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Mechanism of inhibition of platelet aggregation by rutaecarpine, an alkaloid isolated from *Evodia rutaecarpa*

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Abstract

In this study, rutaecarpine was tested for its antiplatelet activities in human platelet-rich plasma. In human platelet-rich plasma, rutaecarpine (40–200 μM) inhibited aggregation stimulated by a variety of agonists (i.e., collagen, ADP, adrenaline and arachidonic acid). The antiplatelet activity of rutaecarpine (120 μM) was not significantly attenuated by pretreatment with the nitric oxide synthase inhibitor N^G-mono-methyl-L-arginine (L-NMMA) (100 μM) or N^G-nitro-L-arginine methyl ester (L-NAME) (200 μM) and with the guanylyl cyclase inhibitor methylene blue (100 μM). In addition, rutaecarpine (40–200 μM) did not significantly affect cyclic AMP and cyclic GMP levels in human washed platelets, whereas it significantly inhibited thromboxane B_2 formation stimulated by collagen (10 μg/ml) and thrombin (0.1 U/ml). Furthermore, rutaecarpine (40–200 μM) inhibited [^{3}H]inositol monophosphate formation stimulated by collagen and thrombin in [^{3}H]myoinositol-loaded platelets. It is concluded that the antiplatelet effects of rutaecarpine are due to inhibition of thromboxane formation and phosphoinositide breakdown.

Keywords: Rutaecarpine; Alkaloid; Platelet, human; cAMP; cGMP; Thromboxane A_2; Phosphoinositide

1. Introduction

Chinese herbs have been widely used as important remedies in oriental medicine. In recent decades, many biologically active constituents have been isolated and their pharmacological actions investigated. The *Evodia rutaecarpa* (Chinese name: Wu-Chu-Yu) is a well-known traditional Chinese medicine. The dried unripened fruit of *Evodia rutaecarpa* has been used as a remedy for gastrointestinal disorders (abdominal pain, dysentery), postpartum hemorrhage, headache and amenorrhea (Li, 1596). It has been claimed to have a remarkable central stimulant effect (Liao et al., 1981), a transient hypertensive effect (Chow et al., 1976; Liao et al., 1981) and positive inotropic and chronotropic effects (Chen et al., 1981). A number of quinazolinocarboline alkaloids have been isolated from *Evodia rutaecarpa*, including rhetsinine, wuchuyine, rutaevine, dehydroevodiamine, evodiamine and rutaecarpine (Chang and But, 1987). The vasodilator effects of evodiamine (Chiou et al., 1992) and the cardiovascular effects of dehydroevodiamine have previously been reported (Yang et al., 1988, 1990; Chiou et al., 1992).

The organic nitrates (i.e., nitroglycerin) and nitrites (i.e., amyl nitrite) and several other nitrogen oxide-containing substances that are capable of undergoing denitration to release nitric oxide (NO) have been collectively termed nitrovasodilators. NO activates guanylyl cyclase, increasing intracellular levels of cyclic GMP, and thereby produces vasodilation (Molina et al., 1987; Thadani, 1992). These nitrovasodilators relax most smooth muscle via an endothelium-independent mechanism (Molina et al., 1987). Recently, Chiou et al. (1994) reported that rutaecarpine exerts a vasodilator effect via an endothelial NO-dependent mechanism on isolated rat mesenteric arteries. Moreover, rutaecarpine is not a nitrogen oxide-containing compound according to the chemical structure of rutaecarpine (Fig. 1). Taken together, the above observations imply that the vasodilator effect of rutaecarpine is not related to the direct release of NO. However, as yet there have not been any published reports correlating the anti-aggregatory ef-

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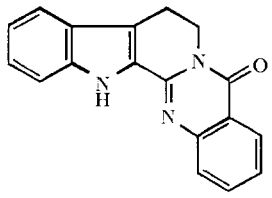

Rutaecarpine

Fig. 1. Chemical structure of rutaecarpine.

fects of rutaecarpine on platelets. Therefore, this investigation was undertaken to study the effects of rutaecarpine on human platelet aggregation.

