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Pharmacological Effects of Rutaecarpine, an Alkaloid Isolated from *Evodia rutaecarpa*

Joen-Rong Sheu

Graduate Institute of Medical Sciences & Department of Pharmacology,, Taipei Medical College, Taipei, Taiwan

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INTRODUCTION

Chinese herbs have been widely used as important remedies in oriental medicine and in recent decades many biologically active constituents of selected herbs have been isolated and evaluated for their pharmacological activity. *Evodia rutaecarpa* (Chinese name: Wu-Chu-Yu) is a well-known traditional Chinese medicine that has been used for a long time in Chinese medical practice. The dried unripened fruit of *Evodia rutaecarpa* is used as a remedy for gastrointestinal disorders (abdominal pain, dysentery), headache, amenorrhea, and postpartum hemorrhage (1). It has also been claimed to have a remarkable central stimulant effect (1), a transient hypertensive effect (1,2), and positive inotropic and chronotropic effects (3). In phytochemical studies a wide variety of compounds, including alkaloids, were found in the fruits of this plant. The alkaloid constituents of this fruit include rutaecarpine, evodiamine, wuchuyine, hydroxyevodiamine (rhesinine), dehydroevodiamine, evocarpine, 1-methyl-2-pentadecyl-4-(1H)-quinolone,1-methyl-2-tridecyl-4-(1H)-quinolone (dihydroevocarpine), 1-methyl-2-undecyl-4-(1H)-quinolone, dihydrorutaecarpine, and 14-formyldihydrorutaecarpine (4). Non-alkaloid constituents include rutaevin, limonin (evodin), evodol, evodinone, evogin, gushuyic and other fatty acids (5). Several of these components are known to possess pharmacological activity. For example, dehydroevodiamine, which is formed by the reduction of evodiamine, induces hypotension, bradycardia, and vasodilation, and it has antiarrhythmic activity (6,7). Evodiamine has a positive inotropic effect on isolated left atria from guinea pigs (8) and an antianoxic action in KCN-induced anoxia in mice (9). The cardiovascular effects of dehydroevodiamine and evodiamine have been reported previously (6,7,10,11). Recently Chiou et al (11) reported that rutaecarpine has a vasodilator effect on isolated rat mesenteric arteries by an endothelium- and nitric oxide (NO)-dependent mechanism. We found that rutaecarpine inhibits aggregation of human platelets by inhibition of phospholipase C activity

Address correspondence to Dr. Joen-Rong Sheu, Graduate Institute of Medical Sciences, Taipei Medical College, 250, Wu-Shing Street, Taipei 110, Taiwan. Tel/Fax: +866-2-27390450, E-mail: sheujr@tmc.edu.tw

(12). In this article, we review *in vitro* and *in vivo* studies of rutaecarpine on cardiovascular function and its pharmacokinetics.

CHEMISTRY

Rutaecarpine (7,8-dihydro-13H-indolo [2′3′:3,4] pyrido [2,1-b] quinazolin-5-one), an alkaloid isolated from the fruit of *Evodia rutaecarpa*, can be synthesized by condensation of iminoketene with amides (13), as shown in Fig. 1. Condensation of N-formyltryptamine (A) with sulfinamide anhydride (B) in a mixture of dry benzene and chloroform at room temperature for 2 h produces, with a 63% yield, 3-indolylethylquinazolin-4-one (C). This product is then heated with concentrated hydrochloric acid in acetic acid at 110°C for 166 h to generate rutaecarpine (D) (13). Rutaecarpine occurs as colorless needles (melting point 259–260°C). It is soluble in alcohol, benzene, chloroform, and ether, but practically insoluble in water. The molecular formula of rutaecarpine is $C_{18}H_{13}N_3O$ and its molecular weight is 287.3.

