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Antagonistic effects of two herbs in Zuojin Wan, a traditional Chinese medicine formula, on catecholamine secretion in bovine adrenal medullary cells

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ABSTRACT

In order to research the target of superior efficacy and lesser side effects, combination of herbal materials has been applied to phytotherapy for thousands of years in China and some other countries. Zuojin Wan (ZJW), a famous traditional Chinese medicine formula, is used in treating gastric diseases in China. It is composed of two herbs, Rhizoma Coptidis (RC) and Fructus Evodiae (FE) in the ratio of 6: 1(w/w). In the present study, we examined the effects of ZJW, RC, FE and active components isolated from these herbs on catecholamine (CA) secretion and intracellular calcium ($[Ca^{2+}]_i$) in cultured bovine adrenal medullary cells. Extracts of ZJW and RC and berberine, palmatine and jatrorrhizine, components of RC, all inhibited CA secretion and rise in $[Ca^{2+}]_i$ induced by acetylcholine (ACh), veratridine (Ver) and/or 56 mM K^+. On the other hand, extract of FE, evodiamine and rutaecarpine, components of FE, stimulated CA secretion and rise in $[Ca^{2+}]_i$ induced by ACh. Furthermore, different proportions of RC and FE caused different responses in CA secretion. The present findings suggest that two herbs in ZJW have opposite effects, i.e., inhibitory effect of RC and stimulatory effect of FE, on CA secretion induced by acetylcholine in cultured bovine adrenal medullary cells.

Introduction

For a long period of time, the pharmaceutical industry has been searching for a single drug that specifically targets a single disease-causing molecule. While the pathogenesis of disease is so complex, the therapeutic effect of single drug may be modest and hampered by various side effects or resistances of drugs in clinic. On the contrast, there are numerous herbal medicines that are considered to be potential agents for their satisfactory curative efficacies with few side effects in traditional Chinese medicine (TCM). It is believed that combination of multiple herbs under the guidance of theories of TCM, called formulae, could hit multiple targets and reduce the adverse effects. For instance, it is reported that the combination of Realgar (tetraarsenic tetrasulfide), Indigo Naturalis, Radix Salviae Miltiorrhizae and Radix Pseudostellariae had exerted a synergic effect in treating human acute promyelocytic leukemia (APL) (Wang et al. 2008). The concept of herbal combination in phytotherapy has been paid more and more attentions (Wagner 2006; Li and Zhang 2008; Ma et al. 2009).

Zuojin Wan (ZJW) is a famous formula of TCM used for treatment of gastric diseases. It is composed of two herbs, Rhizoma Coptidis (RC) and Fructus Evodiae (FE) in the ratio of 6: 1(w/w). It was first recorded in Danxi Xinfa, a famous ancient medicine treatise written hundreds of years ago. Chemical investigations have shown that the active compounds are berberine, palmatine, jatrorrhizine in RC (Sheng et al. 2006). Evodiamine and rutaecarpine were active compounds in FE (Yang et al. 2009) (Fig. 1). ZJW was applied to treat gastrointestinal diseases for its properties of stopping vomiting, decreasing gastric acid and relieving pain. Recent studies revealed that ZJW has various beneficial effects, such as anti-inflammation, anti-ulcer and anti-acid activities and inhibitory effect on the growth of Heliobacter pylori (Chen et al. 2003).

Stress is believed to be an important cause of hyperacidity and ulceration. Previous studies have shown that long-term stress induces an excessive CA secretion which may be an important etiological factor of a variety of changes occurring in the pathogenesis of gastrointestinal diseases (Artz and Fitts 1996). A case report showed that there is an unequivocal relationship

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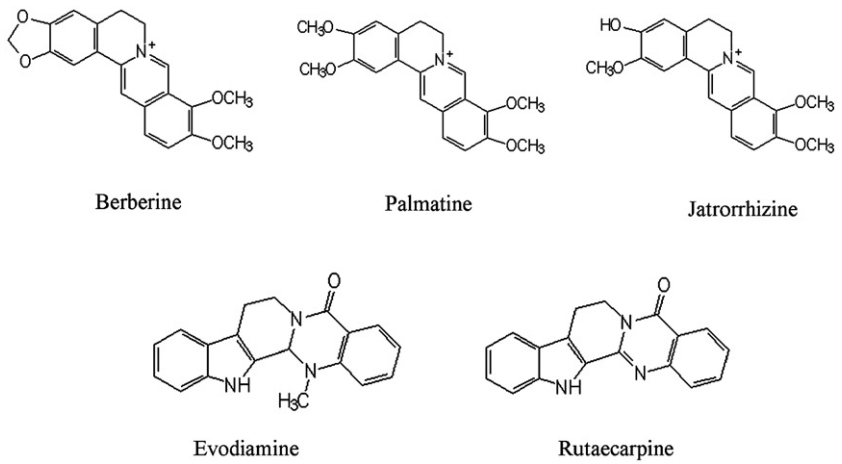

Fig. 1. Structures of active components of Rhizoma Coptidis and Fructus Evodiae.

between stressful events with increased CA release and subsequent severe symptomatic gastrointestinal ischemia which play a pivotal role in the pathogenesis of gastric ulcer (Veenstra et al. 2007). It has also been elucidated that adrenergic system is involved in gastric secretion and pathogenesis of stress-induced ulcer (Sutoo et al. 1998). Accumulating evidence have suggested that decreasing of mucosal blood flow level results in ischemia and hypoxia which is one of causes for the formation of stress gastric ulcer (Guth 1979; Mitchell 2004).

