JPP Journal of Pharmacy and Pharmacology

JPP 2002, 54: 1399–1405

Received April 10, 2002
Accepted June 14, 2002
ISSN 0022-3573

Evodia rutaecarpa protects against circulation failure and organ dysfunction in endotoxaemic rats through modulating nitric oxide release

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Abstract

Using a rat model of septic shock we studied the effects of *Evodia rutaecarpa*, a Chinese herbal medicine with antimicrobial and anti-inflammatory activity, on haemodynamic parameters, biochemical markers of organ function and nitric oxide (NO) production. Anaesthetized rats challenged with a high dosage of endotoxin (*Escherichia coli* lipopolysaccharide; LPS; 50 mg kg^{-1}, i.v.) for 6 h showed a severe decrease in mean arterial pressure. This was accompanied by delayed bradycardia, vascular hyporeactivity to phenylephrine and increase in plasma levels of lactate dehydrogenase, aspartate aminotransferase, bilirubin and creatinine, as well as NOx (NO_2^- plus NO_3^-). Pretreatment with ethanol extract of *E. rutaecarpa* (25, 50 and 100 mg kg^{-1}, i.v.), 1 h before LPS, dose-dependently prevented the circulation failure, vascular hyporeactivity to phenylephrine, prevented liver dysfunction and reduced the NOx over-production in plasma in endotoxaemic rats. A selective inducible NO-synthase (iNOS) inhibitor, aminoguanidine (15 mg kg^{-1}, i.v.), also effectively ameliorated the above pathophysiological phenomenon associated with endotoxaemia so that the normal condition was approached. Endotoxaemia for 6 h resulted in a significant increase in iNOS activity in the liver homogenate, which was attenuated significantly by *E. rutaecarpa* pretreatment. In summary, *E. rutaecarpa*, at the dosages used, exerted these beneficial effects probably through inhibition of iNOS activity and subsequent modulation of the release of NO. These significant results may offer *E. rutaecarpa* as a candidate for the treatment of this model of endotoxaemia.

Introduction

Sepsis syndrome is a complex disease mostly caused by overwhelming bacterial infection. Studies have been conducted using antibacterial agents to prevent or treat sepsis. Recently, interest has been focused on the role of nitric oxide (NO) in septic shock. Large amounts of NO lead to profound vasodilatation and hyporesponsiveness to vasoconstrictors. The cytotoxic effect of NO could also cause tissue injury and organ failure (Hardaway 2000). The recent discovery of the various biological functions of NO has allowed the development of new concepts about the pathophysiology of septic shock, and has provided the bases to design novel therapeutic strategies for the treatment of septic shock, based on the inhibition of NO synthesis (Kilbourn 1999).

Wu-Chu-Yu, the unripe fruit from *Evodia rutaecarpa*, has long been utilized in traditional Chinese medicine for the treatment of bacterial infection (e.g., pneumonia bacteria) and inflammation-related disorders such as eczema, ulcerative stomatitis, etc. (Chang & But 1987). Extracts of *E. rutaecarpa* and its chemical constituents have been shown to display a range of biological activity, such as antinociception (Matsuda et al 1998), anti-inflammation (Chang & But 1987), immune stimulation (Chang et al 1995), and NO inhibition (Chiou et al 1997). Furthermore, the ethanol extract of *E. rutaecarpa* was found to inhibit endotoxin-stimulated NO production in RAW264.7 macrophages (unpublished data). Therefore, it is plausible to hypothesize that *E. rutaecarpa* may have beneficial effects in palliating the severity of sepsis and associated complications. To evaluate the potential therapeutic efficacy and clinical utility of *E. rutaecarpa*, acute

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Funding: This investigation was supported by grant NSC90-2320-B-077-008 to Prof. C. F. Chen from the National Science Council and in part from the National Research Institute of Chinese Medicine, Taipei, Taiwan, Republic of China.

septic insults to rats were provoked by an intravenous bolus of endotoxin. Then, the haemodynamic parameters (blood pressure and heart rate), vascular reactivity to vasoconstrictors, biochemical markers of organ function, and NO level in the plasma before and after *E. rutaecarpa* treatment were evaluated.