While researching the antiplatelet activity of rutaecarpine, we found that rutaecarpine inhibits the aggregation of human platelets in a dose-dependent manner. It has been reported that human platelets can release NO under resting conditions or when stimulated by agonists (i.e., collagen) (Zhou et al., 1995). Therefore, we wondered whether rutaecarpine might also inhibit platelet aggregation through the release of NO. In this paper, we tried to elucidate the inhibitory mechanism of rutaecarpine on platelet aggregation.

2. Materials and methods

2.1. Materials

Rutaecarpine was purchased from Toray Techno, Japan. It was dissolved in dimethyl sulfoxide (DMSO) and stored at −4°C. Human thrombin, ADP, arachidonic acid, collagen (bovine tendon type I), prostaglandin E_1, apyrase, bovine serum albumin, ammonium formate, myoinositol, formic acid, sodium formate, sodium tetraborate, N^G-nitro-L-arginine methyl ester (L-NAME), N^G-monomethyl-L-arginine (L-NMMA), methylene blue, nitroglycerin, elastase (type IV, pig pancrease), heparin, EDTA, sucrose, and Dowex-1 (100–800 mesh: X8, chloride form) were purchased from Sigma, USA. Human fibrinogen (Kabi, Sweden) was used. Myo-2-[^{3}H]inositol was purchased from Amersham, UK. Cyclic AMP, cyclic GMP and thromboxane B_2 enzyme immunoassay (EIA) kits were obtained from Cayman, USA.

2.2. Preparation of human platelet-rich plasma and platelet suspensions

Blood was collected from healthy human volunteers who had not taken any medicine during the preceding 2 weeks and was mixed with 3.8% (w/v) sodium citrate (9 : 1, v/v). Citrated blood was immediately centrifuged at 120 × *g* for 10 min at 25°C, and the supernatant (platelet-rich plasma) was retained. In some experiments, human washed platelet suspensions were prepared by the method of Mustard et al. (1972) and Kornecki et al. (1981). Blood was mixed with acid/citrate/glucose (9 : 1, v/v). After centrifugation at 120 × *g* for 10 min at room temperature, the supernatant (platelet-rich plasma) was supplemented with prostaglandin E_1 (0.5 μM) and heparin (6.4 IU/ml), incubated for 10 min at 37°C and centrifuged at 500 × *g* for 10 min. The platelet pellet was suspended in 5 ml of Tyrode's solution, pH 7.3 (containing NaCl (11.9 mM), KCl (2.7 mM), $MgCl_2$ (2.1 mM), NaH_2PO_4 (0.4 mM), $NaHCO_3$ (11.9 mM) and glucose (11.1 mM)), then apyrase (1 U/ml), prostaglandin E_1 (0.5 μM) and heparin (6.4 IU/ml) were added, and the mixture was incubated for 10 min at 37°C. After centrifugation of the suspension at 500 × *g* for 10 min, the washing procedure was repeated. The washed platelets were finally suspended in Tyrode's solution containing bovine serum albumin (3.5 mg/ml) and adjusted to about 4.5×10^8 platelets/ml. The final concentration of Ca^{2+} in the Tyrode's solution was 1 mM. Elastase-treated platelets were prepared by the method of Kornecki et al. (1986). Elastase (1.25 U/10^8 platelets) was added to the platelet suspension, and the mixture was incubated for 60 min at 37°C in the presence of apyrase (0.5 U/ml). After centrifugation at 500 × *g* for 10 min, the pellet was washed twice with Tyrode's solution and finally suspended in Tyrode's solution containing bovine serum albumin.

2.3. Platelet aggregation

The turbidimetric method (Born and Cross, 1963), with a Lumi-Aggregometer (Chrono-Log), was used to measure platelet aggregation. Platelet-rich plasma (0.4 ml) was prewarmed at 37°C for 2 min (stirring at 1200 rpm) in a silicone-treated glass cuvette. Rutaecarpine or DMSO was added 1 min before the addition of a platelet-aggregation inducer. The reaction was allowed to proceed for at least 6 min and the extent of aggregation was expressed as a percentage of the control (in the absence of rutaecarpine). The degree of aggregation was expressed in light-transmission units.

