plant.\(^8\)

The burst of reactive oxygen species (ROS) seen at the onset of reperfusion is considered one of key factors in the development of myocardial stunning.\(^9\) Previous studies have found that antioxidant treatment improved postischemic ventricular function.\(^10\)–\(^13\) Taking into account the well known antioxidant properties of Ip, it is interesting to assess whether the “mate” treatment would influence the contractile function after ischemia and reperfusion.

It has also been established that nitric oxide (NO) and protein kinase C (PKC) play an important role in the improvement of postischemic recovery achieved by different cardioprotective interventions.\(^14\)–\(^16\)

In the present paper, we examined the actions of an aqueous extract of Ip on systolic and diastolic alterations induced by ischemia and reperfusion in isolated rat heart, assessing the participation of NO and PKC.

Material and methods

Plant material

Aerial parts of Ip St. Hilaire were collected in Posadas (Misiones, Argentina) in May 1998 and authenticated by Dra. Etile Spegazzini. A voucher specimen (LPE 938) was deposited in the herbarium of the Museo de Botánica y Farmacognosia “Carlos Spegazzini” (Universidad Nacional de La Plata, Argentina).

Preparation of the extract

The extract of Ip was prepared as an infusion. Dried and powdered leaves (10 g) were weighted into a 250 ml Erlenmeyer flask and 100 ml of boiling distilled water was added and left to cool down to 40 °C. After being filtered, the aqueous extract was lyophilized (yield = 9% w/w) and dry matter maintained at −20 °C until used. The extract was dissolved in Ringer’s solution immediately before all test were performed.

Isolated heart preparation


Wistar rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg body wt). The heart was rapidly excised and perfused by the non-recirculating Langendorff technique with Ringer’s solution containing (in mmol/l): 118 NaCl, 5.9 KCl, 1.2 MgSO\(_4\), 1.35 CaCl\(_2\), 20 Na\(_2\)CO\(_3\) and 11.1 dextrose. The buffer was saturated with a mixture of 95% O\(_2\)–5% CO\(_2\), had a pH 7.4, and was maintained at 37 °C. The conductive tissue in the atrial septum was damaged with a fine needle to achieve atrioventricular block, and the right ventricle was paced at 280 ± 10 beats/min. A latex balloon tied to the end of a polyethylene tube was passed into the left ventricle through the mitral valve; the opposite end of the tube was then connected to a Statham P23XL pressure transducer. The balloon was filled with water to provide an end-diastolic pressure (LVEDP) of 8–12 mmHg and this volume was unchanged for the rest of the experiment. Coronary perfusion pressure (CPP) was monitored at the point of cannulation of the aorta and adjusted to approximately 60–70 mmHg. Coronary flow (CF), controlled with a peristaltic pump, was 11 ± 2 ml/min. Left ventricular pressure (LVP) and its first derivative (dP/dt) were recorded with a direct writing recorder.

Experimental protocols

After 10 min of stabilization, the following experimental protocols were performed:

Ischemic control hearts (n = 14): Hearts were subjected to 20 min of normothermic global ischemia followed by 30 min of reperfusion. Global ischemia was induced by stopping the perfusate inflow line and the heart was placed in a saline bath held at 37 °C.

Ip extract (n = 8): Hearts were treated 10 min before ischemia and the initial 10 min of reperfusion with a dose of 0.30 mg/min of Ip. The final concentration of Ip in the perfusate was 30 µg/ml.
Other hearts \((n \equiv 8)\) received 1 \(\mu\)M of chelerythrine, a PKC inhibitor, 10 min before ischemia or 1 mM of \(L^\ominus\)-nitro-L-arginine methyl ester (\(L\)\-NAME) \((n \equiv 6)\), a non-selective nitric oxide synthase (NOS) inhibitor, 20 min before ischemia and during the reperfusion period. The \(L\)\-NAME administration was extended through the entire reperfusion time to prevent the activation of the different NOS isoforms.

Systolic and diastolic function

Myocardial contractility was assessed by the left ventricular developed pressure (L VDP), obtained subtracting LVEDP values to the LVP peak values and maximal velocity of contraction \(+dP/dt_{max}\) values. Data were expressed as percentage of their respective preischemic values. The diastolic function was evaluated through the maximal velocity of relaxation \((-dP/dt_{max})\) and LVEDP values. Coronary resistance was calculated as a quotient between CPP and CF.

Assessment of lipid peroxidation

At the end of reperfusion hearts were homogenized in physiological saline solution. After samples were centrifuged and the content of thiobarbituric reactive substances (TBARS) in the supernatant was determined by a spectroscopic technique.\(^\text{17}\) The absorbance at 535 nm was measured and TBARS expressed in nmol MDA/g tissue weight using an extinction coefficient of \(1.56 \times 10^5 M^{-1} cm^{-1}\).

Statistical analysis

Data presented as means \pm SE and repeated measures of one-way analysis of variance (ANOVA) with the Newman–Keul’s test used for multiple comparisons among groups. A \(P\) value < 0.05 were considered significant.

