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REVIEW ARTICLE

Cardiovascular Pharmacological Actions of Rutaecarpine, a Quinazolinocarboline Alkaloid Isolated From *Evodia rutaecarpa*

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Evodia rutaecarpa (Chinese name: Wu-Chu-Yu) is a well-known traditional Chinese medicine and has long been used in Chinese medical practice. Rutaecarpine is an indolopyridoquinazolinone alkaloid isolated from *E. rutaecarpa* and related herbs, which has been shown to have cardiovascular biological effects, such as inotropic and chronotropic, vasorelaxant, antiplatelet aggregation, and anti-inflammatory effects. Furthermore, it has been reported that rutaecarpine has beneficial effects on some cardiovascular diseases. This review was undertaken to summarize data on the cardiovascular pharmacological actions of rutaecarpine published over the recent years, aiming to provide more evidence supporting its use in the treatment of cardiovascular diseases. This review also reveals some interesting and unique pharmacological properties, which may explain its vascular and platelet effects.

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1. Introduction

Herbs have been widely used as important remedies all over the world. Advancement of science and technology in recent decades has made possible not only to purify and characterize the biologically active constituents of herbs, but also to evaluate their biological activities. Rutaceous plants, especially *Evodia rutaecarpa* (whose dried fruit is named "Wu-Chu-Yu" in China), have long been used for the treatment of gastrointestinal disorders, headache, amenorrhea, and postpartum hemorrhage in traditional oriental medicine.[1,2] It has also been reported to have remarkable central stimulant effect[3] and transient positive inotropic and chronotropic effects.[4] Several alkaloids with biological activity have been identified in *E. rutaecarpa*, including three major alkaloids: dehydroevodiamine, evodiamine, and rutaecarpine (Rut).[5] Pharmacological investigations have revealed different extracts of *E. rutaecarpa*, and its chemical constituents display many biological activities related to inflammation, for example, antinociception, anti-inflammation, immune modulation, nitric oxide (NO) inhibition,[6] protection against endotoxin shock in rats and anti-inflammatory activity in human skin.[7,8] A study reveals that rutaecarpine relaxes vascular smooth muscles through the activation of the endothelial Ca^{2+}–NO cyclic guanosine monophosphate cascade and the inhibition of Ca^{2+} influx.[9] In our previous study, we also found that the mechanism of rutaecarpine inhibiting the aggregation of human platelets is mediated through the inhibition of phospholipase C.[10] Furthermore, it has been reported that rutaecarpine has beneficial effects on some cardiovascular diseases.[11–13] This review summarizes the cardiovascular pharmacological effects of rutaecarpine on the basis of *in vitro* and *in vivo* studies, aiming to offer more evidence in the treatment of cardiovascular diseases.

2. 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 *E. rutaecarpa*, has been reported to be synthesized by the condensation of iminoketene with amides[14] as shown in Figure 1. A condensation of *N*-formyltryptamine (A) with sulfinamide anhydride (B) was carried out in a mixture of dry benzene and chloroform at room temperature for 2 hours to give, in 63% yield, 3-indolylethylquinazolin-4-one (C). This product was heated with concentrated hydrochloric acid in acetic acid at 110°C for 166 hours to afford rutaecarpine (D).[14] Rutaecarpine is a colorless needle (melting point, 259–260°C) with the molecular formula $C_{18}H_{13}N_3O$ and molecular weight of 287.3, soluble in alcohol, benzene, chloroform, and ether; however, it is particularly insoluble in water.

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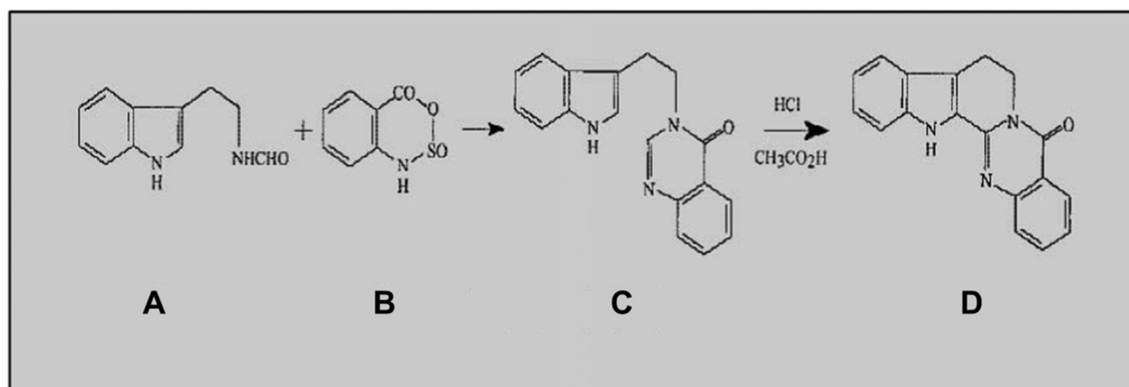