2.4. Measurement of thromboxane B_2 formation

Human washed platelet suspensions (0.4 ml, 4.5×10^8/ml) were preincubated for 2 min in the presence or absence of rutaecarpine before the addition of collagen (10 μg/ml) or thrombin (0.1 U/ml). Six minutes after the addition of agonists, an aliquot of 2 mM EDTA and 50 μM indomethacin was added to the reaction suspension. The vials were then centrifuged in an Eppendorf centrifuge (Model 5414) for 3 min at 14000 rpm. The thromboxane B_2 levels of the supernatant were measured using an EIA kit according to the instructions of the manufacturer.

2.5. Labeling of membrane phospholipids and measurement of the production of [^{3}H]inositol phosphate

Citrated human platelet-rich plasma was centrifuged at $500 \times g$ for 10 min at room temperature, and the platelet pellets were then suspended in 1 ml of a Ca^{2+}-free and bovine serum albumin-free Tyrode's solution containing [^{3}H]inositol (75 μCi/ml). Platelets were incubated at 37°C for 2 h followed by centrifugation. Platelet pellets were finally resuspended in Ca^{2+}-free Tyrode's solution, and the platelet count was adjusted to 5×10^8 platelets per ml. One-milliliter aliquots of platelet suspension were prewarmed at 37°C with 5 mM LiCl in a 3.5-ml cuvette. Rutaecarpine was preincubated with loaded platelets at room temperature for 2 min, and then thrombin (0.1 U/ml) or collagen (10 μg/ml) was added to trigger aggregation. Six minutes later, the reaction was stopped by adding ice-cold trichloroacetic acid (10%, w/v), followed by centrifugation at $1000 \times g$ for 4 min. Aliquots of 1.0 ml of supernatant were transferred to test tubes. Trichloroacetic acid was removed by extraction with 10 ml of ethyl ether 3 times. The mixture was then incubated in 80°C hot water to remove the residual ethyl ether. The inositol phosphates were separated on a Dowex-1 anion exchange column (50%, w/v, 1 ml) as described by Neylon and Summers (1987). In this experiment, only [^{3}H]inositol monophosphate was measured as an index of total inositol phosphate formation, because the levels of inositol bisphosphate and inositol trisphosphate were very low.

2.6. Estimation of platelet cyclic AMP and cyclic GMP

The method of Karniguian et al. (1982) was followed. Platelet suspensions were warmed at 37°C for 1 min, then prostaglandin E_1 (10 μM), rutaecarpine (40, 80 and 200 μM), or DMSO (0.5%) was added and incubated for 3 min. The incubation was stopped by adding 10 mM EDTA and boiling the mixture for 5 min. After cooling to 4°C, the precipitated protein was sedimented by centrifugation in an Eppendorf centrifuge. The supernatant (400 μl) was freeze-dried and the residue was dissolved in 100 μl of distilled water. Fifty microliters of supernatant was used to determine the cyclic AMP content using EIA kits following acetylation of the samples as described by the manufacturer. Cyclic GMP levels were determined by a similar procedure.

2.7. Data analysis

The experimental results are expressed as the means ± S.E.M. and accompanied by the number of observations. Data were assessed by the method of analysis of variance (ANOVA). If this analysis indicated significant differences among the group means, then each group was compared by the Newman-Keuls method. A P value less than 0.05 was considered significant.

3. Results

3.1. Effect of rutaecarpine on platelet aggregation in human platelet-rich plasma

Fig. 2a shows a typical two-wave tracing of platelet aggregation induced by adrenaline (10 μM) in human platelet-rich plasma. Rutaecarpine (40 and 120 μM) inhibited the second wave of aggregation, but did not affect the first wave of aggregation induced by adrenaline (10 μM). Equivalent concentrations of the vehicle DMSO (the final concentration of DMSO in the platelet suspension was fixed to 0.5%) did not significantly affect the platelet aggregation induced by adrenaline (Fig. 2a). From experiments using arachidonic acid (100 μM) to trigger platelet aggregation, we found that rutaecarpine also dose dependently inhibited arachidonic acid-induced platelet aggregation (Fig. 2b). At a higher concentration (200 μM), rutaecarpine almost completely inhibited platelet aggregation induced by arachidonic acid (Fig. 3). Furthermore, rutaecarpine also dose dependently inhibited collagen (10 μg/ml)- and ADP (20 μM)-induced platelet aggregation (Fig. 3). However, even at 200 μM, it did not completely inhibit platelet aggregation induced by collagen, ADP,