Chromaffin cells of the adrenal medulla are derived from multipotent neural crest cells in the developing embryo. They share a common sympathoadrenal progenitor with sympathetic neurons. Thus, adrenal chromaffin cells are considered to be the sympathetic post-ganglionic neurons and have become specialized in the release of CA to the circulating blood flow. Bovine adrenal chromaffin cells have been widely used as a model system for studying the effects of drugs on CA secretion (Mao et al. 2008; Park et al. 2003). In these cells, our previous studies have shown that both ACh-induced Na^+ influx through nicotinic ACh receptor-ion channels and Ver-induced Na^+ influx via voltage-dependent Na^+ channels increase Ca^{2+} influx via activation of voltage-dependent Ca^{2+} channels, a prerequisite for secretion and synthesis of CA. In contrast, high K^+ directly gates voltage-dependent Ca^{2+} channels to increase Ca^{2+} influx without increasing Na^+ influx (Wada et al. 1985). Adrenal medullary cells have been a good model for detailed study of the actions of different drugs on CA secretion (Shinohara et al. 2007).

A previous study reported that berberine and palmatine, components of RC, inhibited catecholamine biosynthesis by modulation of tyrosine hydroxylase activity in PC12 cells (Lee and Kim 1996; Lee et al. 1996; Shin et al. 2000). Yoshizumi et al. (1997) also showed that evodiamine, an active compound extracted from FE, stimulated CA secretion from perfused bovine adrenal medulla, although they did not show any mechanism for it. The potential roles of RC and combination of RC and FE in catecholamine secretion have not been studied. In the present study, we examined the effects of ZJW, RC, FE and their components on CA secretion and rise in $[Ca^{2+}]_i$ in cultured bovine adrenal medullary cells. We found that ZJW, RC and their components inhibited CA secretion and rise in $[Ca^{2+}]_i$ induced by ACh, whereas FE and its components stimulated these responses. It seems that the combination ratio of RC versus FE plays an important role in the regulation of catecholamine secretion in the cells.

Materials and methods

Reagents and materials

Oxygenated Krebs-Ringer phosphate (KRP) buffer was used throughout. Its composition is as follows (in mM): 154 NaCl, 5.6 KCl, 1.1 $MgSO_4$, 2.2 $CaCl_2$, 0.85 NaH_2PO_4, 2.15 Na_2HPO_4 and 10 glucose, adjusted pH to 7.4. Reagents were obtained from the following sources: Eagle's minimum essential medium (MEM) and new born calf serum from Gibco (USA); collagenase from Nitta Zerachin (Osaka, Japan); acetylcholine and veratridine from Sigma (St. Louis, MO, USA); and other chemicals used were of reagent grade obtained from Tianjin (Tianjin, China).

Preparation of the extracts for Chinese herbs

Chemical standards for berberine, palmatine, jatrorrhizine, evodiamine and rutaecarpine were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The purities of the standards were all above 98%. 350 g Fructus Evodiae was decocted three times with 5600 ml water and for 0.5 h each time. The extracts of three times were combined, concentrated, and then dried in vacuum. 400 g Rhizoma Coptidis were decocted three times with 4800 ml water and for 1.5 h each time. The extracts were combined, concentrated, and then dried in vacuum.

Quantitative analysis of various active compounds in the extracts of RC and FE

For quantitative results of alkaloids in Rhizoma Coptidis, the HPLC analyses were performed on an Agilent 1100 series HPLC instrument (Agilent, Waldbronn, Germany) composed of a vacuum degasser, a quaternary pump, an autosampler, a column compartment, and a diode array detector (DAD). The chromatographic separation was carried out on a Waters Symmetry C18 column (5 μm, Ø 3.9 mm × 100 mm) by setting the column temperature at 30 °C. The mobile phase consisted of acetonitrile (A) and water containing 0.05% formic acid (B). A gradient program was used as follows: 12-16% A at 0-10 min, 16-16% A at 10-13 min, then 12% A hold for 7 min. The flow rate was kept at 0.8 ml/min. The detection wavelength was set to monitor at 345 nm. HPLC-UV chromatogram of standard substances and Rhizoma Coptidis sample are shown in Fig. 2A. Quantitative