Materials and Methods

Chemicals

Lipopolysaccharide (LPS; *Escherichia coli* serotype 0111:B4), NaCl, KCl, KH_2PO_4, $MgSO_4$, $CaCl_2$, $NaHCO_3$, glucose, acetylcholine HCl, indometacin, phenylephrine HCl, sulfanilamide, *N*-naphthylenediamine, EDTA, EGTA, Tris-HCl, phenylmethylsulfonyl fluoride and 2-mercaptoethanol were all obtained from Sigma Chemical Company (St Louis, MO). L-[^{3}H]Arginine and L-[^{35}S]methionine were obtained from Amersham (Buckinghamshire, UK).

Preparation of the ethanol extract of *Evodia rutaecarpa*

Dried *Evodia rutaecarpa*, purchased from a local drug store and identified by Mr C. J. Chou (Fellow in Pharmacognosy, Principal Investigator in National Research Institute of Chinese), was ground into powder, mixed with 50% ethanol and stirred continuously at 70°C overnight. After removal of the solid residues by filter paper, the solution was spun at 30 000 *g* for 20 min. The remaining supernatant was further filtered through 0.45-μm millipore filter paper, concentrated under vacuum and lyophilized to afford an ethanolic extract of *E. rutaecarpa*. It was dissolved in normal saline immediately before experiments and was used within 1 week of preparation.

Endotoxaemic rats

All experiments were performed in accordance with the Guidelines for Animal Experiments of the National Research Institute of Chinese Medicine. Food was withheld for a period of 12–15 h before the experiment. Adult male Sprague–Dawley rats (250–300 g) were anaesthetized with sodium pentobarbital (50 mg kg^{-1}, i.p.). Upon completion of the surgical procedure, cardiovascular parameters were allowed to stabilize for 20–30 min. After recording baseline haemodynamic parameters, rats were intravenously treated with either vehicle (saline, control), ethanol extract of *E. rutaecarpa* (25 ~ 100 mg kg^{-1}) or a selective inducible nitric oxide synthase (iNOS) inhibitor (aminoguanidine, 15 mg kg^{-1}), 1 h before endotoxin (*Escherichia coli* LPS). Then rats received LPS (50 mg kg^{-1}, i.v.) as a slow injection over 10 min. The cardiovascular parameters were continuously monitored for 6 h. Normal rats receiving saline only served as the control group (NS-rats). Rats that received LPS as mentioned above were represented as LPS-rats.

Organ bath experiments

At 6 h after endotoxaemia, thoracic aortae were obtained from the rats. After clearing of adhering fat, the thoracic aortae were cut into rings of 3–4 mm length and mounted in organ baths filled with warmed (37°C), oxygenated (95% O_2/5% CO_2) Krebs' solution (pH 7.4) consisting of (mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.17, $CaCl_2$ 2.5, $NaHCO_3$ 25 and glucose 5.6. The endothelium was removed by gently rubbing the intimal surface and was considered denuded when 3×10^{-6} M of acetylcholine failed to relax the phenylephrine-precontracted rings. Indometacin (10^{-5} M) was added to prevent the production of prostanoids. A cumulative concentration–response curve to phenylephrine was obtained for each group of rings. All results were expressed as mg contractile tension per mg tissue wet weight.

Quantification of organ dysfunction and injury

Six hours after the injection of LPS, 1.5 mL of blood was collected from a catheter placed in the left femoral vein. The blood sample was centrifuged (800 *g* for 15 min) to separate serum. All plasma samples were measured within 24 h by a clinical biochemical analyser (FUJI DRI-CHEM 3000, Japan). The following marker enzymes were measured in the plasma as biochemical indicators of multiple organ injury or dysfunction: cell disruption was assessed by measuring the levels of lactate dehydrogenase (LDH) (Bakker et al 1996); liver injury was assessed by measuring the rise in serum levels of aspartate aminotransferase (AST, a non-specific marker for hepatic injury) and bilirubin (a specific indicator of liver dysfunction) (Baue 1993; Hewett et al 1993); renal dysfunction was assessed by measuring the rise in serum levels of creatinine (an indicator of reduced glomerular filtration rate, and hence, renal failure).