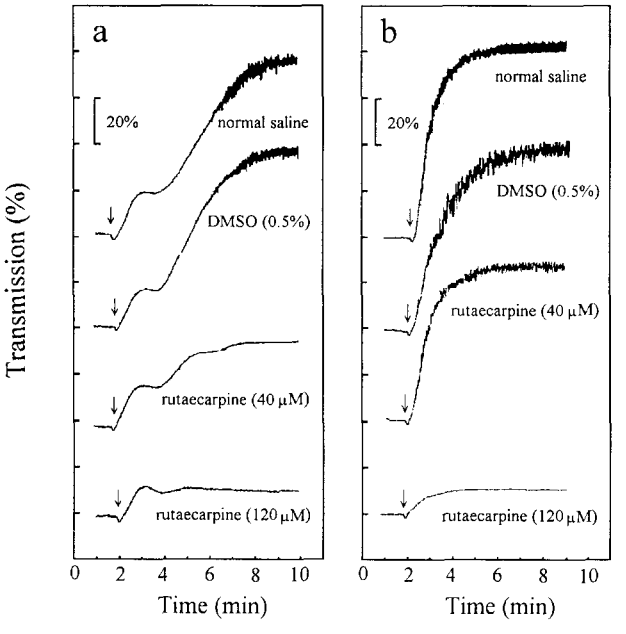

Fig. 2. Typical antiplatelet effect of rutaecarpine on adrenaline (10 μM)- and arachidonic acid (100 μM)-induced aggregation of human platelet-rich plasma. Human platelet-rich plasma was preincubated with normal saline (control), DMSO (0.5%) and rutaecarpine (40 and 120 μM) at 37°C for 1 min. (a) Adrenaline (10 μM; ↓) or (b) arachidonic acid (100 μM; ↓) was then added to induce platelet aggregation. For the detailed experimental procedure, see Section 2.

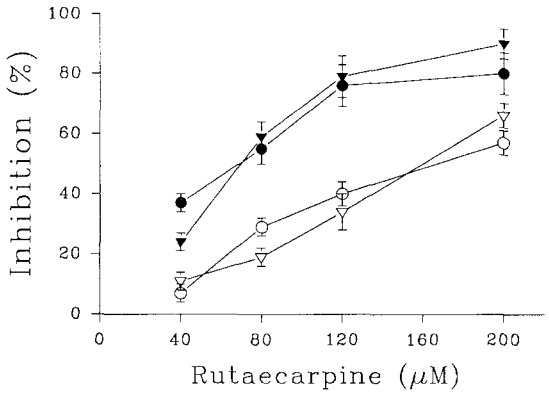

Fig. 3. Dose-inhibition curve of rutaecarpine on collagen (10 μg/ml, ○)-, adrenaline (10 μM, ●)-, ADP (20 μM, ▽)- and arachidonic acid (100 μM, ▼)-induced aggregation of human platelet-rich plasma. Human platelet-rich plasma was preincubated with various concentrations of rutaecarpine at 37°C for 1 min, and then aggregation agonists were added to trigger aggregation. Data are presented as percentage of the control (mean ± S.E.M., $n = 4 \sim 5$).

adrenaline and arachidonic acid (~90%) (Fig. 3). The IC_{50} values for platelet aggregation induced by collagen, adrenaline, ADP and arachidonic acid were estimated to be about (μM) 166.2, 64.8, 159.6 and 76.5, respectively.