IN VITRO PHARMACOLOGY

Effect on Vasodilatation

Rutaecarpine causes concentration-dependent (0.1 μM-0.1 mM) relaxation of isolated rat mesenteric arterial segments precontracted with phenylephrine (11). Phenylephrine-induced contraction of mesenteric arterial segments with intact endothelium is relaxed 90% by 0.1 mM rutaecarpine. Removal of the endothelium markedly attenuates rutaecarpine-induced relaxation (11). Treatment with *L*-N^G-nitroarginine (0.1 mM), a NO synthase inhibitor (14), or methylene blue (10 μM), a guanylyl cyclase inhibitor (15), significantly diminishes but does not completely abolish the vasorelaxing effect of rutaecarpine. Maximal rutaecarpine-induced relaxation of arterial segments is significantly reduced from 87.8 ± 3.7% to 30.6 ± 2.5% by treatment with *L*-N^G-nitroarginine and from 90.2 ± 4.2% to 37.9 ± 2.5% by treatment with methylene blue (11). These findings strongly suggest that release of NO contributes to the relaxing effect of rutaecarpine, but other mechanisms may also be involved. On the other hand, the vasorelaxing effect of rutaecarpine was not significantly attenuated by pretreatment with a muscarinic receptor antagonist, atropine (0.1 μM), a histamine H_1 receptor antagonist (16), triprolidine (0.1 mM), and a selective α_2-adrenoceptor agonist (17), yohimbine (0.3 μM). The data indicate that the vasorelaxing effect of rutaecarpine is endothelium-dependent and is mediated by NO and guanylyl cyclase. Muscarinic receptors, histamine H_1 receptors, and α_2-adrenoceptors are not involved (11).

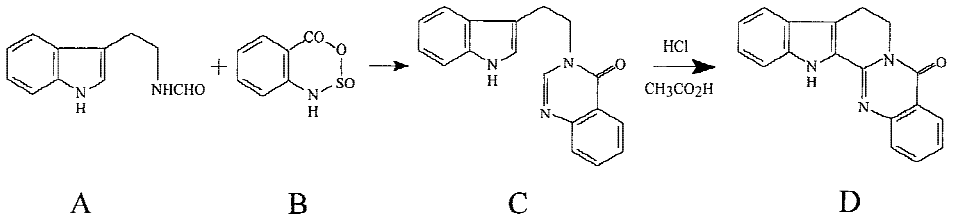

FIG. 1. Chemical synthesis of rutaecarpine.

In addition to NO, the vascular endothelium secretes at least two other mediators that can lead to relaxation of vascular smooth muscle. The other candidates are prostaglandin I_2 (PGI_2) and endothelium-derived hyperpolarizing factor (EDHF). Systematic examination with appropriate antagonists revealed that a cyclooxygenase inhibitor, indomethacin (30 μM), or a nonselective K^+ channel blocker, tetramethylammonium (TEA; 10 mM), had no significant effects. These data support the hypothesis that NO and guanylyl cyclase is the principal endothelium-derived mediator and effector, respectively, responsible for the endothelium-dependent activity of rutaecarpine (18).

The possible role of Ca^{2+} in mediating the effects of rutaecarpine was investigated. Removal of extracellular Ca^{2+} or treatment with 8-(N,N-diethylamino) octyl-3,4,5,-trimethoxybenzoate (TMB-8) (0.1 mM), an intracellular Ca^{+2} antagonist, suggested that influx of extracellular Ca^{2+} mediates rutaecarpine activity (18). In Ca^{2+}-free medium containing EGTA, rutaecarpine failed to induce any vasorelaxation suggesting a dependence on extracellular Ca^{2+} and an important role for transmembrane Ca^{2+} influx.

Several other studies showed that, although pertussis toxin (100 ng/ml) suppressed the ability of histamine to relax arterial segments, it had no effect on rutaecarpine activity (18). Similarly, sodium fluoride (NaF; 1, 2, or 3 mM), an activator of G proteins (19), attenuated the action of acetylcholine but had only a minimal affect on rutaecarpine activity (18). Finally, U73122 (1-[6-{[17β-3-methoxyestra-1,2,3 (10)-trien-17-yl] amino} hexyl]-1H-pyrrole-2,5-dione; 1–10 μM) an inhibitor of phospholipase C (20), also suppressed the action of acetylcholine without affecting rutaecarpine activity (18).