Measurement of plasma Nox

Nitrite (NO_2^-) and nitrate (NO_3^-) are the primary oxidation products formed when NO reacts with oxygen and, therefore, the nitrogen oxides (NOx; NO_2^- plus NO_3^-) concentration in plasma can be used as an indicator of NO synthesis. NO_2^- and NO_3^- in serum were separated by a reverse-phase separation column packed with polystyrene polymer (NO-PAK, 4.6×50 mm, Eicom), and NO_3^- was reduced to NO_2^- in a reduction column packed with copper-related cadmium filings (NO-RED, Eicom). NO_2^- was mixed with a Griess reagent to form a purple azo dye in a reaction coil. The separation and reduction columns and the reaction coil were placed in a column oven that was set at 35°C. The absorbance of the product dye at 540 nm was measured by a flow-through spectrophotometer (NOD-20, Eicom). The mobile phase, which was delivered by a pump at a rate of 0.33 mL min^{-1}, was 10% methanol containing 0.15 M NaCl-NH_4Cl and 0.5 g L^{-1} EDTA-4Na. The Griess reagent, which was 1.25% HCl containing 5 g L^{-1} of sulfanilamide with 0.25 g L^{-1} *N*-naphthylethylene-diamine, was delivered at a rate of 0.1 mL min^{-1}. Total NO metab-

olite levels (NOx) were calculated as the sum of nitrite and nitrate levels.

Nitric oxide synthase activity assay

Livers were removed at 6 h after endotoxaemia and frozen in liquid nitrogen. Livers were stored for no more than 2 weeks at −80°C before assay. Frozen livers were homogenized on ice with a homogenizer in buffer (Tris-HCl 50 mM, EDTA 0.1 mM, EGTA 0.1 M, 2-mercaptoethanol 12 mM and phenylmethylsulfonyl fluoride 1 mM, pH 7.4). Conversion of [^{3}H]-L-arginine to [^{3}H]-L-citrulline was measured in the homogenates as described previously (Chiou et al 2000).

Statistics

All data are expressed as mean±s.e.m. of n observations. Student's *t*-test was used to determine the significance of difference for means. $P < 0.05$ was considered statistically significant. For simultaneous multiple comparisons, the statistical significance of differences between groups was analysed by one-way analysis of variance followed by Dunnett's multiple comparisons test; n represents observed animal number or the tested sample number.

Results

Effects of *E. rutaecarpa* on haemodynamic parameters in normal rats

Baseline values (T_0) for mean arterial pressure and heart rate were not significantly different between vehicle-, *E. rutaecarpa*- and aminoguanidine-pretreated rats (Table 1).

Table 1 Alterations in mean arterial pressure and heart rate in normal rats that received vehicle, *Evodia rutaecarpa* or aminoguanidine alone measured at baseline (T_0), 3 h (T_3) and 6 h (T_6) after drug administration.

	T_0	T_3	T_6
Mean arterial pressure (mmHg)			
Vehicle (n = 4)	96±2	104±8	89±6
Evodia rutaecarpa 100 mg kg^{-1} (n = 6)	99±4	110±6	105±4
Aminoguanidine (15 mg kg^{-1}) (n = 6)	94±3	105±7	97±7
Heart rate (beats/min)			
Vehicle (n = 4)	379±9	381±8	383±5
Evodia rutaecarpa 100 mg kg^{-1} (n = 6)	385±7	391±9	401±11
Aminoguanidine 15 mg kg^{-1} (n = 6)	390±5	393±11	391±4

Data are expressed as mean±s.e.m. of n observations.

In rats receiving saline vehicle only, mean arterial pressure and heart rate remained stable until 6 h. *E. rutaecarpa* and a selective iNOS inhibitor, aminoguanidine, when injected into rats, had no significant effect on the haemodynamic parameters during the 6-h observation period.

Effect of *E. rutaecarpa* on the circulatory failure caused by LPS in anaesthetized rats

A high dosage of LPS (50 mg kg^{-1}, i.v.) caused severe circulatory shock in the rats. As shown in Figure 1, LPS caused a rapid (within 30 min) but mild fall in mean arterial pressure that was sustained for 2 h (from 97±4 mmHg to 87±3 mmHg). After 3 h, there was a second and substantial further fall in mean arterial pressure to 43±4 mmHg until 6 h. This delayed hypotension was associated with a significant drop in heart rate observed starting at 5 h. Endotoxaemic rats that had been pretreated with *E. rutaecarpa* (100 mg kg^{-1}) 1 h before LPS maintained significantly higher mean arterial pressure values. The delayed bradycardia was also prevented by *E. rutaecarpa*. All rats challenged with LPS survived until 6 h.

