Mechanism of Relaxation of Rat Aorta by Scopoletin; an Active Constituent of *Artemisia Capillaris*

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In the present work, we examined the mechanism of vasorelaxant effect of scopoletin, an active constituent of *Artemisia capillaris* on rat thoracic descending aortic rings. Scopoletin induced a concentration-dependent relaxation in rat thoracic descending aortic rings pre-contracted with phenylephrine (EC_{50} = 238.94±37.4 μM), while it was less effective in rat thoracic descending aortic rings precontracted with high potassium solution (KCl 30 mM). Vasorelaxation by scopoletin was significantly inhibited after endothelial removal, but recovered at high concentration. Pretreatment of rat thoracic descending aortic rings with N^G-nitro-L-arginine (100 μM), a nitric oxide synthase inhibitor, and atropine (1 μM), a muscarinic receptor antagonist, significantly inhibited scopoletin-induced relaxation of rat thoracic descending aortic rings. Neither indomethacin (3 μM), an inhibitor of cyclooxygenase, nor propranolol (1 μM), a β-adrenoceptor antagonist, modified the effect of scopoletin. The combination of N^G-nitro-L-arginine (100 μM) and miconazole (10 μM), an inhibitor of cytochrome P 450, did not modify the effect of scopoletin, when compared with pretreatment with N^G-nitro-L-arginine(100 μM) alone. Vasorelaxant effect of scopoletin was inverted by pretreatment with diltiazem (10 μM), a Ca^{2+}-channel blocker, at low concentration, while restored at high concentration. Apamin (K_{Ca}-channel blocker, 1 μM), 4-aminopyridine (4-AP, K_V-channel blocker, 1 mM), and tetrodotoxin (TTX, Na^+-channel blocker 1 μM) potentiated the vasorelaxant effect of scopoletin, but glibenclamide (K_{ATP}-channel blocker, 10 μM), tetraetylammonium(TEA, non-selective K-channel blocker, 10 mM) did not affect the relaxation of scopoletin. Free radical scavengers (TEMPO, catalase, mannitol) did not modify vascular tone. These results suggest that nitric oxide, Ca^{2+}-channels play a role in endothelium-dependent relaxations to scopoletin in rat aortas, that apamin, 4-AP, TTX but not glibenclamide, TEA potentiated relaxation to scopoletin mediated by these channels, and that free radicals do not concern to the vasorelaxant effect of scopoletin.

Key words : *Artemisia capillaris*, scopoletin, endothelium-derived relaxing factor(EDRF), nitric oxide(NO), endothelium-derived hyperpolarizing factor(EDHF), potassium channel, calcium channel, β-adrenoceptor.

Introduction

Artemisia capillaris (Compositae) is perennial herb growing in east Asia. The aerial part have been used to treat jaundice, hypertension, fever, etc in oriental medicine[1]. *Artemisia capillaris* extract possess choleretic, diuretic, antifebrile, depressant, antibacterial properties and the herb inhibits enterokinesia of intestine, falls down serum cholesterol and β-riboprotein[2]. Scopoletin, a active constituent of *Artemisia capillaris*[3], have a free radical scavenging property[6),7),8),9)], is a known reactants with peroxidase and is a naturally fluorescent occurring compound[6]. But H_2O_2 oxidizes scopoletin to a nonfluorescent product. Therefore scopoletin is widely used in a peroxidase assay for H_2O_2[6),7),8),9)]. Scopoletin have been shown a relaxant effect in most human smooth muscle but the mechanism is not well known[4),5)]. The purpose of our study is to manifest the relaxant mechanism of scopoletin on the rat thoracic aorta. In general, endothelium plays a important role in modulating vascular tone. Endothelial cells release various vasoactive substances, including nitric oxide(NO), prostacyclin, endothelin etc[10]. Many vasodilator substances, especially acetylcholine and bradykinin, induce an effect via the NO production of endothelial cells. Endothelium-derived relaxing factor(EDRF) has been identified as NO which is produced from L-arginine by endothelial nitric oxide synthase (eNOS) in the vascular endothelial cells. When the intracellular $[Ca^{2+}]i$ is increased, the activity of eNOS is increased and endothelial NO production is enhanced[11]. NO cause smooth muscle relaxation by increasing cyclic GMP formation[12]. Prostacyclin activates adenylate cyclase so that increase cyclic AMP formation. In this way, prostacyclin relaxes vascular smooth

