Contribution of potassium channels, beta2-adrenergic and histamine H1 receptors in the relaxant effect of baicalein on rat tracheal smooth muscle

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Abstract

Objective(s): Baicalein, a compound extracted from a variety of herbs, showed various pharmacological effects. This study evaluated the relaxant effects of baicalein and its underlying molecular mechanisms of action on rat's isolated tracheal smooth muscle.

Materials and Methods: Tracheal smooth muscle were contracted by 10 μM methacholine or 60 mM KCl and the effects of cumulative concentrations of baicalein (5, 10, 20 and 40 mg/ml) and theophylline (0.2, 0.4, 0.6 and 0.8 mM) were evaluated. To examine the possible mechanism(s) of the relaxant effect of baicalein, its effect was also evaluated on incubated tissues with atropine, indomethacin, diltiazem, N(G)-Nitro-L-arginine methyl ester (L-NAME), glibenclamide, propranolol and chlorpheniramine.

Results: A concentration-dependent and significant relaxant effect was seen for baicalein in non-incubated tissues contracted by KCl or methacholine (P<0.01 to P<0.001). No significant difference was seen between the relaxant effects of high concentrations of baicalein and theophylline. The relaxant effects of all concentrations of baicalein in incubated tissues with glibenclamide, propranolol and chlorpheniramine were significantly lower than non-incubated tissues (P<0.05 to P<0.001). Additionally, the EC50 values of baicalein in incubated tissue with propranolol was significantly higher than non-incubated condition (P<0.05).

Conclusion: A potent relaxant effect comparable to the effect of theophylline was shown for baicalein, which was probably mediated via inhibition of histamine (H1) receptors, stimulation of beta2-adrenergic receptors and potassium channels activation.

Introduction

Flavonoids comprise a large group of naturally existing polyphenolic compounds widely distributed throughout the plant kingdom (1). The flavonoids, baicain and its aglycone, baicalein (5,6,7-trihydroxy-2-phenyl-4H-1-benzopyran-4-one) are found in edible medicinal plants, Scutellaria baicalensis Georgi, Scutellaria viscidula Bge, Scutellaria lixiangensis Diels, Scutellaria amoenaec H. Wright, Scutellaria rehderiana Diels, Scutellaria hypericifoliaevel, Oroxyllum indicum L. Kurz and Plantago major in abundant quantities (2-6). The anti-inflammatory and antioxidant effects of these flavonoids were demonstrated in various disease models (3, 7, 8). Flavonoids also modulate vascular tone and the potency of relaxant effect of flavonoids were reported as follow; flavonol > flavones > flavanol (1).

Various neurotransmitters, mediators and drugs influence airway smooth muscle (ASM) which most of them are mediated by cell surface receptors. Therefore, various bronchodilators and bronchoconstrictors have now been identified (9). Although, the vasodilatory effects of baicalein have been reported (10) but so far, the relaxant effect of baicalein has not been shown on the ASM. Within such a context, the aim of the present study was evaluation of the relaxant effects of baicalein and its underlying molecular mechanisms of action in rat's isolated tracheal smooth muscle (TSM).

Materials and Methods

Materials

Baicalein (C15H10O5) with CAS Number 491-67-8 was purchased from Sigma Chemical Co Ltd. Potassium chloride (KCl) was obtained from Merck (Darmstadt, Germany). Methacholine, atropine, chlorpheniramine, indomethacin, diltiazem, glibenclamide, propranolol, and N(G)-Nitro-L-arginine methyl ester (L-NAME) were also purchased from Sigma Chemical Co, Ltd.

Animals

Fifty-six young male Wistar rats (200–250 g) purchased from the Animal House, Faculty of Medicine, Mashhad University of Medical Sciences (Mashhad, Iran). The animals were maintained under controlled condition at 12/12 hr light/dark cycle and 22 ± 2 °C. Water and food ad libitum was always accessible to animals. The Ethics Committee of Mashhad University of Medical Sciences (Code; 941083) confirmed the study protocol. The study was carried out according the regulations of the Institute of Laboratory Animals Resources Commission on Life Sciences (11).