Table 2 summarizes the haemodynamic changes measured at baseline (T_0) and at 6 h after LPS administration (T_6) in endotoxaemic rats pretreated with vehicle (normal saline), *E. rutaecarpa* or aminoguanidine. Both mean arterial pressure and heart rate were significantly reduced at 6 h (T_6) after endotoxaemia and these changes were blunted dose-dependently by *E. rutaecarpa* (25, 50 and 100 mg kg^{-1}) treatment. On the other hand, endotoxaemic rats that had been pretreated with aminoguanidine also maintained significantly higher mean arterial pressure values when compared with rats treated with vehicle. The negative chronotropic effect of LPS was somewhat attenuated by aminoguanidine, but this was not statistically significant.

E. rutaecarpa attenuates the LPS-induced vascular hyporeactivity to phenylephrine ex-vivo

In endothelium-denuded aortic rings obtained from normal rats (receiving normal saline for 6 h, n = 6), phenylephrine (10^{-9} to 10^{-5} M) caused a dose-dependent increase in vascular tone (Figure 2). Phenylephrine-evoked contractions were dramatically suppressed in aortic rings obtained from endotoxaemic rats (LPS-rats; n = 7) after 6 h of endotoxaemia as compared with normal rats (NS-rats; $P < 0.05$ at 3×10^{-9} M, $P < 0.01$ at 10^{-8} to 10^{-5} M). Pretreatment of LPS-rats with *E. rutaecarpa* (100 mg kg^{-1}) significantly reversed the vascular hyporeactivity to phenylephrine ex-vivo ($P < 0.01$, n = 8) as compared with LPS-rats. However, the contractions were still less than in NS-rats ($P < 0.01$ at 3×10^{-8} to 10^{-5} M). Treatment of LPS-rats with aminoguanidine (15 mg kg^{-1}, n = 6) also significantly enhanced the diminished contractile response to phenylephrine. However, the ameliorating effect by aminoguanidine was less pronounced than *E. rutaecarpa*. Treatment of

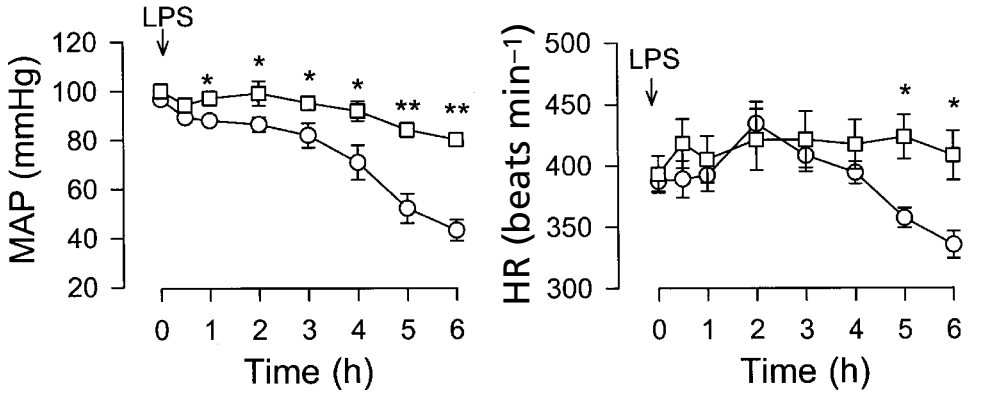

Figure 1 Time-course of mean arterial pressure (MAP) and heart rate (HR) changes in endotoxaemic rats that had received either vehicle (○, normal saline, n = 6) or *Evodia rutaecarpa* (□, 100 mg kg^{-1}, n = 7) 1 h before LPS (50 mg kg^{-1}, i.v.). Data are expressed as mean±s.e.m. $^{*}P < 0.05$ and $^{**}P < 0.01$, compared with control.