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muscle. Endothelin is a vasocontractile peptide released from endothelial cells by diverse chemical and physical factor[10]. It is not confined to blood vessels, and its physiological role is not clear. Besides this, endothelium- derived hyperpolarising factor(EDHF) is related to the vasorelaxation. What EDHF is and how to produce EDHF are not yet identified with certainty. However, EDHF is released from endothelial cells and induce hyperpolarization of the vascular smooth muscle cells via the opening of potassium channels[13]. The vasodilator effect of EDHF is mediated by the activation of potassium channel on the vascular smooth muscle. Although the subtype of channel has not been identified definitively, calcium activated rather than ATP- sensitive potassium channels are likely involved in the EDHF-induced relaxation[14),15]. Although scopoletin is known to have nonspecific vasorelaxant effect, the vasorelaxant mechanism is not known in detail. Therefore, the purpose of this study is to elucidate the vasorelaxant mechanism of scopoletin on the rat thoracic aorta.

Material and methods

1. Tissue preparation

Male Sprague-Dawley rats, weighing 250-300g, were killed by cervical dislocation and thoracic aorta was removed and placed in oxygenated normal physiological salt solution containing (in mM) NaCl, 136.9; KCl, 5.40; $MgCl_2$, 1.0; $CaCl_2$, 1.5; $NaHCO_3$, 23.8; EDTA 0.001-0.01; and glucose, 5.5. The aorta was cleaned of loosely adhering fat and connective tissue and cut into rings of 2-3 mm width. Extreme care was taken to avoid damage during the isolation process. Rings were mounted between parallel stainless steel wires in organ baths at 37 ℃. The bath medium contained normal physiological salt solution which was aerated With 95 % O_2 and 5 % CO_2 to maintain the pH at 7.4.

2. Measurement of tension

The aortic rings were suspended horizontally between two stainless steel wire in organ baths filled with 10 ml normal physiological salt solution. One of the stainless steel wire was anchored to the organ bath and the other was connected to a force transducer (FT03 Grass instrument Co.) for recording of isometric tension. The rings were stretched progressively to the optimal tension (1.0 g) and allowed to equilibrated for at least 60 min prior to the execution of the experiments. And the rings were washed by replacing the fresh normal physiological solution every 10 min. After the equilibration period, the artery rings were constricted with Iso 65.4 mM K solution (composition (mM): NaCl, 76.9; KCl, 65.4; $MgCl_2$ 1.0; $CaCl_2$, 1.5; $NaHCO_3$, 23.8; EDTA, 0.001-0.01; glucose, 5.5) for twice time and the contraction was allowed to stabilize over a period of 10 min. In some preparation, the endothelium was removed mechanically by gently abrading the intimal surface of the aortic rings with the needle. The integrity of endothelium was assessed with acetylcholine-induced relaxation on the phenylephrine-preconstricted aortic rings. And then, the aortic rings were exposed again to phenylephrine. Once the plateau of the contraction elicited by phenylephrine was achieved, a cumulative concentration-relaxation curve for scopoletin (10^{-5}-10^{-3} M) was made. In some experiments, rings were incubated with an appropriate treatment for 30 min before the addiction of scopoletin.

3. Drugs used

Scopoletin, acetylcholine chloride (ACh), L-phenylephrine hydrochloride, N^G-nitro-L-arginine (L-NNA), indomethacin, propranolol, atropine sulfate, diltiazem hydrochloride, glibeclamide, tetraethylammonium chloride (TEA), 4-aminopyridine (4-AP), apamin, (±)miconazole, tetrodotoxin were obtained from Sigma Chemical Co. (St. Louis, Mo., USA). Scopoletin was dissolved in DMSO to make one molar stock solution, and diluted with ethanol to make lower molar solution. The solvent was not above 1 % of organ bath medium and induced a little contraction. (7.00± 2.60%). Indomethacin and glibenclamide were dissolved in 100 % ethanol. (±)Miconazole was dissolved in DMSO. Neither DMSO nor ethanol affected the response of the tissue at the concentration used. All other drugs were dissolved in distilled water. Drug concentrations are reported as the final molar concentration (in M) in the bath.