Incubation of platelets with proteolytic enzymes such as pronase, α-chymotrypsin or elastase results in the exposure of specific binding sites (glycoprotein IIb/IIIa) for fibrinogen. Subsequently, the addition of fibrinogen in the absence of any aggregation inducer causes platelet aggregation (Kornecki et al., 1981, 1986; Niewiarowski et al., 1981). As shown in Fig. 4, we found that rutaecarpine (40 and 120 μM) did not significantly inhibit fibrinogen (200 μg/ml)-induced aggregation of elastase-treated platelets, indicating that the anti-aggregation effect of rutaecarpine is not directly due to interference with the binding of fibrinogen to its specific receptor on the platelet membrane surface.

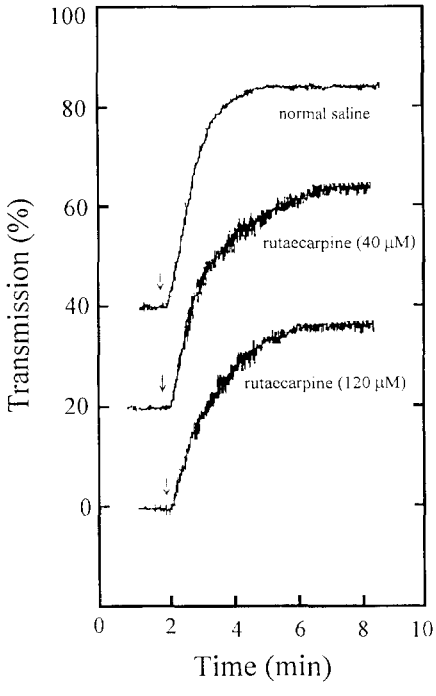

Fig. 4. Effect of rutaecarpine on fibrinogen (200 μg/ml)-induced platelet aggregation of elastase-treated human platelets. The elastase-treated human platelet suspension (4.5×10^8/ml) was preincubated with rutaecarpine (40 and 120 μM) at 37°C for 1 min. Fibrinogen (200 μg/ml, ↓) was then added to induce platelet aggregation. For the detailed experimental procedure, see Section 2.

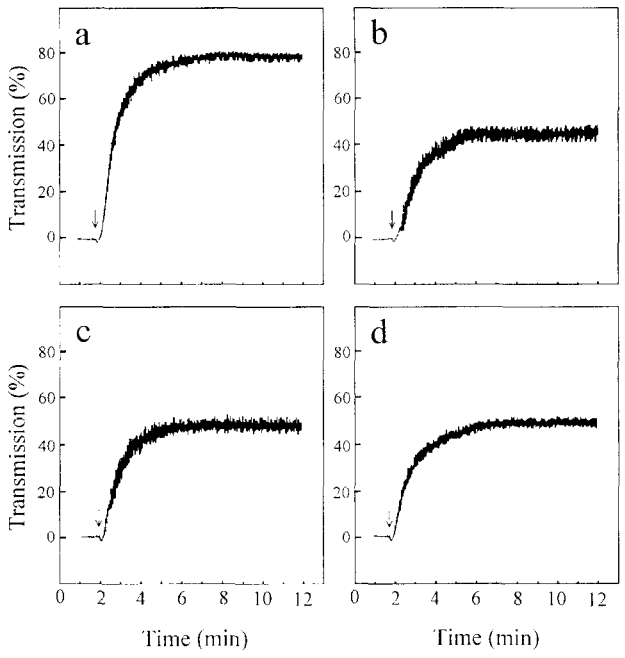

Fig. 5. Antiplatelet effect of rutaecarpine on collagen (10 μg/ml)-induced platelet aggregation with or without preincubation with N^G-mono-methyl-L-arginine (L-NMMA) (100 μM) or methylene blue (100 μM). Human platelet-rich plasma was preincubated with (a) normal saline and (b) rutaecarpine (120 μM) followed by the addition of collagen (10 μg/ml) to induce platelet aggregation, or platelets were preincubated with (c) L-NMMA (100 μM) and (d) methylene blue (100 μM) 3 min prior to the addition of rutaecarpine (120 μM), followed by the addition of collagen (10 μg/ml) to induce platelet aggregation.