Table 2 Alterations in mean arterial pressure and heart rate in endotoxaemic rats pretreated either with vehicle, *Evodia rutaecarpa* or aminoguanidine 1 h before LPS (50 mg kg^{-1}, i.v.).

	T_0	T_6
Mean arterial pressure (mmHg)		
Vehicle (n = 6)	93±5	48±4
Evodia rutaecarpa 25 mg kg^{-1} (n = 6)	95±4	64±5*
Evodia rutaecarpa 50 mg kg^{-1} (n = 6)	100±4	77±6**
Evodia rutaecarpa 100 mg kg^{-1} (n = 6)	96±2	84±2**
Aminoguanidine 15 mg kg^{-1} (n = 6)	91±3	69±6**
Heart rate (beats/min)		
Vehicle (n = 6)	383±14	335±9
Evodia rutaecarpa 25 mg kg^{-1} (n = 6)	387±6	364±6*
Evodia rutaecarpa 50 mg kg^{-1} (n = 6)	393±11	375±11*
Evodia rutaecarpa 100 mg kg^{-1} (n = 6)	400±13	408±20**
Aminoguanidine 15 mg kg^{-1} (n = 6)	407±11	356±9

Haemodynamic parameters were measured at baseline (T_0) and at 6 h after LPS (T_6) administration. Data are expressed as mean±s.e.m. of n observations. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with vehicle at the same time point.

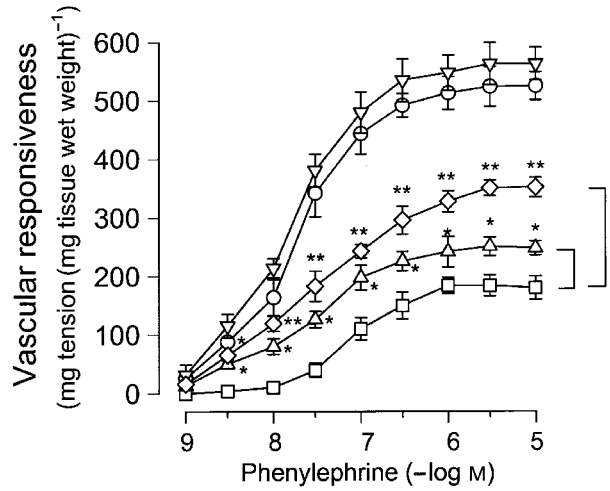

Figure 2 Effect of *Evodia rutaecarpa* and aminoguanidine on the endotoxin-induced vascular hyporeactivity to phenylephrine in rat endothelium-denuded aortic rings ex-vivo. Results are concentration–response curves to phenylephrine (10^{-9} to 10^{-5} M) obtained from normal-saline-treated rats (○, NS-rats, n = 6), endotoxaemic rats (□, LPS-rats, n = 7), and two groups of rats pretreated either with *Evodia rutaecarpa* (◇, 100 mg kg^{-1}, n = 8) or aminoguanidine (△, 15 mg kg^{-1}, n = 6) 1 h before LPS (50 mg kg^{-1}, i.v.), respectively. Data from rats treated with normal saline and *Evodia rutaecarpa* are also shown (▽). Data are expressed as mean±s.e.m. $^{*}P < 0.05$ and $^{**}P < 0.01$, compared with LPS-rats.

NS-rats with aminoguanidine did not alter the contractile responses to phenylephrine (data not shown).

Effect of *E. rutaecarpa* on plasma NOx induction and multiple organ dysfunction caused by LPS

Table 3 illustrates the changes in plasma NOx, LDH, AST, bilirubin and creatinine concentrations measured at baseline (T_0) and at 6 h after LPS (T_6) administration in rats pretreated either with vehicle (normal saline) or *E. rutaecarpa* in endotoxaemic rats, respectively. In endotoxaemic rats receiving vehicle, plasma NOx significantly increased from 13±2 μM at T_0 to 557±30 μM measured at 6 h (T_6) after LPS. *E. rutaecarpa* alone had no significant effect on NOx concentrations measured at T_0, however, it dose-dependently counteracted the NO-stimulating effect of LPS in endotoxaemic rats measured at T_6.