4. Data analysis

All data in the text and figures are presented as means ± standard error of the mean (S.E.M.). Changes in tension are shown as percentage of the contraction induced by phenylephrine or KCl. Statistical significance was assessed by Student's paired and unpaired t-test. A P value of less than 0.05 was considered statistically significant. "n" denotes the number of the rings assessed.

Results

1. Direct effect of scopoletin on the rat aortic rings

To examine the direct effect of scopoletin on vascular contraction, scopoletin alone was inserted in aortic rings mounted organ bath. Scopoletin induced relaxation of aortic rings at resting tension. (Emax = 5.0±1.02 %, n = 6, data not

shown). But scopoletin induced great concentration -dependent relaxation of phenylephrine constricted rat aortic rings (IC_{50} = 232.63±49.0 μM, Emax = 92.22±3.90 %, n = 6). Relaxation was significantly inhibited by endothelium -denudation (IC_{50} = 462.92±20.24 μM, Emax = 89.09±2.96 %, n=6). Endotheilum denudation shifted the dose-response curve rightward. But scopoletin produced the same degree of relaxation between endothelium-intact and denuded aortic rings (Fig. 1 & 2).

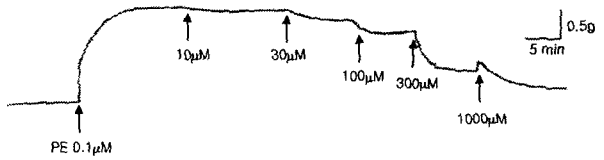

Fig. 1. Representative trace shows relaxant response of scopoletin in rat aortic rings precontracted with 0.1 μM phenylephrine.

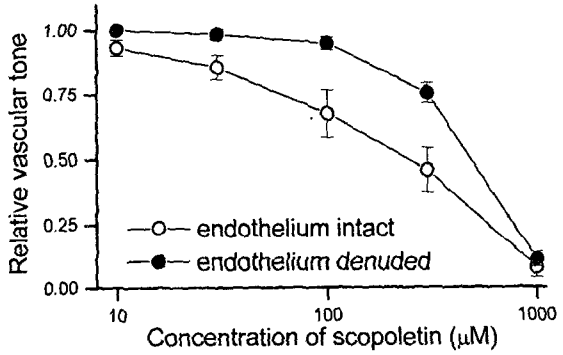

Fig. 2. Concentration response curve for relaxation induced by scopoletin in rat aortic rings with and without endothelium. The aortic rings were precontracted with 0.1 μM phenylephrine. Each point represents the mean of six experiments with S.E.M. shown by vertical bar.

2. Effect of propranolol on scopoletin-induced relaxation of rat aortic rings

To examine the hypothesis that scopoletin produces the relaxation via stimulation of β-adrenoceptor, propranolol (β -adrenoceptor antagonist, 1 μM.) was incubated for 10 min before following relaxation by scopoletin. Propranolol did not affect the scopoletin-induced relaxation on aortic rings (data not shown).

3. Effect of atropine on scopoletin-induced relaxation

To examine that muscarinic receptor is concerned to relaxation of scopoletin, atropine (muscarinic receptor antagonist. 1 μM) was incubated for 10 min before following relaxation by scopoletin. Incubation with atropine inhibited relaxation of aortic rings by scopoletin. Atropine shifted dose-response curve rightward (IC_{50} = 263.07±43.5 μM, 388.20 ±35.7 μM. Emax = 79.32±2.10 %, 72.04±6.12 %, control and incubation with atropine, respectively) (Fig. 3).