Figure 1 The chemical synthesis of rutaecarpine.

3. Pharmacokinetic Studies

Effects of rutaecarpine on the pharmacokinetics have been reported by Ko et al.[15] An intravenous administration of rutaecarpine (2 mg/kg) in mice revealed that the curves of concentration in plasma versus time exhibited a biexponential decline with the administration.[15] The pharmacokinetic parameters of rutaecarpine in rats after administration of intravenous bolus (2 mg/kg) dose were as follows (mean ± standard error of the mean; $n = 6$): the half-life ($t_{1/2}$), 29.29 ± 4.25 (minutes); clear rate, 63.46 ± 5.39 mL/min/kg; volume, 655.15 ± 43.93 mL/kg; and the area under the curve, 32.93 ± 3.39 μg/min/mL. The effects of rutaecarpine on the pharmacokinetics of acetaminophen in rats have also been investigated by Lee et al.[16]

3.1. Blood pressure

Hypertension is one of the common global cardiovascular diseases. Persistent high blood pressure could induce pathological alterations in many tissues and organs, including blood vessels, heart, brain, and kidney, and then result in severe complications, such as atherosclerosis, coronary heart diseases, stroke, renal dysfunction, and others. Although effective control of blood pressure in the clinic can be achieved with a range of antihypertensive agents, such as diuretics, calcium channel blockers, β-adrenoceptor antagonists, and angiotensin-converting enzyme inhibitors, the need of novel antihypertension drugs with low side effects and low costs still attracts particular attention in cardiovascular research.

Rutaecarpine has been used widely in China for hundreds of years to treat hypertension.[17] It was previously reported that rutaecarpine produced a sustained hypotensive effect in phenol-induced and two-kidney, one-clip (2K1C) hypertensive rats with a novel antihypertensive mechanism by stimulating the synthesis and release of calcitonin gene—related peptide (CGRP), a principal transmitter in capsaicin-sensitive sensory nerves, and CGRP, in turn, can relax vascular smooth muscle and reduce the peripheral resistance.[18,19]

The hypotensive effect and the mechanism of intracellular Ca^{2+} ($[Ca^{2+}]i$) regulation, underlying rutaecarpine-induced vasodilation has been reported by Wang et al.[20] An intravenous bolus injection of rutaecarpine (10 μg/kg, 30 μg/kg, or 100 μg/kg) in anesthetized Sprague—Dawley rats produced a dose-dependent hypotensive effect. The maximum hypotension induced by rutaecarpine (100 μg/kg) was 25 ± 7 mmHg.[20] As determined by the Fura-2/AM (INVITROGEN, USA) method, rutaecarpine (10 μM), in the presence of extracellular Ca^{2+}, suppressed the KCl (30 mM)—induced increment of $[Ca^{2+}]i$ of cultured vascular smooth muscle cells (VSMC).[20] Rutaecarpine (10μM) also attenuates the norepinephrine-induced peak rise of $[Ca^{2+}]i$ in VSMC placed in Ca^{2+}-free solution. On the other hand, rutaecarpine (1μM and 10 μM) increases the level of $[Ca^{2+}]i$ of cultured endothelial cells (ECs) in the presence of extracellular Ca^{2+}.[20] Therefore, rutaecarpine acts on both VSMC and EC directly. In VSMC, it reduces $[Ca^{2+}]i$ through the inhibition and release of Ca^{2+} influx from intracellular stores. In EC, rutaecarpine augments EC $[Ca^{2+}]i$ by increasing Ca^{2+} influx, possibly leading to NO release.[20] The paradoxical regulation of Ca^{2+} in both VSMC and EC acts simultaneously to cause vasorelaxation, which could account, at least in part, for the hypotensive action.[20] A study has also revealed that a chronic treatment of rutaecarpine (10 mg/kg/d or 40 mg/kg/d) or losartan (20 mg/kg/d) for 4 weeks in the hypertensive rats resulted in a sustained dose-dependent attenuation of increases in blood pressure and increased lumen diameter.[21] The result of the recent study showed that, compared with the crude rutaecarpine, administration of the solid dispersion of rutaecarpine significantly increased the blood concentration of rutaecarpine in a dose-dependent manner with a sustained hypotensive effect.[22]