All biochemical variables significantly increased in all groups at 6 h (T_6) after endotoxaemia. The increased plasma levels of LDH and AST after endotoxaemia for 6 h were prevented by *E. rutaecarpa*. However, the difference was statistically significant only starting at 50 mg kg^{-1} of

Table 3 Alterations in plasma levels of NOx ($NO_2^- + NO_3^-$), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), bilirubin and creatinine in endotoxaemic rats pretreated either with vehicle, *Evodia rutaecarpa* or aminoguanidine 1 h before LPS (50 mg kg^{-1}, i.v.).

	NOx (μM)		LDH (U $dL^{-1} \times 100$)		AST (U dL^{-1})		Bilirubin (mg $dL^{-1} \times 100$)		Creatinine (mg $dL^{-1} \times 100$)	
	T_0	T_6	T_0	T_6	T_0	T_6	T_0	T_6	T_0	T_6
Vehicle (n = 6)	13±2	557±30	18±4	80±8	6±5	124±17	40±17	191±35	42±10	112±21
Evodia rutaecarpa										
25 mg kg^{-1} (n = 6)	15±3	387±54*	17±3	71±3	12±9	116±23	35±15	89±22*	39±5	93±8
50 mg kg^{-1} (n = 6)	14±2	362±19**	16±6	58±6*	11±4	90±10*	41±13	74±16**	36±8	89±13
100 mg kg^{-1} (n = 6)	11±3	234±7**	18±5	37±8**	10±6	66±5**	48±23	52±12**	43±9	82±10
Aminoguanidine										
15 mg kg^{-1} (n = 6)	13±4	378±46*	16±3	62±5	6±4	79±28*	31±15	86±25*	35±6	87±11

Biochemical markers were measured at baseline (T_0) and at 6 h after LPS (T_6) administration. Data are expressed as mean±s.e.m. of n observations. *$P < 0.05$ and **$P < 0.01$, compared with vehicle at the same time point.

E. rutaecarpa. Indeed, the plasma level of bilirubin was more sensitive to a lower dose of *E. rutaecarpa* extract (25 mg kg^{-1}). Although *E. rutaecarpa* also blunted the increased plasma levels of creatinine at 6 h after endotoxaemia, this effect was not statistically significant.

Effect of aminoguanidine on plasma NOx and biochemical parameters of organ function

As shown in Table 3, after 6 h of endotoxaemia (T_6) a striking increase in plasma NOx and the biochemical markers of organ function were observed as compared with T_0. Aminoguanidine (15 mg kg^{-1}), when administered 1 h before LPS, substantially suppressed the NOx levels in endotoxaemic rats observed at T_6 as compared with the vehicle group. Evaluating the effect of aminoguanidine on multiple organ function in endotoxaemic rats also showed a marked reduction in plasma concentrations of biochemical markers. As shown in Table 3, LPS-induced increases in plasma AST and bilirubin were significantly less in aminoguanidine-pretreated rats, while amelioration of the effect of aminoguanidine on LDH and creatinine was not statistically significant.

E. rutaecarpa inhibits iNOS activity in endotoxaemic rats

Results obtained from organ function study indicated that *E. rutaecarpa* was most effective in improving liver damage in endotoxaemic rats. Thus, liver tissue was chosen to further assay the iNOS activity before and after *E. rutaecarpa* treatment. A small but detectable background level of iNOS activity in liver homogenates obtained from NS-rats was 0.18±0.07 pmol min^{-1} (mg protein)$^{-1}$. After 6 h of endotoxaemia, there was a substantial increase in iNOS activity in liver homogenates (Figure 3). Pretreating the LPS-rats with *E. rutaecarpa* significantly reduced the iNOS activity by 33.4±5.6% ($P < 0.05$).