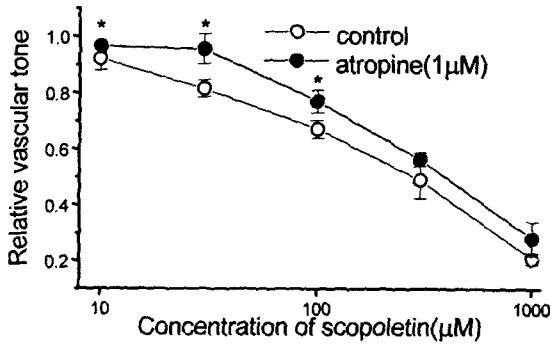

Fig. 3. Concentration response curve for relaxation induced by scopoletin in rat aortic rings with endothelium. The effect of atropine (1 μM) on scopoletin-induced relaxation of rat aortic rings. Atropine significantly inhibited relaxant effect of scopoletin. Each point represents the mean of six experiments with S.E.M. shown by vertical bar. *: Significantly different (P < 0.05) from control values.

4. Effect of N^G-nitro-L-arginine on scopoletin-induced relaxation.

We were convinced of that scopoletin-induced relaxation is endothelium-dependent through prior experiment, so we tested the effect of N^G-nitro-L-arginine (nitric oxide synthase inhibitor. 100 μM) on the scopoletin-induced relaxation to confirm that NO is related to endothelium-dependent relaxation of scopoletin. Incubation with L-NNA inhibited the relaxation of scopoletin, and shifted dose-response curve to the right 2.02 fold (IC_{50} = 217.32±21.7, 440.45±22.6 μM. control and incubation with L-NNA, respectively) with no reduction in maximum response (Fig. 4).

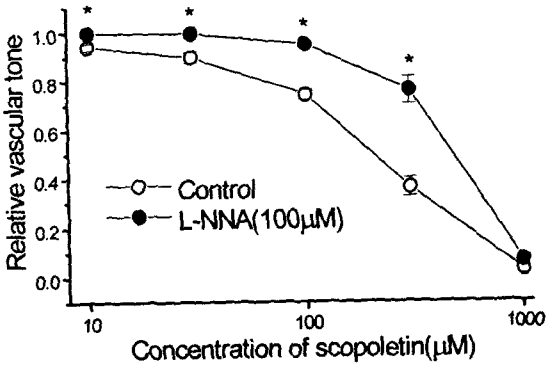

Fig. 4. Concentration response curve for relaxation induced by scopoletin in rat aortic rings with endothelium. The effect of L-NNA (100 μM) on scopoletin-induced relaxation of rat aortic rings. L-NNA significantly inhibited relaxant effect of scopoletin. Each point represents the mean of six experiments with S.E.M. shown by vertical bar. *: Significantly different (P < 0.05) from control values.

The Effect of L-NNA was equal to that of endothelial denudation. Therefore pretreatment with L-NNA completely inhibited endothelium-dependent relaxation of rat aorta by scopoletin. In addition, we investigated the probability that endothelium-dependent relaxation may be induced via another EDRFs except NO. But indomethacin (inhibitor of cyclooxygenase. 10 μM) did not affect scopoletin -induced relaxation of rat aorta, and combination of L-NNA and indomethacin did not further inhibit scopoletin-induced relaxation compared with pretreatment with L-NNA alone (data not shown).

5. Calcium channel & calcium ion

To investigate role of calcium ion on scopoletin-induced relaxation of rat aorta, we assessed the effect of scopoletin on KCl-preconstricted rat aorta. Relaxant response was reduced than phenylephrine-constricted rat aorta. IC_{50} values were 238.94±37.4 μM and 399.64±35.8 μM in PE & KCl respectively (Fig. 5A). Pretreatment with diltiazem (calcium channel blocker. 10 μM) induced contraction at concentration under 100 μM but restored relaxant effect at concentration above 100 μM (Fig. 5B).