Preparation of tracheal ring

Tracheal rings of rats were prepared, mounted in 10 ml organ bath containing Krebs-Henseliet solution (KHS),...
and maintained at 37 ± 0.5 °C with isometric tension of 1 g as previously described (12, 13). In all experiments, contraction responses were measured using an isometric transducer (MLT0202, AD Instruments, Australia) which was connected to a power lab system (Power Lab 8/30, ML870, AD Instruments, Australia).

**Measurement of tracheal smooth muscle relaxation**

TSM relaxation was examined according to the method described previously (12, 13). Briefly, TSM was contracted by 10 μM methacholine for 7 min or 60 mM KCl for 5 min and the cumulative concentrations of baicalein (5, 10, 20 and 40 mg/ml) (14), theophylline (0.2, 0.4, 0.6 and 0.8 mM) as a positive control, or 1 ml of normal saline (NS) as a negative control were added to the tissue bath every 5 min (Figure 1).

The concentration-response curves of the relaxant effect of baicalein was constructed in each experiment and its effective concentration causing 50% of maximum response (EC50) was calculated as previously described (12).

**Experimental groups**

In order to examine the possible mechanism(s) of the relaxant effect of baicalein (15), its relaxant effect was evaluated in various groups as described in Table 1.

**Statistical analysis**

The results were described as the mean±SEM. The comparison of the results was done using One-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test. Statistical significance was considered at P<0.05.

**Table 1.** The protocol of the study and the methods of evaluating of various mechanisms of the relaxant of effect of baicalein on tracheal smooth muscle

| Contraction agent | Condition | Incubating substance | Mechanisms |
|-------------------|-----------|----------------------|------------|
| 60 mM KCl         | Non-incubated tissues (n=7) | 1 μM atropine (n=5) | Muscarinic receptor inhibition |
|                   | Incubated tissues | 1 μM indomethacin (n=7) | Cyclooxygenase inhibition |
| 10 μM methacholine| Non-incubated tissues (n=6) | 1 μM chlorpheniramine (n=6) | Histamine (H1) receptor inhibition |
|                   | Incubated tissues | 5 μM diltiazem (n=6) | Calcium channel blocking |
|                   |                 | 1 μM glibenclamide (n=7) | Potassium channel opening |
|                   |                 | 1 μM propranolol (n=6) | β-adrenoceptor stimulation |
|                   |                 | 300 μM L-NAME (n=6) | Inhibition of nitric oxide synthesis |

L-NAME: N(G)-Nitro-L-arginine methyl ester
Results

The relaxant effects of baicalein on tracheal smooth muscle contracted by KCl

Concentration-dependent and significant relaxant effect was seen for baicalein in the tissues contracted by KCl (P<0.05 for 5 mg/ml and P<0.001 for 10, 20 and 40 mg/ml). The relaxant effects of 5 and 10 mg/ml of baicalein were significantly lower than theophylline (P<0.05 and P<0.01, respectively, Figure 2a).

Baicalein showed concentration-dependent and significant relaxant effects in incubated TSM with atropine (P<0.05 for 10 mg/ml and P<0.001 for 20 and 40 mg/ml, Figure 2b) and indomethacin (P<0.05 for 5 mg/ml and P<0.001 for 10, 20 and 40 mg/ml, Figure 2c). No significant difference was observed in the relaxant effects of baicalein between non-incubated and incubated tissue with atropine or indomethacin (Figure 2).

There was no significant difference in EC50 values of baicalein between non-incubated tissues concentrated by KCl (7.5±2.73) and incubated with atropine (12.5±2.49) or indomethacin (8.1±3.58, Figure 2d).

The relaxant effects of baicalein on tracheal smooth muscle contracted by methacholine

Concentration-dependent and significant relaxant effect was observed for baicalein in the tissues contracted by methacholine (P<0.05 for 5 mg/ml and P<0.001 for 10, 20 and 40 mg/ml, Figure 3a). There was no significant difference between the relaxant effects of 10, 20 and 40 mg/ml of baicalein and theophylline, but the relaxant effect of 5 mg/ml of baicalein was significantly lower than theophylline (P<0.05, Figure 3a).