Chiou et al. (1994) previously reported that rutaecarpine appears to cause vasorelaxation by releasing NO from endothelial cells. Therefore, we wondered whether rutaecarpine might also inhibit platelet aggregation by releasing NO from platelets. As shown in Fig. 5, the antiplatelet activity of rutaecarpine was not significantly attenuated by pretreatment with the NO synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA) (100 μM) (Fig. 5c) or N^G-nitro-L-arginine methly ester (L-NAME) (200 μM) (data not shown), or by the guanylyl cyclase inhibitor methylene blue (100 μM) (Fig. 5d), suggesting that platelet-derived NO does not play a significant role in the antiplatelet activity of rutaecarpine. Pretreatment with L-NMMA (50 μM), L-NAME (100 μM) and methylene blue (50 μM) clearly potentiated collagen (5 μg/ml)-induced platelet

aggregation (data not shown), suggesting that sufficient dosages of these inhibitors were employed in Fig. 5.

3.2. Effect of rutaecarpine on cyclic AMP and cyclic GMP levels in human platelets

The level of cyclic AMP in unstimulated platelets was low (0.45 ± 0.17 pmol/10^8 platelets, $n = 6$). Prostaglandin E_1 (10 μM) increased the cyclic AMP level to 6.43 ± 1.56 pmol/10^8 platelets ($n = 6$). Rutaecarpine (40, 80 and 200 μM) resulted in no significant increase in platelet cyclic AMP level (0.77 ± 0.19, 0.72 ± 0.23 and 0.86 ± 0.27 pmol/10^8 platelets, $n = 6$, respectively). Furthermore, DMSO (0.5%) did not interfere with the level of cyclic AMP in unstimulated platelets (0.36 ± 0.11 pmol/10^8 platelets, $n = 6$). Similar results were found for cyclic GMP. The level of cyclic GMP in unstimulated platelets was very low, but when nitroglycerin (100 μM) was present in the platelet suspension, the cyclic GMP level increased from the resting level of 0.28 ± 0.08 to 1.81 ± 0.37 pmol/10^8 platelets ($n = 6$). Neither DMSO (0.5%; 0.25 ± 0.10 pmol/10^8 platelets, $n = 6$) nor rutaecarpine (40, 80, and 200 μM) (0.41 ± 0.13, 0.38 ± 0.11, and 0.47 ± 0.19 pmol/10^8 platelets, $n = 6$, respectively) produced significant increases in platelet cyclic GMP levels.

3.3. Effect of rutaecarpine on thromboxane B_2 formation

As shown in Table 1, the resting platelets showed no significant thromboxane B_2 formation as compared with thrombin- or collagen-activated platelets. The vehicle control DMSO (0.5%) did not significantly increase the level of thromboxane B_2. Prostaglandin E_1 (5 μM) inhibited thromboxane B_2 formation of thrombin-activated platelets by 85% (data not shown). Furthermore, in the presence of various concentrations of rutaecarpine (40–200 μM), there were significant effects on thromboxane B_2 formation of platelets stimulated by thrombin (0.1 U/ml) or collagen (10 μg/ml) (Table 1). Therefore, rutaecarpine exerts an inhibitory effect on thromboxane A_2 formation.

Table 1
Effect of rutaecarpine on thrombin (0.1 U/ml)- and collagen (10 μg/ml)-induced thromboxane B_2 formation in human washed platelets

Treatment	Thromboxane B_2 (ng/ml)	
Resting	8.2 ± 1.2	(4)
DMSO	13.8 ± 2.5	(4)
Thrombin	165.3 ± 19.2	(4)
+ rutaecarpine 40 μM	109.3 ± 15.2 [a]	(4)
+ rutaecarpine 80 μM	105.4 ± 11.3 [a]	(4)
+ rutaecarpine 200 μM	62.8 ± 8.6 [c]	(4)
Collagen	185.7 ± 25.4	(4)
+ rutaecarpine 40 μM	124.5 ± 10.8 [a]	(4)
+ rutaecarpine 80 μM	95.6 ± 11.4 [b]	(4)
+ rutaecarpine 200 μM	70.1 ± 7.3 [c]	(4)

To human washed platelet suspensions (4.5×10^8/ml), rutaecarpine (40, 80 and 200 μM) was added 2 min before the addition of thrombin (0.1 U/ml) or collagen (10 μg/ml). The reaction was terminated 6 min after the addition of thrombin or collagen and the platelet supernatants were collected for the determination of the thromboxane B_2 level. Data are presented as means ± S.E.M. (n). [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$ as compared with thrombin (0.1 U/ml) or collagen (10 μg/ml).