3.2. Blood vessels

Rutaecarpine is a natural vasodilator; it widens blood vessels allowing increased blood flow to the genitals. Rutaecarpine caused concentration-dependent (0.1 μM—0.1mM) relaxation of isolated rat mesenteric arterial segments, which were precontracted with phenylephrine.[9] The phenylephrine-induced contraction was relaxed 90% in endothelium-intact mesenteric arterial segments by 0.1mM rutaecarpine. Removal of the endothelium markedly attenuated the rutaecarpine-induced relaxation.[9] Treatment with the NO synthase inhibitor[23], L-N^G-nitroarginine (0.1 mM), or a guanylyl cyclase inhibitor[24], methylene blue (10 μM), significantly diminished but did not completely abolish the vasorelaxing effect of rutaecarpine. Maximal relaxations in response to rutaecarpine were significantly reduced from 87.8 ± 3.7% to 30.6 ± 2.5% in L-N^G-nitroarginine-treated rings and from 90.2 ± 4.2% to 37.9 ± 2.5% in methylene blue-treated arterial rings.[9] These findings strongly suggest that NO is responsible, albeit not completely, for the relaxing effect of rutaecarpine. On the other hand, the vasodilator effect of rutaecarpine was not significantly attenuated by pretreatment with muscarinic receptor antagonist, atropine (0.1 μM), histamine H_1 receptor antagonist[25], triprolidine (0.1mM), and selective α_2-adrenoceptor agonist[26], yohimbine (0.3 μM). It is concluded that the vasorelaxing effect of rutaecarpine appears to be endothelium dependent and to involve NO and guanylyl cyclase.

In addition, the vascular endothelium secretes a number of vasoactive substances, among which NO, prostaglandin I_2 (PGI_2),

and endothelium-derived hyperpolarizing factor are three likely candidates as mediators that could lead to the relaxation of vascular smooth muscles. Systemic examination with appropriate antagonists revealed that the cyclooxygenase (COX) inhibitor, indomethacin (30 μM), or the nonselective K^+ channel blocker, tetramethylammonium (10 mM) had no significant effects, suggesting that NO and guanylyl cyclase were likely the endothelial mediators and effectors responsible for the endothelium-dependent actions of rutaecarpine.[9]

For experiments designed to study the possible roles of Ca^{2+} in the actions of rutaecarpine, both removal of extracellular Ca^{2+} and treatment with the $[Ca^{2+}]i$ antagonist, 8-(*N*,*N*-diethylamino) octyl-3,4,5,-trimethoxybenzoate (0.1mM), suggested that influx of extracellular Ca^{2+} was the major factor contributing to the action of rutaecarpine.[25] Because the vasorelaxant of rutaecarpine appeared to be largely dependent on extracellular Ca^{2+}, as rutaecarpine failed to induce any relaxation in Ca^{2+}-free, ethylene glycol tetraacetic acid (EGTA)-containing medium, indicating the possible involvement of a transmembrane Ca^{2+} influx. Moreover, pertussis toxin (100 ng/mL) suppressed the relaxation potency of histamine but had no effects on the action of rutaecarpine.[24] Sodium fluoride (1 mM, 2 mM, or 3 mM), the G protein activator[26], attenuated the action of acetylcholine, but only minimally affected rutaecarpine.[25] 1-[6-{[17b-3-methoxyestra-1,2,3(10)-trien-17-yl]amino}hexyl]-1H-pyrrole-2,5-dione (U73122) (1−10 μM), the phospholipase C inhibitor,[27] suppressed the actions of acetylcholine but had few effects on rutaecarpine.[25]