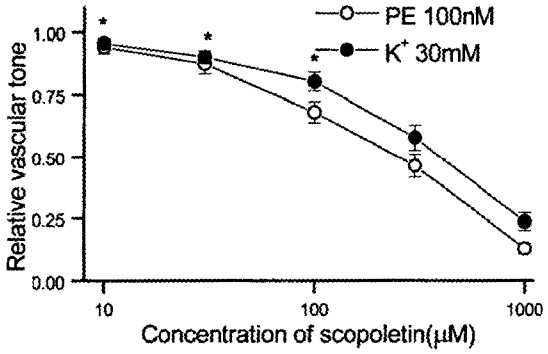

Fig. 5-A. Concentration response curve for relaxation induced by scopoletin in rat aortic rings with endothelium. The effect of high potassium solution (30 mM) on scopoletin-induced relaxation of rat aortic rings. When aortic rings were contracted with high potassium solution, relaxant effect of scopoletin was significantly inhibited. Each point represents the mean of six experiments with S.E.M. shown by vertical bar. *: Significantly different (P < 0.05) from control values.

6. Effect of miconazole on scopoletin-induced relaxation

To investigate whether scopoletin induce relaxation via production of EDHF (endothelium-derived hyperpolarizing factor) or not, we carried experiment with miconazole (inhibitor of cytochrome P450. 1 μM) pretreated. Combination of L-NNA and miconazole did not further inhibit scopoletin-induced relaxation compared with pretreatment with L-NNA alone (data not shown).

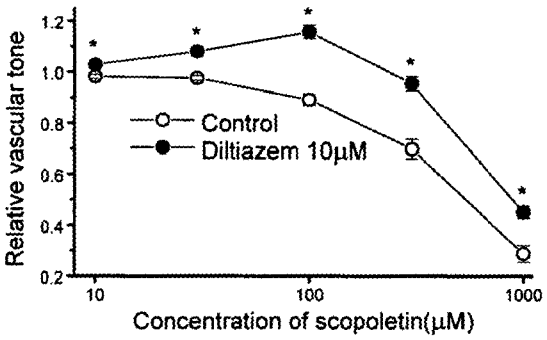

Fig. 5-B. Concentration response curve for relaxation induced by scopoletin in rat aortic rings with endothelium. The effect of diltiazem (10 μM) on scopoletin-induced relaxation of rat aortic rings. Diltiazem converted relaxant effect of scopoletin and induced contraction from 10 μM to 100 μM. Each point represents the mean of six experiments with S.E.M. shown by vertical bar. *: Significantly different (P < 0.05) from control values.

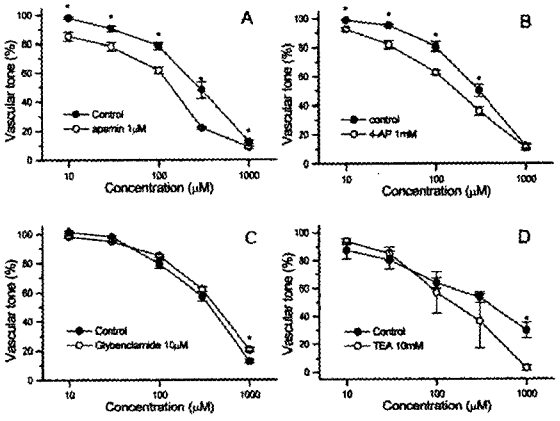

Fig. 6. Concentration response curve for relaxation induced by scopoletin in rat aortic rings with endothelium. A, The effect of apamin (1 μM) on scopoletin-induced relaxation of rat aortic rings. B, The effect of 4-aminopyridine (1 mM) on scopoletin-induced relaxation of rat aortic rings. C, The effect of glibenclamide (10 μM) on scopoletin-induced relaxation of rat aortic rings. D, The effect of tetraethylammonium (10 mM) on scopoletin-induced relaxation of rat aortic rings. Apamin and 4-aminopyridine potentiated the relaxant effect of scopoletin but glibenclamide and tetraethylammonium did not modify relaxant effect of scopoletin. Each point represents the mean of six experiments with S.E.M. shown by vertical bar. *: Significantly different (P < 0.05) from control values.