The data strongly suggest that rutaecarpine induces an endothelium- and NO-dependent vasorelaxation or dilation in rat arterial segments, previously made to contract with phenylephrine. Since these responses are inhibited by removal of extracellular Ca^{2+}, the vasorelaxation induced by rutaecarpine is dependent primarily on the influx of Ca^{2+} and not on the mobilization of intracellular Ca^{2+} ($[Ca^{+2}]_i$). G_i proteins or G protein-phospholipase C coupling pathways are probably not involved because pertussis toxin, sodium fluoride, and U73122 had no effect on rutaecarpine-induced endothelium-dependent vasodilation (18).

Effect on Platelet Aggregation

In platelet-rich plasma, rutaecarpine (40–200 μM) inhibits human platelet aggregation stimulated by a variety of agonists, including collagen, ADP, epinephrine, and arachidonic acid (21). At a concentration of 200 μM, rutaecarpine almost completely inhibits platelet aggregation induced by arachidonic acid (Fig. 2). Rutaecarpine inhibits platelet aggregation induced by collagen (10 μg/ml), epinephrine (10 μM), ADP (20 μM), or arachidonic acid (100 μM) in a concentration-dependent manner (Fig. 2). However, at the highest concentration tested (200 μM), rutaecarpine is unable to completely inhibit platelet aggregation induced by these agonists. The greatest inhibition, about 90%, is observed with 200 μM arachidonic acid. The IC_{50} values for inhibition of platelet aggregation induced by collagen, epinephrine, ADP, and arachidonic acid are estimated to be 166.2, 64.8, 159.6, and 76.5 μM, respectively.

The inhibitory activity of rutaecarpine (120 μM) on platelets is not significantly attenuated by pretreatment with N^G-mono-methyl-*L*-arginine (L-NMMA) (100 μM) or N^G-nitro-*L*-arginine methylester (L-NAME) (200 μM), both NO synthase inhibitors, or

FIG. 2. Dose-inhibition curve of rutaecarpine on collagen (10 μg/ml, ○)-, epinephrine (10 μM, ●)-, ADP (20 μM, ▽)- and arachidonic acid (100 μM, ▼)-induced aggregation of human platelet-rich plasma. Human platelet-rich plasma was preincubated with various concentrations of rutaecarpine at 37°C for 1 min and then agonists were added to stimulate aggregation. Data are presented as percent of control aggregatioin (mean ± S.E.M., n = 4–5). From Ref. 21.

with methylene blue (100 μM), a guanylyl cyclase inhibitor. Rutaecarpine (40–200 μM) also did not significantly affect cyclic AMP or cyclic GMP levels in human ashed platelets. However, rutaecarpine (40–200 μM) significantly inhibited thromboxane B_2 (TxB_2) synthesis stimulated by collagen (10 μg/ml) or thrombin (0.1 U/ml) (21). Additional studies determined whether inhibition of TxB_2 formation was due to inhibition of thromboxane synthetase or phospholipase A_2 (PLA_2). Sheu *et al* (12) observed that rutaecarpine (100 and 200 μM) did not significantly affect thromboxane synthetase activity in aspirin-treated platelet microsomes, indicating that inhibition of TxB_2 synthesis by rutaecarpine, at least in part, is not due to inhibition of thromboxane synthetase. Furthermore, rutaecarpine (100 and 200 μM) did not significantly affect PLA_2 activity (measured as the release of [^{3}H]arachidonic acid) in [^{3}H]arachidonic acid-labeled resting platelets while significantly inhibiting [^{3}H]arachidonic acid release in collagen-activated platelets (12). These results indicate that rutaecarpine inhibits TxA_2 synthesis in activated-platelets probably through intracellular pathways other than by direct inhibition of PLA_2 activity in platelet membranes.