Therefore, rutaecarpine induced an endothelium/NO-dependent vasodilatation in rat aorta precontracted by phenylephrine. These responses could be inhibited by the removal of extracellular Ca^{2+} in the medium. This vasodilatation induced by rutaecarpine depended primarily on the influx of Ca^{2+} and not on the mobilization of $[Ca^{2+}]i$. Because pertussis toxin, even though sodium fluoride and U73122 did not affect rutaecarpine-induced endothelium-dependent vasodilatation; it is speculated that G_i proteins or G protein-phospholipase C coupling pathways were probably not involved in the action of rutaecarpine on vascular ECs.[25] The depressor and vasodilator effects of rutaecarpine have also been studied by Hu et al,[28] in rat model, where they suggest that the depressor and vasodilator effects of rutaecarpine are related to the stimulation of endogenous CGRP release through the activation of vanilloid receptors.

3.3. Cerebral protection

To improve the disorders caused by cerebral injuries because of traffic accidents, cerebral metabolic activator and cerebrovasodilators have received more attention in this field. Currently available cerebral metabolic activators and cerebrovasodilators, which are used for the treatment of post disorders of cerebral infarction and cerebral hemorrhage, as well as cerebroarteriosclerosis, are recognized as having antianoxic action that is effective against ischemia.[29]

Brain tissue has a very high oxygen requirement as compared with other tissues and is quite sensitive to lower oxygen conditions caused by ischemia. Cyanidine compound, such as KCN, is known to interfere with cytochrome oxidase in mitochondria, thereby inhibiting cellular respiration.[30] In KCN-induced anoxia studies, all the mice in the control group, which received the KCN (30 mg/kg, intravenously) injection through the tail vein, had respiratory arrest after about 1 minute of repeated convulsive attacks, leading to death.[25] In mice treated with rutaecarpine at 50 mg/kg, intraperitoneally, there was a significant life-prolonging effect as compared with the controls. The mean survival duration was 142.1 ± 15.7 seconds with the survival rate of 5 out of 10 (mortality, 50%) for rutaecarpine-treated rats as compared with the control groups, whose mean survival duration was 69.4 ± 13.0 seconds, with 1 out of 10 survival rate (mortality, 90%).[27] These results suggest that rutaecarpine has an antianoxic action in the KCN-induced anoxia model.

3.4. Antithrombotic

Thrombosis, arterial or venous, is the most common cause of death in the United States, with about 2 million deaths per year attributable to such conditions as myocardial infarction, pulmonary embolism, and cerebrovascular thrombosis. Intravascular thrombosis is one of the generators of a wide variety of cardiovascular diseases. The initiation of an intraluminal thrombosis is believed to involve platelet adherence and aggregation. Platelets cannot be aggregated by themselves in normal circulation. However, when a blood vessel is damaged, platelets adhere to the disrupted surface, and the adherent platelets release some biologically active constituents and aggregate.[31] Thus, platelet aggregation may play a crucial role in the atherothrombotic process. Indeed, antiplatelet agents (i.e., aspirin and triflavin) have been shown to reduce the incidence of thrombosis *in vivo*.[32,33] It has been reported that platelet thrombi were induced by irradiation of filtered light in microvasculature of mice pretreated with fluorescein sodium intravenously.[34] We used this model to evaluate the *in vivo* antithrombotic effect of rutaecarpine on platelet plug formation. Additionally, we also tested its antithrombotic activity in experimental acute pulmonary thrombosis of mice.[35]

In anesthetized mice, pretreatment of fluorescein sodium (10 μg/kg and 20 μg/kg) or the combination of fluorescein sodium (20 μg/kg) with heparin (1.5 U/g), aspirin (250 μg/g), and rutaecarpine (200 μg/g) did not significantly change the baseline blood pressure within 2 hours (data not shown). The latent period in inducing platelet plug formation was shortened as the administered dose of fluorescein sodium was increased.[36] When the fluorescein sodium was given at 10 μg/kg or 20 μg/kg, the occlusion time required was 127 ± 25 seconds and 54 ± 9 seconds, respectively. Rutaecarpine (200 μg/g) and aspirin (250 μg/g) significantly prolonged the occlusion times induced by fluorescein sodium in venous. On a molar basis, rutaecarpine was about twofold more potent than aspirin at inhibiting fluorescein sodium-induced platelet plug formation in microvessels of mice. However, heparin (0.75 U/g and 1.5 U/g) and a lower concentration of aspirin (150 μg/g) and rutaecarpine (100 μg/g) showed no significant effects on occlusion times.[36]