Results

Figure 1 (upper panel) shows the effects in our preparation of 20 min of global ischemia followed by 30 min of reperfusion. In ischemic control hearts a decrease in L VDP to values of 57 \pm 6\% from baseline was detected at the end of the reperfusion period. The infusion of 30 \(\mu\)g/ml of Ip extract improved postsischemic recovery, reaching L VDP values near 100\%. Figure 1 (lower panel) shows that PKC blockade with chelerythrine did not modify whereas NO inhibition with \(L\)\-NAME abolished the protective effect of Ip on systolic function.

Similar pattern was obtained when \(+dP/dt_{max}\) values were analyzed. Figure 2 (upper panel) shows that Ip extract improved this parameter of contractility reaching at the end of reperfusion period values higher than those observed in untreated hearts (95 \pm 10\% vs. 53 \pm 6\%). The improvement of \(+dP/dt_{max}\) after Ip treatment was preserved when PKC was blocked but it was lost by NOS inhibition (Fig. 2, lower panel).

Examining \(-dP/dt_{max}\) an improvement of relaxation velocity after treatment with Ip was evident (90 \pm 12\% vs. 57 \pm 8\% in ischemic control hearts) (Fig. 3, upper panel). This beneficial effect on relaxation was not modified by chelerythrine treatment whilst it was lost when NO synthesis was inhibited (Fig. 3, lower panel). The increase of diastolic stiffness detected during reperfusion in ischemic control hearts was significantly attenuated by Ip treatment (LVEDP = 20 \pm 6 mmHg vs.}
42 ± 4 mmHg at the end of reperfusion period (Fig. 4, upper panel). The PKC blockade did not change and NOS inhibition abolished the improvement of diastolic stiffness conferred by Ip (Fig. 4, lower panel).

The Ip treatment did not produce the increase of coronary resistance detected in ischemic control hearts. This vascular protection was not affected by PKC blockade but it was lost by L-NAME administration (Fig. 5).

TBARS content detected during the reperfusion period in ischemic control hearts was significantly diminished when hearts were treated by Ip extract. PKC blockade did not modify whereas NOS inhibition decreased the attenuation of lipid peroxidation obtained after Ip treatment (Fig. 6).

Discussion

The present study describes for the first time the protection conferred by an Ip extract against systolic and diastolic alterations of myocardial stunning in isolated rat heart. Other important findings in this paper are an attenuation of coronary resistance increase and lipid peroxidation of cardiac tissue, both events induced by ischemia and reperfusion.

The heart needs oxygen avidly and it is susceptible to oxidative stress, which occurs during post-ischemic reperfusion. The formation of ROS is increased at the beginning of reperfusion, depending on the duration and severity of the preceding ischemia. With ischemia, the antioxidant defenses are eroded and a new danger exists as elevated H2O2 become increasingly capable of generating the destructive hydroxyl radical. Oxidative stress, in turn, causes oxidation of thiol groups and lipid peroxidation leading first to reversible damage, and eventually to necrosis and/or apoptosis. The administration of antioxidants is able to limit the evolution of myocardial damage reducing ROS-induced lipid peroxidation.
Recently, we demonstrated that Ip extract is a potent inhibitor of enzymatic and non-enzymatic lipid peroxidation in rat liver microsomes. In the present study, the Ip treatment decreased significantly the lipid peroxidation of heart, indicating that Ip extract is protecting to myocardial tissue against ROS-dependent damage produced by ischemia and reperfusion.

In addition to physiological actions, NO has been involved as mediator of the cardioprotection against ischemia-reperfusion injury. In this study, the attenuation of systolic and diastolic dysfunction conferred by Ip treatment was abolished when NO production was inhibited, indicating that NO is involved in the events that lead to the protection.

The interaction between NO and superoxide anion plays an important role in vascular pathophysiology. Endothelial function is controlled by a balance between the production of NO and ROS. In states in which NO production is not altered, its bioavailability may be reduced because of oxidative inactivation by excessive production of superoxide anion in the vascular wall. During ischemia and reperfusion situations in which oxidative stress and increase of NO production occur, in addition to myocardial dysfunction, a coronary microvascular dysfunction (called “endothelial stunning”) takes place. In this study, a decrease of coronary resistance after Ip treatment and its abolishment when NOS were inhibited indicates that NO is also the possible mediator of the vasorelaxing effect obtained with Ip extract. However, from our data we could not distinguish whether the observed protective action of “mate” extract is due to a direct stimulation of NOS and/or
to an indirect effect through a diminution of ROS content by its recognized scavenger activity.

An increase of cGMP, a reduction of mitochondrial calcium uptake and an increase of mitochondrial K\(_{\text{ATP}}\) channel open probability have been suggested as possible mediators of the cardioprotection achieved by NO.

In a previous paper we demonstrated that PKC plays a crucial role in the heart protection afforded by the application of one cycle of ischemia and reperfusion before a 20-min ischemic period (ischemic preconditioning, IP). In the present study, the improvement obtained after IP treatment was not modified when PKC was inhibited, suggesting that the cardioprotective action achieved by IP extract is not mediated by PKC activation. Thus, we showed that despite the fact that IP treatment and IP are protecting the postischemic function in a similar magnitude, both interventions involve different mechanisms.

In conclusion, IP extract compounds cause a significant attenuation of endothelial and myocardial stunning and lipid peroxidation. These beneficial effects are mediated through pathways that involve the NO participation.

References