On the other hand, rutaecarpine (50–100 μM) dose-dependently inhibits collagen (10 μg/ml)-stimulated increases in $[Ca^{2+}]_i$ levels of Fura 2-labeled platelets and phosphoinositide breakdown in [^{3}H]myoinositol-labeled platelets (Fig. 3). Collagen (10 μg/ml) induces an increase in inositol monophosphate (IP) formatioin that reaches a maximum after about 2 min. In the presence of rutaecarpine (50, 100, and 200 μM), IP formation in collagen-stimulated platelets is markedly and dose-dependently decreased at all incubation times (Fig. 3). The IC_{50} value of rutaecarpine was estimated to be 142 μM in this assay and this value is close to the IC_{50} value (166 μM) of rutaecarpine-induced inhibition of collagen-stimulated platelet aggregation (21). Taken together, the data suggest that the antiplatelet activity of rutaecarpine is probably due to inhibition of phospholipase C activity, leading to a reduction of phosphoinositide breakdown and $[Ca^{2+}]_i$ mobilization and a decrease in TxA_2 synthesis in platelets stimulated by agonists.

Uterotonic Effect

The effects of rutaecarpine on uterotonic activity were evaluated *in vitro* using isolated rat uterus. Rats in proestrus (determined by vaginal smear) were pretreated with 100 μg

FIG. 3. Time course of the effect of rutaecarpine on formation of inositol monophosphate in [^{3}H]myoinositol-labeled washed human platelets. Platelets were labeled with [^{3}H]myoinositol and stimulated with collagen (10 μg/ml) in the absence (circles) or presence of various concentrations of rutaecarpine (triangles, 50 μM; squares, 100 μM; diamonds, 200 μM) for different periods of time (1, 2, 3, and 5 minutes). Data are presented as mean ± S.E.M. (n = 4). *p < 0.05, **p < 0.01, and ***p < 0.001 as compared with the respective control at different incubation times (circles). From Ref. 12.

estradiol (intramuscular injection in peanut oil) 24 h prior to the experiment (22). In this study, the middle one-third segment of an isolated uterine horn was mounted in an organ bath and isometric contractions were recorded. In these *in vitro* experiments, the uterotonic effect of rutaecarpine on the isolated rat uterus is not blocked by atropine (30 nM) but is blocked by methysergide (3 nM). These results suggest that rutaecarpine may be a serotoninergic agonist. The effective *in vitro* uterotonic dose is estimated to be less than 1 μg/ml (22).

If the experimental data of the uterotonic activity of rutaecarpine in rats can be extrapolated to the human situation, then the presence of uterotonic alkaloids such as rutaecarpine in unripe fruit of *Evodia rutaecarpa* provides the basis for rational use of this drug in traditional Chinese medicine for the treatment of "female reproductive disorders."

IN VIVO PHARMACOLOGY

Hypotensive Effect

The hypotensive effect and mechanisn of $[Ca^{2+}]_i$ regulation underlying rutaecarpine-induced vasodilation was reported by Wang et al (23). An intravenous bolus injection of rutaecarpine (10, 30, or 100 μg/kg) in anesthetized rats produces dose-dependent hypotension. Mean arterial pressure (MAP) before rutaecarpine treatment was 95 ± 6 mm Hg and the maximal hypotension induced by rutaecarpine (100 μg/kg) lowered MAP to 25 ± 7 mm Hg (23). The mechanism for this effect was studied in cultured vascular smooth muscle cells (VSMC) and cultured endothelial cells labeled with Fura-2 to detect changes in $[Ca^{2+}]_i$. In the presence of extracellular Ca^{2+}, rutaecarpine (10 μM) suppresses KCl (30 mM)-induced increases in $[Ca^{2+}]$, in VSMC (23). In Ca^{2+}-free solution, rutaecarpine (10 μM) attenuates the norepinephrine-induced peak rise of $[Ca^{2+}]_i$ in VSMC. In cultured endothelial cells, on the other hand, rutaecarpine (1 and 10 μM) increases the level of $[Ca^{2+}]_i$ in the presence of extracellular Ca^{2+} (23). The data suggests that rutaecarpine acts directly on both VSMC and endothelial cells. In VSMC, rutaecarpine reduces $[Ca^{2+}]_i$ by inhibition of Ca^{2+} influx and Ca^{2+} release from intracellular stores. In endothelial cells, rutaecarpine increases $[Ca^{2+}]_i$ by increasing Ca^{2+} influx, possibly leading to NO release.