Furthermore, we demonstrated the effect of rutaecarpine in preventing death because of acute pulmonary embolism in mice. Acute pulmonary thromboembolism was induced according to the one previously described.[35] Various doses of rutaecarpine (25 μg/g and 50 μg/g), heparin (1.5 U/g), and aspirin (20 μg/g) were administered by injection into the tail vein. Four minutes later, adenosine diphosphate (ADP) (0.7 mg/g) was injected into the contralateral vein.[37] The mortality of mice in each group after injection was determined within 10 minutes. As shown in Table 1,[31] rutaecarpine and aspirin significantly lowered the mortality of mice challenged with adenosine diphosphate (ADP) (0.7 mg/g). Rutaecarpine (50 μg/g) and aspirin (20 μg/g) reduced the mortality from 81% to 35% and 30%, respectively (Table 1). In contrast, heparin (1.5 U/g) showed no significant effect in reducing mortality (81% and 80%) in ADP-treated mice.[36] Therefore, rutaecarpine is an effective antithrombotic agent in preventing the thromboembolism in these two *in vivo* models.

3.5. Platelet aggregation

Platelets' primary physiological role is in hemostasis,[38] and their activation is a complex, multicomponent process with independent

Table 1 Effect of aspirin and rutaecarpine on mortality and platelet count of acute pulmonary thrombosis caused by intravenous injection of ADP in experimental mice

$10^3/mm^3$	No. of deaths	Total no.	Mortality %	Platelet count
				Mean ± SEM (n)
Control	0	5	0	223 ± 28 (6)
ADP (0.7 mg/g)	17	21	81	147 ± 19* (12)
+ Aspirin (μg/g)				
20	6	20	30	168 ± 21 (10)
50	6	20	30	189 ± 23 (10)
+ Rutaecarpine (μg/g)				
25	15	20	75	173 ± 19 (8)
50	7	20	35	195 ± 20 (8)

* $p < 0.05$ as compared with the control group (normal saline). See Ref. 31.
SEM = standard error of the mean.

and redundant pathways.[39–43] Dysregulated platelet activation can result in a host of thrombotic events, such as stroke, or myocardial infarction. This is especially relevant as, together, these diseases represent almost 40% of the total mortality in the United States.[44] Therefore, understanding the signaling pathways that underlie platelet activation has been invaluable in aiding drug discovery for treating platelet-dependent disease states.

A study conducted by Sheu et al,[37] revealed that, rutaecarpine (40–200 μM) inhibited aggregation in human platelet-rich plasma stimulated by a variety of agonists [i.e., collagen, ADP, epinephrine, and arachidonic acid (AA)], as shown in Figure 2.[10,37] At 120 μM concentration, rutaecarpine almost completely inhibited platelet aggregation induced by AA (Figure 2). Furthermore, rutaecarpine also dose dependently inhibited collagen (10 μg/ml)- and ADP (20 μM)-induced platelet aggregation.[37] However, even at 200 μM, it did not completely inhibit platelet aggregation induced by collagen, ADP, and epinephrine (Figure 2). The inhibition concentration (IC_{50}) values for platelet aggregation induced by collagen, epinephrine, ADP, and AA were estimated to be about 166.2 μM, 64.8 μM, 159.6 μM, and 76.5 μM, respectively.[37] The antiplatelet activity of rutaecarpine (120 μM) was not significantly attenuated by pretreatment with the NO synthase inhibitor N^G-mono-methyl-*L*-arginine (100 μM) or N^G-nitro-*L*-arginine methylester (200 μM) and with the guanylyl cyclase inhibitor methylene blue (100 μM). In addition, rutaecarpine (40–200 μM) did not significantly affect cyclic adenosine monophosphate (AMP) and cyclic GMP levels in human washed platelets, whereas it (40–200 μM) significantly inhibited thromboxane B_2 (TxB_2) formation stimulated by collagen (10 μg/mL) and thrombin (0.1 U/mL).[37] Further characterized whether or not the inhibition of TxB_2 formation was because of the inhibition of thromboxane synthetase or phospholipase A_2 (PLA_2). Sheu et al[10] found that rutaecapine (100 μM and 200 μM) did not significantly affect thromboxane synthetase activity in aspirin-treated platelet microsomes, indicating that the inhibition of TxB_2 formation by rutaecarpine, at least in part, is not because of the inhibition of thromboxane synthetase in platelets (Table 2).[10] Furthermore, rutaecarpine (100 μM and 200 μM) did not significantly affect the PLA_2 activity in [^{3}H] AA-labeled resting platelets.[10]