In incubated TSM with diltiazem, baicalein showed a concentration-dependent and significant relaxant effect (P<0.001 for 10, 20 and 40 mg/ml). There was no significant difference between the relaxant effects of baicalein in non-incubated and incubated tissues with diltiazem (Figure 3b).

Concentration-dependent and significant relaxant effect were seen for baicalein in incubated tissues with...
Figure 4. Concentration-response curves of the relaxant effect (mean±SEM) of baicalein on methacholine (10 μM) induced contraction of tracheal smooth muscle in non-incubated (n=6) and incubated tissues with (a) glibenclamide (1 μM, n=7), (b) propranolol (1 μM, n=6), (c) chlorpheniramine (1 μM, n=6). (d) EC_{50} values of baicalein induced relaxation obtained on contracted TSM of rat with methacholine (10 μM) in non-incubated (n=6) and incubated tissues with diltiazem (n=6), L-NAME (n=6), glibenclamide (n=7), propranolol (n=6), and chlorpheniramine (n=6). TSM relaxation was presented as percent change in proportion to maximum contraction due to 10 μM methacholine. *: P<0.05, **: P<0.01, ***: P<0.001 compared to non-incubated tissues.

L-NAME (P<0.05 for 10 mg/ml and P<0.001 for 20 and 40 mg/ml, Figure 3c). The relaxant effects of 20 mg/ml of baicalein in incubated tissues with L-NAME was significantly lower than non-incubated TSM (P<0.05 Figure 3c).

In incubated TSM with glibenclamide, a concentration-dependent and significant relaxant effect was seen for baicalein (P<0.001 for 10, 20 and 40 mg/ml, Figure 4a). The relaxant effects of all concentrations of baicalein in incubated tissues with glibenclamide were significantly lower than non-incubated TSM (P<0.05 for 5 mg/ml and P<0.01 for 10, 20 and 40 mg/ml, Figure 4a).

Only two last concentrations of baicalein showed significant relaxant effects in incubated tissues with propranolol (P<0.01 and P<0.001 for 20 and 40 mg/ml, respectively, Figure 4b) and incubated tissues with chlorpheniramine (P<0.01 and P<0.001 for 20 and 40 mg/ml, respectively, Figure 4c). The relaxant effects of all concentrations of baicalein in incubated tissues with propranolol and chlorpheniramine were significantly lower than non-incubated TSM (P<0.05 to P<0.001, Figure 4b and 4c).

No significant difference was observed in EC_{50} values of baicalein between non-incubated and contracted tissues by methacholine (6.9±2.75) and incubated tissue with chlorpheniramine (13.1±4.295), diltiazem (7.0±2.93), glibenclamide (10±1.355) or L-NAME (9.5±4.51). The EC_{50} values of baicalein in incubated tissue with propranolol (15.8±2.47) was significantly higher than non-incubated condition (P<0.05, Figure 4d).

Comparison of the relaxant effects of baicalein in tissues contracted by KCl with those of methacholine

There was no significant difference in the relaxant effects of various concentrations of baicalein obtained in KCl-induced contraction with those in TSM contracted by methacholine (Figure 5).

Discussion

The results of this study showed concentration-dependent relaxant effects of baicalein in non-incubated TSM contracted by KCl or methacholine, which may indicate possible bronchodilatory effect of this agent in airway of patient with airway constriction.

In TSM incubated with atropine, indomethacin, diltiazem and L-NAME, there were no significant difference in the relaxant effects of baicalein between non-incubated and incubated tissues. These results indicated that the relaxant effect of baicalein is not due to muscarinic receptors, arachidonic acid metabolism and cyclooxygenase pathways, calcium channel blocking and NO production.