The combined effect of the paradoxical regulation of Ca^{2+} in VSMC and endothelial cells by rutaecarpine causes vasodilation, which could, at least in part, account for its hypotensive action (23).

Antianoxic Effect

Cerebral metabolic activators and cerebrovasodilators have been receiving attention for their utility in the improvement of disorders following traumatic cerebral injuries caused by accidents. Currently available cerebral metabolic activators and cerebrovasodilators used for the treatment of disorders following cerebral infarction and cerebral hemorrhage, as well as cerebral arteriosclerosis, are recognized as having antianoxic action (24). Brain tissue has a very high oxygen requirement compared to other tissues and is quite sensitive to low oxygen tension during ischemia.

Cyanide compounds, such KCN, are known to interfere with cytochrome oxidase in mitochondria and thereby inhibit cellular respiratioin (25). In a mouse model of KCN-induced anoxia, all mice in a control group that received an injection of KCN (30 mg/kg, i.v.) through a tail vein had respiratory arrest after about 1 min of repetitive convulsions which led to death (26). In contrast, rutaecarpine (50 mg/kg, i.p.) pretreatment significantly prolongs the life of mice following injection of KCN and decreases their mortality rate. The mean duration of survival was 142.1 ± 15.7 s in mice pretreated with rutaecarpine and 5 out of 10 of these mice survived (mortality rate = 50%). In comparison, the mean duration of survival was 69.4 ± 13.0 s in control mice and only 1 out of 10 control mice survived (mortality rate = 90%) (26). These results demonstrate an antianoxic activity of rutaecarpine in the KCN-induced anoxia model.

Antithrombotic Effect

Intravascular thrombosis is involved in the pathogenesis of several cardiovascular diseases. The initiation of intraluminal thrombosis is believed to involve platelet adherence and aggregation. In the normal circulation, platelets do not aggregate in the absence of stimulation. When a blood vessel is damaged, platelets adhere to the disrupted surface and then release several biologically active mediators that promote platelet aggregation (27). Platelet aggregation probably plays a crucial role in the growth of an atherosclerotic lesion, unstable angina, and in acute myocardial infarction. Antiplatelet agents such as aspirin and triflavin (an Arg-Gly-Asp-containing peptide from the venom of the snake *Trimeresurus flavoviridis*) are known to reduce the incidence of thrombosis *in vivo* (28,29).

Platelet thrombi in the microvasculature of mice pretreated with fluorescein sodium can be induced *in vivo* by irradiation with filtered light (30). This mouse model of mesenteric venule thrombosis was used to evaluate the *in vivo* antithrombotic and antiplatelet effects of rutaecarpine. In addition, the antithrombotic and antiplatelet activity of rutaecarpine was studied in an experimental model of acute pulmonary thrombosis in mice (31).

The baseline blood pressure in anesthetized mice was not significantly affected for 2 h following treatment with fluorescein sodium (10 and 20 μg/kg) or the combination of fluorescein sodium (20 μg/kg) with heparin (1.5 U/g), aspirin (250 μg/g), or rutaecarpine (200 μg/g) (data not shown). Irradiation with filtered light induced the formation of platelet plugs with a latent period that decreased with an increasing dose of fluorescein

sodium (Table 1). Platelet plug formation, or the occlusion time, was 127 ± 25 and 54 ± 9 s with fluorescein sodium at 10 μg/kg and 20 μg/kg, respectively. The occlusion time induced by irradiation of mice pretreated with fluorescein sodiuim (10 μg/kg) was significantly prolonged to 201 ± 20 s and 193 ± 19 s in mice that were treated with rutaecarpine (200 μg/g) and aspirin (250 μg/g), respectively (Table 1). On a molar basis, rutaecarpine was about 2-fold more potent than aspirin at inhibiting fluorescein sodium-induced platelet plug formation in mesenteric venules of mice. Heparin (0.75 and 1.5 U/g) and a lower dose of aspirin (150 μg/g) or rutaecarpine (100 μg/g) had no significant effects on occlusion times (Table 1).