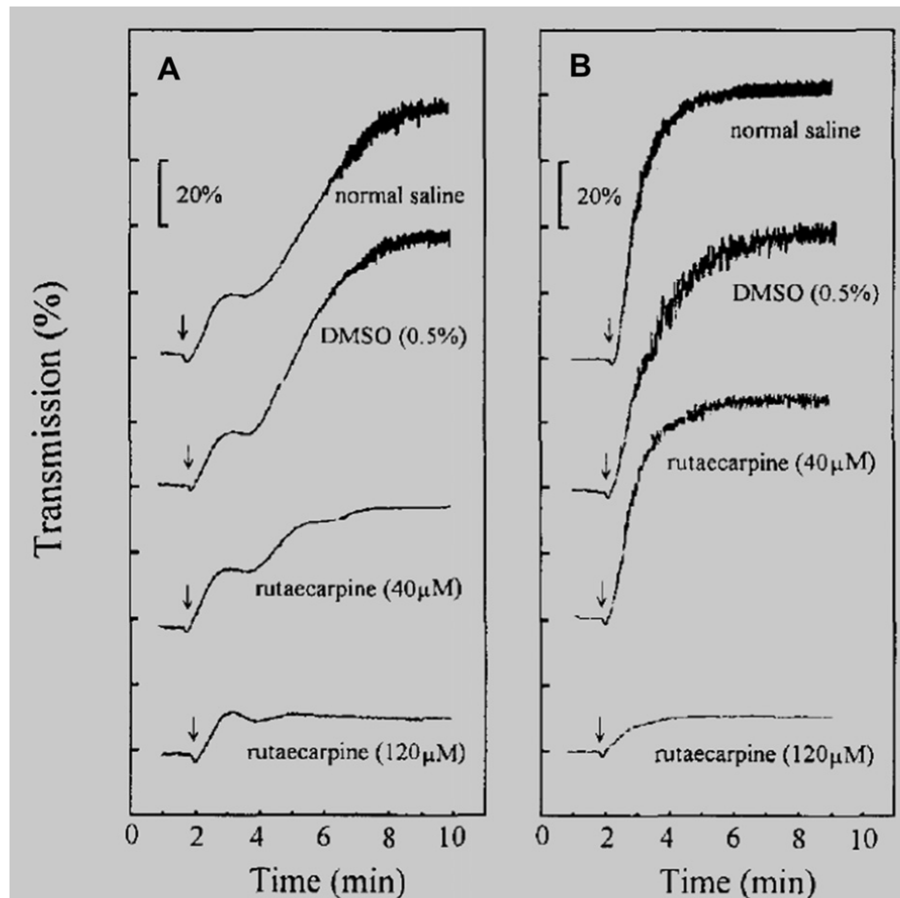

Figure 2 Antiplatelet effect of rutaecarpine on adrenaline (10 μM)- and arachidonic acid (100 μM)–induced aggregation of human platetet-rich plasma. Human platelet-rich plasma was preincubated with normal saline (control), dimethyl sulfoxide (DMSO) (0.5%), and rutaecarpine (40 μM and 120 μM) at 37°C for 1 minute. (A) adrenaline (10 μM: ↓) or (B) arachidonic acid (100 μM: ↓) was then added to induce platelet aggregation. For the detailed experimental procedure, see Ref. 10.