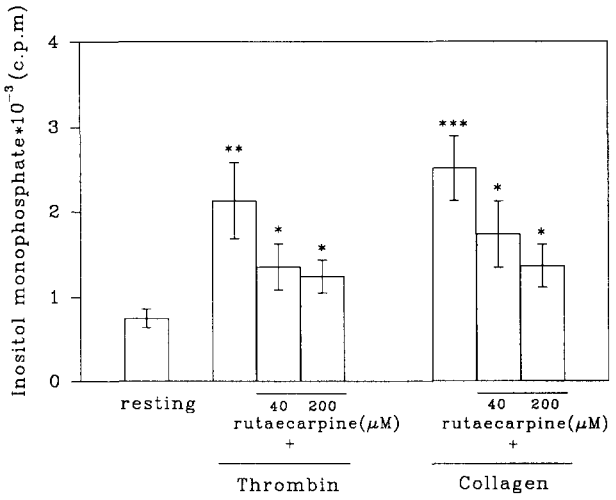

Fig. 6. Effect of rutaecarpine on thrombin- and collagen-induced inositol monophosphate formation in human washed platelets. Platelets were labeled with [^{3}H]inositol and stimulated with thrombin (0.1 U/ml) or collagen (10 μg/ml) in the presence of various concentrations of rutaecarpine. For the detailed experimental procedure see Section Section 2. Data are presented as means ± S.E.M. ($n = 4$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ as compared with resting.

3.4. Effect of rutaecarpine on phosphoinositide breakdown in human platelets

Phosphoinositide breakdown is observed in platelets activated by many agonists (Broekman et al., 1980). In this study, we found that thrombin (0.1 U/ml) and collagen (10 μg/ml) induced the rapid formation of radioactive inositol monophosphate, inositol bisphosphate and inositol trisphosphate in human platelets loaded with [^{3}H]inositol. We measured [^{3}H]inositol monophosphate formation only as an index of the total inositol phosphate formation. As shown in Fig. 6, thrombin (0.1 U/ml) and collagen (10 μg/ml) caused about 2.8- and 3.3-fold rises respectively in inositol monophosphate formation, compared to that in resting platelets (2130 ± 450 cpm, thrombin-treated platelets; 2510 ± 380 cpm, collagen-treated platelets vs. 750 ± 110 cpm, resting platelets). In the presence of prostaglandin E_1 (70 μM), thrombin did not cause a significant rise in inositol monophosphate formation (1.1-fold) (data not shown). In the presence of rutaecarpine (40 and 200 μM), inositol monophosphate formation in thrombin- and collagen-stimulated platelets was markedly decreased. These results indicate that rutaecarpine interferes with the phosphoinositide breakdown in platelets stimulated by thrombin (0.1 U/ml) and collagen (10 μg/ml).

4. Discussion

Evodia rutaecarpa has been used for a long time in Chinese medical practice. Several bioactive components have been isolated from *Evodia rutaecarpa*. For example, dehydroevodiamine has hypotensive, bradycardiac, vasodilator and antiarrhythmic effects (Yang et al., 1988, 1990). Evodiamine, a compound that is reduced to form dehydroevodiamine, can exert a positive inotropic effect on the isolated left atria of the guinea pig (Shoji et al., 1986), and an antianoxic action in KCN-induced anoxia in mice (Yamahara et al., 1989). Rutaecarpine exerts vasodilator effects on isolated rat mesenteric arteries in a dose-dependent manner (Chiou et al., 1994). However, little information relating to the effects of rutaecarpine on platelet aggregation has been reported.