To evaluate the effect of baicalein on histamine (H1) receptors, beta2-adrenergic receptors and potassium channel activation, the relaxant effects of baicalein were examined on TSM incubated with chlorpheniramine, propranolol and glibenclamide, respectively. The relaxant effects of all concentrations of baicalein in incubated tissues with chlorpheniramine, propranolol and glibenclamide were significantly lower than non-incubated tissues. These results indicated inhibitory effect of baicalein on histamine (H1) receptors, its stimulatory effect on beta2-adrenergic receptors and its opening effect on potassium channels are responsible in its relaxant effects on TSM. Lower relaxant effect of some concentrations of baicalein on incubated tissues with chlorpheniramine and propranolol compared to the effects obtained in tissues incubated with other agent also support this mechanism of action for baicalein (Table 2). In addition, EC_{50} baicalein in incubated tissues with propranolol was significantly higher than that of non-incubated TSM, which supports the stimulatory effect of this agent on beta2-adrenergic receptors. Taken together, these findings suggest the possible inhibitory effect of baicalein on histamine (H1) receptors, its...
Contractile mechanisms mediated by protein kinase at higher concentrations through inhibition of the Baicalein relaxed the arterial smooth muscle partially release of arachidonic acid derived vasoconstrictor lipoxygenase, resulting in reduced biosynthesis and bronchodilation by maxi-K channel opening. denuded arteries (14). Therefore, bacalein could induce involved in baicalein-induced relaxation in endothelium-glibenclamide-sensitive potassium channels was not dependent potassium channels, while activation of the activation effect on potassium channels.

Baicalein have been thought to be as the inhibitory agent for chemical mediator release from mast cells in vitro and allergic immediate phase reactions in skin and airway in vivo (16). The bronchoconstrictory effect of histamine is mediated via H1 receptors (9). Until now, the relaxant effect of baicalein on TSM has not been reported, while baicalin showed anti-asthmatic activity in isolated tracheal muscle from asthmatic guinea pigs (17).

Biological activity studies have indicated that baicalein has a beta1-adrenergic receptors antagonistic effect (6), while the relationship between baicalein and beta2-adrenergic receptors is unknown. Probably, baicalein stimulates beta2-receptors and increases production of cyclic adenosine monophosphate (cAMP), which leads to the characteristic cellular response via the activation of protein kinase A (PKA). In ASM cells, PKA phosphorylates certain potassium channel opener, leads to potassium efflux from the cell, membrane hyperpolarization, and relaxation.

The predominant K1 channel in ASM is the maxi-K channel, which may be opened by cAMP, but also through direct coupling of beta2-receptors via Gs proteins (9). Thus, beta2-agonists may cause bronchodilatation via a direct effect of maxi-K channels as well as through an increase in cAMP (9). Glibenclamide is known to block ATP-dependent potassium channels. The present study suggests that baicalein probably activates ATP-dependent potassium channels, while activation of glibenclamide-sensitive potassium channels was not involved in baicalein-induced relaxation in endothelium-denuded arteries (14). Therefore, bacalein could induce bronchodilation by maxi-K channel opening.

In other smooth muscles, baicalein inhibited lipoxygenase, resulting in reduced biosynthesis and release of arachidonic acid derived vasoconstrictor products such as aortic smooth muscle cells (18). Baicalein relaxed the arterial smooth muscle partially at higher concentrations through inhibition of the contractile mechanisms mediated by protein kinase C (14). On the other hand, baicalein increased vasoconstricting sensitivity to adrenergic agonist in isolated rat arteries (18, 19). It is suggested that baicalein induces a contractile response at low concentrations and inhibits the endothelium-dependent relaxation, probably through inhibition of endothelial NO formation or release (14). This flavonoid impaired the endothelium independent relaxation by NO donors and attenuates NO-mediated aortic relaxation and cyclic GMP increases, likely through inhibition of NO-dependent guanylate cyclase activity (18). Baicalein reduced both acetylcholine and cyclopiazonic acid induced relaxation. It may also have little influence on the nifedipine-sensitive calcium channels or caffeine-sensitive intracellular calcium release in arterial smooth muscle cells (14). However, the current study did not show the effect of baicalein on calcium channel blocking, NO formation, arachidonic acid metabolism and cyclooxygenase pathways.

**Conclusion**

The present study provides novel information about the tracheal smooth muscle relaxant effect of baicalein. The relaxant effect of baicalein on TSM probably mediated through inhibition of histamine (H1) receptors, stimulation of beta2-adrenergic receptors and potassium channels activation.

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**Conflicts of Interest**

The authors declare that there are no conflicts of interest.