Table 2 Effect of rutaecarpine on thromboxane synthetase activity

Treatment	Thromboxane B_2 (ng/mL)
	Mean ± SEM (n) ($n = 4$)
PBS	1527.1 ± 14.0 (4)
DMSO (0.5%)	1535.1 ± 28.2 (4)
Imidazole (1mM)	1329.4 ± 45.1* (4)
Rutaecarpine (μM)	
100	1606.9 ± 39.1 (4)
200	1632.8 ± 60.7 (4)

* $p < 0.001$ as compared with the phosphate buffered saline (PBS) group. See Ref. 10.
0.1-mL volumes of aspirin-treated platelet microsomes were aliquoted into tubes, followed by the addition of dimethyl sulfoxide (DMSO) (0.5%), imidazole (1 mM), or rutaecarpine (100 μM and 200 μM), at 25°C for 3 minutes. Then, 2 μL of Prostaglandin H_2 (PGH_2) solution was added, vortexed, and incubated for 3 minutes at 25°C. Lastly, 10 μL $FeCl_2$ solution was added followed by centrifugation (3000 g, at 4°C for 10 minutes). The supernatant thromboxane B_2 level was assayed by using an ELISA kit.
SEM = standard error of the mean.

These results indicate that rutaecarpine inhibited TxA_2 formation in activated platelets, may be through other intracellular secondary pathways rather than by directly affecting PLA_2 activity on platelet membrane.

On the other hand, rutaecarpine (50–100 μM) dose dependently inhibited both the increase in the $[Ca^{2+}]$i level of Fura 2–loaded platelets (Figure 3)[10] and phosphoinositide breakdown stimulated by collagen (10 μg/mL) in $[^3H]$ myoinositol-loaded platelets at different incubation times.[10] Collagen (10 μg/mL) induced a time-related increase in inositol monophosphate (IP) formation, which caused about 1.3-fold rise in IP formation occurring during the initial 1 minute and reached a maximal IP formation approximately 2 minutes after collagen addition. In the presence of rutaecarpine (50 μM, 100 μM, and 200 μM), IP formation in collagen-stimulated platelets was markedly and dose dependently decreased at different incubation times, respectively.[10] The IC_{50} value of rutaecarpine was estimated to be about 142 μM in this reaction. This IC_{50} value of rutaecarpine at inhibiting collagen-induced inositol phosphate formation is close to the IC_{50} value (166 μM) at inhibiting collagen-induced platelet aggregation.[36] It is concluded that the antiplatelet activity of rutaecarpine may possibly be the result of the inhibition of phospholipase C activity, leading to reduce phosphoinositide breakdown, followed by the inhibition of TxA_2 formation, and then the inhibition of $[Ca^{2+}]$i mobilization of platelet aggregation stimulated by agonists.

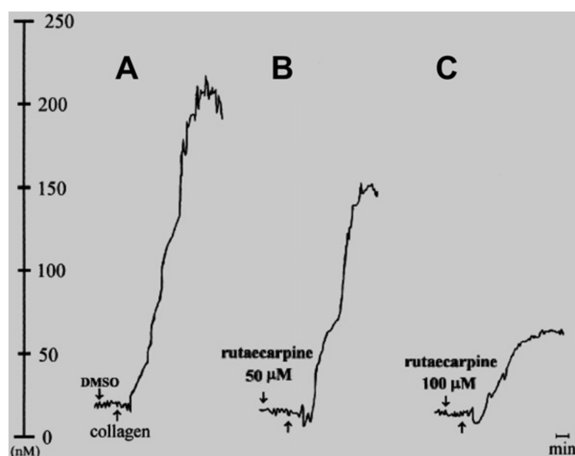

Figure 3 Effect of rutaecarpine on collagen-induced intracellular Ca^{2+} mobilization of Fura 2-AM–loaded human platelets. Platelet suspensions were incubated with Fura 2-AM (5 μM) at 37°C for 30 minutes, followed by the addition of collagen (10 μg/mL) in the presence of (A) Dimethyl sulfoxide (DMSO) (0.5%), control; (B) rutaecarpine (50 μM); and (C) (100 μM), which was added 2 minutes before the addition of collagen (10 μg/mL). For the detailed experimental procedure, see Ref. 10.