7. Effect of potassium channel blockers on scopoletin-induced relaxation

We assessed if scopoletin induced relaxation via efflux of potassium ion through potassium channel on cell membrane. But potassium channel blockers did not inhibit scopoletin -induced relaxation. Tetraethylammonium, non selective K^+ channel blocker (TEA 10 mM), and glibenclamide, K_{ATP} channel blocker (10 μM) did not affect relaxant effect of scopoletin. On the contrary, apamin, K_{Ca} channel blocker (1 μM) and 4-aminopyridine, K_V channel blocker (4-AP 1 mM) potentiated relaxant effect of scopoletin (Fig. 6 & Table 1).

Table 1. IC_{50} values of relaxant effect of scopoletin in control groups and in potassium channel blocker incubation groups.

	control	after incubation
apamin (1μ M)	280.77±23.0	137.05±34.2
4-aminopyridin (1mM)	292.61±15.0	168.59±16.5
glibenclamide (10μ M)	329.86±46.0	418.16±26.6
tetraethylammonium (10mM)	427.72±126.0	152.73±32.0

In addition, scopoletin reduced contractile response of phenylephrine to rat aorta. Incubation with scopoletin 300 μM reduced contractile response about 50 % compared with control group. Contractile response was restored by several washing. Incubation with tetrodotoxin (sodium channel blocker. 1 μM) potentiated relaxant effect of scopoletin. Scopoletin have free radical scavenging property so that may resist against inactivation of NO. But several free radical scavengers (TEMPO 300 μM, catalase 1000 unit, mannitol 1 μM) did not modify tension of phenylephrine-constricted rat aortic rings. So, we could exclude hypothesis that scopoletin induce relaxation of rat aorta via free radical scavenging property.

Discussion

Endothelial cells release a factor that relaxes the underlying vascular smooth muscle and this was later shown to be mainly nitric oxide or a related compound[16]. Endothelial cells constitutively express a NO synthase that generates NO using L-arginine as a substrate. The relaxation of vascular smooth muscle by NO involves the stimulation of soluble guanylate cyclase and consequently the increased formation of cyclic GMP. The latter activates cyclic GMP-dependent protein kinase which leads to an increased extrusion of Ca^{2+} from the cytosol in vascular smooth muscle, and to the inhibition of contractile machinery. Cyclic GMP-dependent protein kinase phosphorylates K^+ channel to induce hyperpolarization and thereby inhibits vasocontriction[17]. Therefore if production of NO is reduced, relaxation of vessels will be decreased. Though production of NO is completely disappeared, Endothelium-dependent relaxation is preserved about 40~50 %[18]. This fact means that other relaxant agent is released from endothelium. At present prostacyclin and endothelium-derived hyperpolarizing factor are known as other relaxant agent[19]. Prostacyclin is primarily produced by endothelial cells in the vascular wall. however, unlike NO its vasodilator activity is determined by the expression of specific receptors in vascular smooth muscle. Hence, in arterial bed that do not express such receptors, prostacyclin does not participate in endothelium-dependent vasodilation. Prostacyclin-receptors are coupled to adenylate cyclase to elevate cyclic AMP levels in vascular smooth muscle. In turn stimulates ATP-sensitive K^+ channel to cause hyperpolarization of the cell membrane and inhibit the development of contraction. Cyclic AMP also increases the extrusion of Ca^{2+} from the cytosol in vascular smooth muscle, and to the inhibition of contractile machinery[19]. In addition to the complementarity of their respective mechanisms of action in target tissues, prostacyclin facilitates the release of NO by endothelial cells. Futhermore, the action of prostacyclin in vascular smooth muscle is potentiated by NO. Endothelium derived hyperpolarizing factor (EDHF) also contributes to endothelium-dependent vasodilation as production of NO is inhibited[20]. EDHF activates various K^+ channels, thus induces hyperpolarization of vascular smooth muscle cells. Hyperpolarization inhibits vasoconstriction by closing voltage-sensitive Ca^{2+} channels, impairing the receptor-dependent activation of phospholipase C and the subsequent release of Ca^{2+} from intracellular stores as well as by reducing the Ca^{2+} sensitivity of the contractile proteins. At present, scopoletin is known only as a non-specific spasmolytic agent[4]. In the past studies, there were the following conclusion; 1. Scopoletin have the relaxant effect, but this effect does not reduced nor disappeared by atropine (antagonist of muscarinic receptor) or hexamethonium (antagonist of nicotinic receptor). Therefore the relaxant effect of scopoletin is not likely to be mediated via cholinergic mechanisms[5]. 2. Depressor effect of scopoletin is not modified by mepyramine. Therefore effect of scopoletin is not mediated via histamine H1 receptor[4]. 3. Scopoletin has a relaxant or spasmolytic effect on the most of smooth muscles. Probably the effect of scopoletin is related with membrane stabilizing activity or non-specific spasmolytic action[5]. 4. Scopoletin inhibits electrical stimulation-evoked response of peripheral nerves, so scopoletin has a adrenergic neuron blocking action. But this action is not mediated via the mechanism that guanetidine showing a effect ; blocking the release of adrenergic neurotransmitter, because adrenergic neuron blocking action is not restored by dexamphetamine[4]. 5. Depressor effect of scopoletin is related with smooth muscle relaxant property and membrane stabilizing action coupled non-specific spasmolytic property[4,5]. Scopoletin has effects as to not only blood vessels but also other tissues as followings; 1. Scopoletin suppresses inducible NOS mRNA, subsequently decreases induction of iNOS protein[21,22]. Therefore scopoletin inhibits production of inducible NO. 2. Scopoletin has a choleretic effect so that improves a condition like jaundice[23,24]. 3. Scopoletin has a reactive oxygen-scavenging property, antioxidation effect and free radical scavenging effect. Thus scopoletin maintains glutathione content and superoxide dismutase and reduces production of malondialdehyde from CCl_4-intoxicated primary cultured rat hepatocytes. So