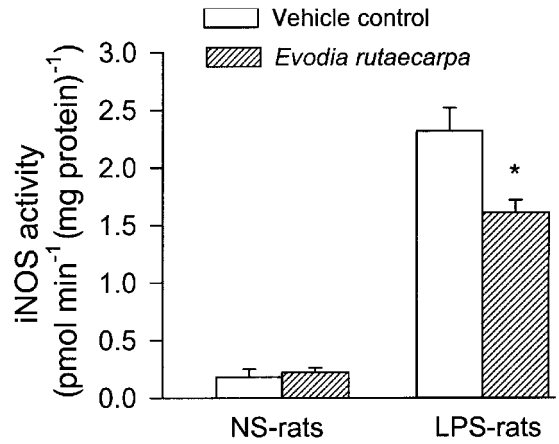

Figure 3 Effect of *Evodia rutaecarpa* on iNOS activity in liver homogenates obtained form normal rats receiving vehicle (NS-rats, n = 6) or subjected to 6 h of endotoxaemia (LPS-rats, n = 6) ex-vivo. *Evodia rutaecarpa* (100 mg kg^{-1}, i.v.) was administered 1 h before LPS (50 mg kg^{-1}, i.v.). Data are expressed as mean±s.e.m. *$P < 0.05$, compared with vehicle control.

Discussion

This study demonstrates that *Evodia rutaecarpa*, a Chinese herbal medicine with anti-inflammatory and antimicrobial activity, has a protective effect against the delayed circulatory failure associated with endotoxic shock. In particular, we show that *E. rutaecarpa* attenuates the fall in blood pressure and heart rate and the vascular hyporeactivity to phenylephrine elicited by prolonged periods of endotoxaemia in rats. Furthermore, the beneficial effects of *E. rutaecarpa* were associated with an inhibition of plasma levels of LDH, AST, bilirubin and NOx.

Arterial blood pressure followed a biphasic time course after LPS administration, with an initial acute drop followed by a progressive decline until the end of the experiments. In fact, NOx formation was already significantly observed in the early phase (increasing from basal levels to

195.3±21.5 μM within 3 h) after exposure to a high dosage of endotoxin (50 mg kg^{-1}). The delayed hypotension was associated with a further rise in plasma NOx, suggesting a significant activation of the L-arginine : NO pathway during endotoxaemia (Schoonover et al 2000). Effective prevention by aminoguanidine in the reduced blood pressure also confirmed that NO has a clear detrimental effect in endotoxaemia.

The bradycardia produced by endotoxin may reflect altered baroreceptor responses (Karzai et al 1995), increased vagal activity (Halinen 1976) and a reduced chronotropioc response to β-adrenoceptor agonists (Parratt 1973). Besides, NO decreases the spontaneous beating rate of cardiac myocytes (Balligand et al 1993) and isolated cells from cardiac pacemaker tissue (Han et al 1994), by reducing the L-type calcium current in these cells. An enhanced NO production following iNOS expression has been shown to reduce the beating rate of cardiac myocytes exposed to interleukin-1β or other cytokines (Kumar et al 2001). Additionally, excess NO may indirectly reduce heart rate in endotoxaemic conditions by inhibiting both the release (Schwarz et al 1995) and the biological activity (Macarthur et al 1995) of catecholamines. Treatment with *E. rutaecarpa* maintained heart rate at the normal level. Our results showed that a decrease in plasma NOx was also observed with *E. rutaecarpa* treatment. In view of the negative chronotropic action of NO found in previous studies, we speculate that *E. rutaecarpa* may prevent bradycardia through modulating NO release.

Evidence in the literature suggests that induction of iNOS activity by LPS causes prolonged production of large amount of NO that contributes significantly to the vascular hyporeactivity characteristic of endotoxic shock (Thiemermann & Vane 1990; Erol & Kosay 2000). In this regard, experiments examining the role of NO in the decreased vascular contractility in endotoxaemic rats have used aminoguanidine which shown some beneficial effects in this study. Here we showed that pretreatment of LPS-rats with aminoguanidine also attenuated the vascular hyporeactivity to phenylephrine in aortic rings ex-vivo, suggesting a possible mechanistic link to NO inhibition.