3.6. Uterotonia

Rutaecarpine was evaluated for *in vitro* uterotonic activity using isolated rat uterus. Proestrus (determined by vaginal smear) rats were pretreated with 100 μg of estradiol (intramuscular injection in peanut oil) 24 hours before experiment.[38] The middle one-third segment of the isolated uterine horn was used for study.[38] In the *in vitro* situations, rutaecarpine on isolated rat uterus contraction was blocked by methysergide. If the biological data (on rats) from uterotonic activity can be extrapolated into the human situation, the presence of the uterotonic alkaloids (i.e., rutaecarpine) in the unripe fruit of *E. rutaecarpa* can form the basis for the rational use of this drug in traditional Chinese medicine for the treatment of female reproductive disorders (such as postpartum hemorrhage). Cherian[45] has reported that the intake of a large quantity of unripe papaya fruit and subsequent ingestion of papaya latex could cause uncontrolled uterine contractions leading to abortion, depending on the estrogen levels in the tissues. Sewram et al[46] have also demonstrated that the compounds of oleanonic acid (1.83 μg/μL) and 3-*epi*oleanolic acid (1.77 μg/μL) from the extract of the wood *Ekebergia capensis* Sparrm exhibits varying degrees of agonist activity on uterine smooth muscle with minor changes in the molecular structure affecting its intrinsic activity on uterine muscle in guinea pig.

3.7. COX, PG, and cytochrome P450

The formation of PGs begins with the liberation of AA from membrane phospholipids, and the liberated AA is converted to PGs by COX and PG isomerases. Inhibition of COX is one of the major mechanisms by which nonsteroidal anti-inflammatory drugs exert their analgesic and anti-inflammatory effects. Rutaecarpine reduced the production of PGE(2) in RAW264.7 cells treated with lipopolysaccharide (LPS) in a dose-dependent manner when added to the culture media at the time of stimulation. However, the inhibition of total cellular COX activity under the same experimental condition was observed only at high concentrations of rutaecarpine.[47] However, rutaecarpine reduced the total cellular COX activity in macrophages treated with LPS only at high concentrations.[48] It was reported that LPS-induced production of PG in macrophages is highly associated with the expression of inducible isoform of COX (COX-2).[48]

Strong and selective inhibitory activity on COX-2 has been claimed as the origin of the anti-inflammatory activity of rutaecarpine.[49] A series of substituted rutaecarpines were prepared by using Fischer indole synthesis as the key step, and their inhibitory activities on COX-1 and 2 as well as selectivity on COX-2 have been evaluated. Rutaecarpine inhibited COX-2- and COX-1-dependent phases of PGE2 generation in bone marrow–derived mast cells in a concentration-dependent manner with IC_{50} values of 0.28 μM and 8.7 μM, respectively.[49] It inhibited COX-2-dependent conversion of exogenous AA to PGE2 in a dose-dependent manner by the COX-2-transfected human embryonic kidney 293 (HEK293) cells.

Cytochrome P450–dependent monooxygenase is the primary enzyme responsible for the oxidoreductive metabolism of a variety of endogenous and exogenous compounds, including steroids, drugs, and chemical carcinogens. Medicinal and herbal drug-dependent inhibition and induction of P450s are a major cause of drug interactions[50]; therefore, it is important to determine the effects of xenobiotics on P450s *in vivo* and *in vitro*. Identification of the role of individual P450s involved in the biotransformation of

a therapeutic agent can be useful in the interpretation and prediction of its pharmacological and toxicological actions. From several previous studies, rutaecarpine is known to induce the activity of hepatic cytochrome Ps.[51,52]

4. Conclusions

Many herbal preparations have been claimed to be effective in treating diseases but, in most cases, the active ingredient(s) in many herbal mixtures are unknown and the mechanism of action is obscure. Furthermore, it has been suggested that, for future drug development, herbs may be an important source of new compounds. It is, therefore, important for pharmacologists to identify the active substance(s) from effective herbal preparations and explore its mechanism of action. The presentation of this article is an example: Wu-Chu-Yu is a plant material that has been used to treat several diseases, including hypertension. Rutaecarpine is a pure chemical isolated from *E. rutaecarpa*, and this phytochemical has been shown in this presentation to have hypotensive and antithrombotic effects. This review also revealed some interesting unique pharmacological properties, which may explain its vascular and platelet effects.

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