scopoletin expresses hepatoprotective activity[25),26)]. 4. Scopoletin enhances eosinophil activation and has eosinophils release superoxide[27)]. Scopoletin has diverse action, but used to only limited field; assay of hydrogen peroxide. To seek a new applications of scopoletin, we carried out our experiment. Scopoletin induced dose-dependent relaxation of rat aorta. This relaxant effect was come out after 10-20 second latency period. This fact means that the relaxant effect of scopoletin is not likely to induced by gene expression or suppression of gene expression. Relaxant effect of scopoletin was more efficient in endothelium intact aorta. But scopoletin is thought to have both endothelium-dependent and independent relaxant effect because L-NNA treatment failed in complete inhibition of relaxation. In general, β-adrenoceptor of sympathetic nerve play a important role to relaxation of blood vessels. Propranolol, antagonist of β-adrenoceptor, did not modify scopoletin induced relaxation, so scopoletin-induced relaxation is not related to β-adrenoceptor. Indomethacin did not modify scopoletin-induced relaxation. This experiment means that prostacyclin is not related. And combination of L-NNA and indomethacin did not modify relaxation compared with L-NNA alone. Miconazole is known to inhibit production of EDHF, also did not efficiently inhibit scopoletin-induced relaxation. EDHF is other vasodilative substance released from endothelial cells, but action of EDHF is feed back negatively by NO in physiological condition[28)]. The effect of EDHF is come out compensatory in pathological condition that production of NO is inhibited. And combination of miconazole and L-NNA did not more inhibit relaxation compared with L-NNA alone. Atropine, antagonist of muscarinic receptor, inhibited scopoletin-induced relaxation, this fact is opposite to past studies. We think that it is natural to inhibit relaxant effect of scopoletin because action of scopoletin is induced by production of NO. This fact means that scopoletin produces NO via stimulation of endothelial muscarinic receptors. But fail to complete inhibition is due to other relaxant effect of scopoletin. Pretreatment with diltiazem, calcium channel blocker, induced inverse effect of scopoletin. So to speak, socopoletin induced further contraction below 100 μM concentration. It is probably due to inhibition of calcium influx into endothelial cells[29),30)], subsequently NO production is suppressed and stimulation of muscarinic receptor on smooth muscle cells not endothelial cells. But above 100 μM concentration, relaxant effect of scopoletin was restored, and this effect may be endotheilum-independent. We incubated several potassium channel blockers to prevent relaxant effect of scopoletin. Glibenclamide and TEA did not modify scopoletin-induced relaxation. Contrary to our presumption, 4-AP and apamin potentiate relaxant effect of scopoletin. Generally speaking, the receptor-binding agonists cause an increase in $[Ca^{2+}]i$ by mobilization of Ca^{2+} from intracellular stores, which subsequently leads to the opening of Ca^{2+}-dependent K^+ channels in endothelial cells. The opening of the Ca^{2+}-dependent K^+ channel increases K^+ efflux, hyperpolarizing the endothelial cells. This hyperpolarization provides the driving force for transmembrane Ca^{2+} influx into endothelial cells and thus causes synthesis and release of NO[31)]. In smooth muscle cells, closing of K^+ channel depolarize cells and exhibit contraction of smooth muscle. But our results is opposite to general truth, suggesting that scopoletin also may have contractile effect on smooth muscle via closing of K^+ channel. Although scopoletin have contractile effect, contraction is not exhibited because relaxant effect is predominant than contractile effect. This results remain to be elucidative. Incubation with tetrodotoxin potentiated relaxant effect of scopoletin. This effect of tetrodotoxin is shown because depolarization is inhibited by closing Na^+ channel. In physiological condition, free radical exhibits relaxant effect on blood vessels but, in pathological condition, a large amount of free radical is produced, contracts and inhibits relaxation of blood vessels. Scopoletin has free radical scavenging effect[32),33)] so that may prevent inactivation of NO by free radicals[34)]. But this effect of scopoletin is not affect to the relaxant response of scopoletin because other potential free radical scavengers (TEMPO, catalase, mannitol) did not modify relaxant effect of scopoletin nor tension of blood vessels. We did not deal with endothelium-independent relaxation of scopoletin. This part remains to be elucidative. In conclusion, scopoletin exhibits dose-dependent relaxant effect on rat thoracic aorta and this effect is consisted of endothelium-dependent and independent relaxation. Endothelium-dependent relaxant effect of scopoletin is expressed by NO production via stimulation of muscarinic receptor on endothelial cells.