**References**

1. Deep M, Gilani A-UH, Mustafa MR. Effects of flavonoids on vascular smooth muscle of the isolated rat thoracic aorta. Life Sci 2003; 74:603-612.
2. Dinda B, SilSarma I, Dinda M, Rudrapaul P. Oroxylum
indicum (L.) Kurz, an important Asian traditional medicine: from traditional uses to scientific data for its commercial exploitation. J Ethnopharmacol 2015; 161:255-278.
3. Reina E, Al-Shibani N, Allam E, Gregson KS, Kowolik M, Windsor LJ. The effects of Plantago major on the activation of the neutrophil respiratory burst. J Tradit Complement Med 2013; 3:260-272.
4. Vaadala S, Ponneri N, Karmam VS, Pamuru RR. Baicalein, a flavonoid, causes prolonged estrus and suppressed fertility output upon prenatal exposure in female mice. Iran J Basic Med Sci 2019; 22:452.
5. Dijiong W, Xiaowen W, Linlong X, Wenbin L, Huijin H, Baodong Y, Yuhong Z. Iron chelation effect of curcumin and baicalein on aplastic anemia mouse model with iron overload. Iran J Basic Med Sci 2019; 22:660-668.
6. Li W, Du L, Li M. Alkaloids and flavonoids as α1-adrenergic receptor antagonists. Curr Med Chem 2011; 18:4923-4932.
7. Park KS, Chang I-M. Anti-inflammatory activity of aucubin by inhibition of tumor necrosis factor-α production in RAW 264.7 cells. Planta medica 2004; 70:778-779.
8. Zhou Y-J, Wang H, Sui H-h, Li L, Zhou C-L, Huang J-J. Inhibitory effect of baicalin on allergic response in ovalbumin-induced allergic rhinitis guinea pigs and lipopolysaccharide-stimulated human mast cells. Inflamm Res 2016; 65:603-612.
9. Barnes PJ. Pharmacology of airway smooth muscle. Am J Respir Crit Care Med 1998; 158:123-132.
10. Huang Y, Tsang S-Y, Yao X, Chen Z-Y. Biological properties of baicalein in cardiovascular system. Cardiovasc Hematol Disord Drug Targets 2005; 5:177-184.
11. Clark JD, Gebhart GF, Gonder JC, Keeling ME, Kohn DF. The 1996 guide for the care and use of laboratory animals. ILAR J 1997; 38:41-48.
12. Saadat S, Naghdi F, Ghorani V, Rakhshandeh H, Boskabady MH. Histamine (H1) receptors, cyclooxygenase pathway and nitric oxide formation involved in rat tracheal smooth muscle relaxant effect of berberine. Iran J Allergy Asthma Immunol 2019; 18:320-331.
13. Saadat S, Yazavoli M, Ghalanmehzad Z, Aslani MR, Boskabady MH. The relaxant effect of crocin on rat tracheal smooth muscle and its possible mechanisms. Iran J Pharm Res 2019; 18:1358-1370.
14. Chen Z-Y, Su Y-L, Lau C-W, Law W-I, Huang Y. Endothelium-dependent contraction and direct relaxation induced by baicalein in rat mesenteric artery. Eur J Pharmacol 1999; 374:41-47.
15. Mokhtari-Zaer A, Khazdair MR, Boskabady MH. Smooth muscle relaxant activity of Crocus sativus (saffron) and its constituents: possible mechanisms. Avicenna J Phytomed 2015; 5:365-375.
16. Kimata M, Shichijo M, Miura T, Serizawa I, Inagaki N, Nagai H. Effects of luteolin, quercetin and baicalein on immunoglobulin E-mediated mediator release from human cultured mast cells. Clin Exp Allergy 2000; 30:501-508.
17. Prasad R, Lawania RD, Gupta R. Role of herbs in the management of asthma. Pharmacogn Rev 2009; 3:247.
18. Huang Y, Wong CM, Lau C-W, Yao X, Tsang SY, Su YL, et al. Inhibition of nitric oxide/cyclic GMP-mediated relaxation by purified flavonoids, baicalein and baicalin, in rat aortic rings. Biochem Pharmacol 2004; 67:787-794.
19. Chen B, Senthilkumar R, Rong F, Guo Q. Cardioprotective potential of baicalein: A short review of in vitro and in vivo studies. Pharm Anal Acta 2014; 5:280-284.