A further effect of *E. rutaecarpa* was a marked reduction in the LPS-induced cell disruption assessed by measuring the levels of lactate dehydrogenase (LDH) and a tendency to limit the development of liver injury and dysfunction caused by endotoxin. *E. rutaecarpa* at the dosage used, however, did not significantly affect the rise in the serum levels of creatinine and, hence, the renal injury caused by endotoxin in the rats. We predict that a pronounced effect might be observed if the dosage of *E. rutaecarpa* was further increased. An increase in the plasma levels of LDH demonstrated the development of tissue hypoxia, presumably secondary to impairment in tissue oxygen extraction (Baue 1993). Animal models of sepsis and humans with septic shock have also demonstrated increased markers of oxidant stress (Goode & Webster 1993; Goode et al 1995). NO is able to inhibit mitochondrial function, either directly or through interaction with free radicals resulting in the formation of peroxynitrite ($ONOO^-$) and causing further enzymatic dysfunction and cellular damage (Huie & Padmaja 1993; Zingarelli et al 1997). Since hyperlactataemia may reflect the development of tissue anaerobiosis, one may hypothesize that *E. rutaecarpa* partially corrected the endotoxin-induced tissue dysoxia, but this issue remains speculative. A distinct possibility is that of an improvement in tissue perfusion due to the higher blood pressure and heart rate afforded by *E. rutaecarpa*. Furthermore, serum nitrate (an index for the systemic production of NO) correlated well with multiple organ failure score in a recent study of critically ill patients, implying that NO is somehow associated with the pathophysiology of multiple organ failure (Groeneveld et al 1996; Deitch & Goodman 1999). These abnormalities were significantly blunted by *E. rutaecarpa*, which might reflect a reduction in NO-mediated cytotoxicity.

What, then, is the mechanism by which *E. rutaecarpa* affords the beneficial effects on liver function in endotoxic shock in this study? Clearly, *E. rutaecarpa* attenuates the iNOS activity in the liver of rats subjected to prolonged periods of endotoxaemia. *E. rutaecarpa* seems not to act through inhibition of endothelial NOS, for this extract did not cause an increase in blood pressure in control rats. It has been proposed that the maintenance of a basal NO synthesis is critical for organ perfusion and survival in endotoxic shock and that the deleterious effects of NOS inhibitors (such as L-NAME) might be related to the blockade of constituted NOS (Wright et al 1992). Furthermore, selective inhibition of NO synthesis by iNOS inhibitors not only produces beneficial haemodynamic and metabolic effects, but also improves the survival rate in rodent endotoxic shock (Liaudet et al 1996). Thus, the ameliorating effect on circulatory failure by *E. rutaecarpa* may be attributable to selective interference of iNOS induction.

Increasing evidence suggests that liver failure in endotoxaemia largely involves tumour necrosis factor (TNF) that contributes to early apoptosis followed by necrosis (Nowak et al 2000). Thus, there has been considerable interest in the possibility of using monoclonal antibodies to TNF, or agents that inhibit the release of TNF, in the treatment of organ failure associated with severe sepsis. Considerable experimental evidence suggests that *E. rutaecarpa*, at high concentration ranges, has an inhibitory effect on the secretion of TNF by mononuclear cells (Chang et al 1995). This effect of *E. rutaecarpa* may, to some extent, provide a scientific basis for its clinical application in the treatment of sepsis.

Results of this study indicate that the beneficial effects of *E. rutaecarpa* take place, at least in part, through NO modulation. However, *E. rutaecarpa* is a mixture containing a diverse number of constituents, thus we predict that other actions may be involved. Many chemical constituents of *E. rutaecarpa* have been identified, including alkaloids, limonoids and flavonoids (Chang & But 1987). Flavonoids are naturally occurring plant products that when consumed medicinally, have mild (if any) side-effects and are, therefore, referred to as tender drugs. They demonstrate various types of pharmacological activity in-vitro and in-vivo, including anti-lipid peroxidation and anti-inflammatory activity. Additionally, flavonoid pretreat-

ment has been reported to protect mice from lethal shock induced by endotoxin by reducing the serum tumour TNF-α level and the plasma level of lipid peroxides (Takahashi et al 2001). Further separation and identification of active components in ethanol extract of *E. rutaecarpa* is underway.

Conclusion

In conclusion, this study demonstrated that *Evodia rutaecarpa* prevents circulatory failure, liver dysfunction and membrane disturbance in rats with endotoxic shock. The mechanisms by which it exerts these beneficial effects is uncertain and warrants further investigation, but may involve, partly, the prevention of NO over-production. We propose that *Evodia rutaecarpa* may be useful in the therapy of circulatory shock or of disorders associated with inflammation.

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