Conclusion

Many studies reported that scopoletin induced nonspecific relaxation on most of smooth muscle. And other studies announced that scopoletin inhibited the synthesis of inducible NO in RAW 264.7 cells stimulated INF-γ and LPS. We conducted experiments to reveal whether scopoletin induced relaxation through the synthesis of NO or not in rat thoracic descending aorta. And we arrived at result as below.

1. Scopoletin induced concentration-dependent relaxation in rat thoracic descending aorta precontracted with phenylephrine 0.1 μM, and when endothelial cells were removed, relaxant

effect of scopoletin was inhibited significantly.

2. Pretreatment with propranolol and pretreatment with miconazole did not modify the effect of scopoletin.
3. Pretreatment with atropine significantly inhibited the relaxant of scopoletin.
4. Pretreatment with L-NNA significantly inhibited the relaxant effect of scopoletin.
5. The relaxant effect of scopoletin was siginficantly decreased in rat thoracic aorta precontracted with high potassium solution (KCl 30 mM).
6. Pretreatment with diltiazem inverted the relaxant effect of scopoletin, but at high concentration the relaxant effect was restored.
7. The relaxant effect of scopoletin was potentiated by Pretreatment with tetrodotoxin, pretreatment with 4-aminopyridine, and pretreatment with apamin, but pretreatment with glibenclamide and pretreatment with tetraethylammonim did not modify the effect of scopoletin.
8. Pretreatment with scopoletin significantly decreased contractile force of phenylephrine, and several free radical scavengers (TEMPO, catalase and mannitol) did not modify the effect of scopoletin.

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