The antithrombotic and antiplatelet effects of rutaecarpine were also demonstrated in a mouse model of acute pulmonary embolism (described in Ref. 31). In this study, rutaecarpine (25 or 50 μg/g), heparin (1.5 U/g), or aspirin (20 μg/g) were administered by injection into a tail vein. Four minutes later, ADP (0.7 mg/g) was injected into the contralateral vein. The mortality rate of mice in each group after ADP injection was determined after 10 min. Rutaecarpine and aspirin significantly lowered the mortality rate of mice challenged with ADP. The mortality rate in untreated mice was 81%. It was unchanged with heparin pretreatment (mortality rate = 80%) but reduced to 35% and 30% with rutaecarpine (50 μg/g) and aspirin, respectively (Table 2). Taken together, the results from these two *in vivo* model systems suggest that rutaecarpine is an effective antithrombotic agent in the prevention of thrombosis and thromboembolism.

PHARMACOKINETIC STUDIES

Pharmacokinetic studies of rutaecarpine were reported by Ko et al (32). Plasma concentration curves of rutaecarpine (2 mg/kg) after intravenous administration in mice revealed a biexponential decline in concentration with time following administration (32). Pharmacokinetic parameters of rutaecarpine (2 mg/kg) after intravenous bolus dosing in rats were determined to be as follows (mean ± S.E.M.; n = 6): half-life ($t_{1/2}$) = 29.29 ± 4.25 (min); clearance (CL) = 63.46 ± 5.39 ml/min/kg; volume of distribution = 655.15 ± 43.93 ml/kg; and area under the curve (AUC) = 32.93 ± 3.39 μg min/ml.

TABLE 1. *Effect of fluorescein sodium, heparin, aspirin, and rutaecarpine on the occlusion time after light irradiation of mesenteric venules of mice pretreated with fluorescein sodium*

	Dose of fluorescein sodium (μg/kg)	
	10	20
Normal saline	127 ± 25 (6)	54 ± 9 (6)
Heparin (U/g)		
0.75	129 ± 24 (4)	ND
1.5	95 ± 19 (4)	50 ± 13 (4)
Aspirin (μg/g)		
150	88 ± 23 (5)	45 ± 6 (5)
250	193 ± 19* (6)	42 ± 5 (5)
Rutaecarpine		
100 μg/g	105 ± 28 (6)	51 ± 5 (6)
200 μg/g	201 ± 20* (6)	46 ± 3 (6)

Values are elapsed time in seconds of platelet plug formation following irradiation of mesenteric venules. The mean ± S.E.M. and (n) are presented. ND, not determined. *$p < 0.05$ compared to control (normal saline).

TABLE 2. *Dose-response of rutaecarpine on mortality rate in acute pulmonary thrombosis induced by intravenous injection of ADP in mice*

	Number of deaths	Total number of mice	Mortality (%)
Normal saline	0	5	0
ADP (0.7 mg/g)	17	21	81
+ rutaecarpine (μg/g)			
25	15	20	75
50	7	20	35
+ heparin (1.5 U/g)	16	20	80
+ aspirin (20 μg/g)	6	20	30

CONCLUSIONS

Many Western ethical drugs were originally derived from herbs and other plants. Aspirin, digitalis, penicillin, and quinine are only some of the better known examples. In many parts of the world herbal medicine has been the main method for treating diseases and disorders for thousands of years. Many herbal preparations are claimed to be effective in treating diseases but, in most cases, the active ingredient(s) in these herbal mixtures are unknown and the mechanism of action is obscure. It has been suggested that herbs may be an important source of new compounds, possibily with fewer side effects, in future drug development. It is therefore important for pharmacologists to identify active substance(s) from effective herbal preparations and explore their mechanism(s) of action. The review of the pharmacology and activity of rutaecarpine is an example of this type of work.

Wu-Chu-Yu (*Evodia rutaecarpa*) is a plant material that has been used to treat several diseases, including hypertension. Rutaecarpine is a pure chemical isolated from *Evodia rutaecarpa* and the antihypertensive and antithrombotic effects of this phytochemical have been reviewed in this presentation. Rutaecapine exhibits interesting pharmacologic properties that may explain its vascular and platelet effects.

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