The principal objective of this study was to ascertain whether or not nitric oxide (NO) is involved in the inhibition by rutaecarpine of agonist (i.e., collagen, ADP, adrenaline and arachidonic acid)-induced human platelet aggregation. The reason for this approach was based on the observation that the vasorelaxing effect of rutaecarpine appears to be endothelium-dependent and to involve NO and guanylyl cyclase. It has been reported that platelets contain an L-arginine/NO pathway and NO synthase activity in the cytosolic fraction, which may be associated with platelet aggregation (Radomski et al., 1990a,b). From the above results, we found that the antiplatelet activity of rutaecarpine was not affected by pretreatment with the NO synthase inhibitor L-NMMA or L-NAME and the guanylyl cyclase inhibitor methylene blue (Fig. 5), indicating that platelet-derived NO does not play a significant role in the antiplatelet activity of rutaecarpine (Fig. 5). Furthermore, Radomski et al. (1990b) have demonstrated that the activation of human platelets is associated with the generation of NO, and that inhibition of platelet aggregation is mediated by a cyclic GMP-dependent mechanism. Rutaecarpine did not affect the level of cyclic GMP in human platelets. Taken together, the above results suggest that the anti-aggregation effect of rutaecarpine is not exerted through the NO pathway.

In addition, the importance of cyclic AMP in modulating platelet reactivity is well established (Karniguian et al., 1982). Elevated cyclic AMP inhibits most platelet responses, including a decrease in the intracellular Ca^{2+} concentration by promoting the uptake of Ca^{2+} into the dense tubular system (Zavoico and Feinstein, 1984). From this observation we suggest that the antiplatelet activity of rutaecarpine is not due to an increase in the level of cyclic AMP. Thromboxane A_2 is an important mediator of release reactions and aggregation of platelets (Hornby, 1982). The aggregation and ATP release of platelets induced by arachidonic acid are due to thromboxane A_2 formation (Hamberg et al., 1975). Formation of thromboxane B_2, a stable metabolite of thromboxane A_2, induced by collagen and thrombin was markedly inhibited by rutaecarpine. This indicates that the antiplatelet effect of rutaecarpine is due, at least partly, to the inhibition of thromboxane A_2 formation.

It has been demonstrated that phosphoinositide breakdown could induce thromboxane A_2 formation via the release of arachidonic acid by diglyceride lipase (Bell et al., 1979) or by endogenous phospholipase A_2 from membrane phospholipids (Mckean et al., 1981). Furthermore, stimulation of platelets by agonists (i.e., thrombin) results in phospholipase C-catalyzed hydrolysis of the minor plasma membrane phospholipid phosphatidylinositol 4,5-bisphosphate, with concomitant formation of inositol 1,4,5-trisphosphate and diacylglycerol (Abdel-latif et al., 1977; Kirk et al., 1981). There is strong evidence that inositol 1,4,5-trisphosphate induces the release of Ca^{2+} from intracellular stores (Berridge, 1983). Diacylglycerol activates protein kinase C, inducing protein phosphorylation and a release reaction. Although phosphoinositide turnover is believed to be an earlier event than thromboxane synthesis, thromboxane A_2 can also activate platelets, leading to phosphoinositide breakdown (Macintyre et al., 1985). In this study, both thromboxane B_2 formation and phosphoinositide breakdown in thrombin- and collagen-activated platelets were inhibited by rutaecarpine, suggesting that inhibition of platelet aggregation by rutaecarpine is related to inhibition of thromboxane A_2 formation or phosphoinositide breakdown.

In conclusion, platelet aggregation may play a pathophysiological role in a variety of thromboembolic disorders, including myocardial infarction, cerebrovascular diseases and atherosclerosis. Therefore, prevention of platelet aggregation by drugs should provide effective prophylactic and/or therapeutic means of treating such disorders. The present observation that rutaecarpine inhibits platelet aggregation, possibly by mechanisms involving the inhibition of thromboxane formation and phosphoinositide breakdown, suggests that novel approaches could be taken in the design of more clinically effective anti-aggregatory agents.

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