

# Effect of *Scutellariae radix* (*Huangqin*) on preventing rhinovirus-provoked asthmatic inflammation in cultured human bronchial epithelia

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## KEY MESSAGES

1. Infection of human bronchial epithelial cells with rhinovirus has minimal effect on the electrophysiological properties and ion transport processes of these epithelia.
2. The *Scutellariae radix* extract and its major flavonoids could not suppress rhinovirus replication but could promote the secretion of at least two pro-inflammatory cytokines (IL-6 and

IL-8) in human bronchial epithelia.

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## Introduction

*Scutellariae radix* (SR), also known as *Huangqin*, is the dried root of *Scutellaria baicalensis* Georgi (Lamiaceae). It is listed in the Chinese Pharmacopoeia and is one of the most widely used Chinese herbal medicines against bacterial infection of the respiratory and the gastrointestinal tracts. The main components of SR (and of all *Scutellaria* species) are baicalein, baicalin, and wogonin. Rhinovirus (RV) infection is the major cause of common colds and the most frequent trigger (up to 85%) for asthma exacerbation.<sup>1</sup> The cellular mechanism by which viral respiratory infections may induce asthma is complex. The bronchial epithelium is the primary target for respiratory viral infections. The viruses must pass through the epithelial barrier to enter the body. At the same time, the viruses infect and replicate in the bronchial epithelial cells. Such infection is associated with airway inflammation, which is partly due to the major basic protein (MBP), a product of the eosinophils. The MBP is increased in upper respiratory viral infections associated with asthma exacerbations,<sup>2</sup> causing virus-induced cellular damage.

The airway surface epithelium itself is responsible for the synthesis and release of cytokines that cause the selective recruitment, retention, and accumulation of various inflammatory cells.<sup>3</sup> Certain inflammatory cytokines alter the fluid and electrolyte transport of the airway epithelium. Therefore, asthma can be considered a disease of the bronchial epithelium, which could contribute to the pathophysiology of airway inflammation. Damage of the surface airway epithelium in

asthmatic inflammation is due to the secretion of eosinophil-derived, highly toxic cationic proteins, such as MBP. To simply mimic the damage seen in asthma inflammation, the bronchial epithelium can be challenged with highly charged cationic proteins, such as poly-L-arginine. This study aimed to examine the antiviral activity and therapeutic potential of SR and its three major flavonoids (baicalein, baicalin, and wogonin) in the RV39-infected human bronchial epithelial cell lines (16HBE14o-). In addition, the SR effect on cellular immune responses to experimental RV39 challenge in cells treated with poly-L-arginine, a surrogate of MBP was investigated.

## Methods

This study was conducted from December 2010 to November 2011. Cultured human bronchial epithelial cell lines (16HBE14o-) were infected with RV39. Some of the epithelial cells were treated with poly-L-arginine as a surrogate of MBP. Detection and quantification of RV39 RNA in the epithelial cells were performed by quantitative reverse transcription polymerase chain reaction. Cytokine release was quantified by a cytokine antibody array or enzyme-linked immunosorbent assay. The effect of the SR extract and its major flavonoids (baicalein, baicalin, and wogonin) on RV39 mRNA expression in infected 16HBE14o- epithelia was measured by quantitative real-time polymerase chain reaction.

## Results

At 72 hours post-infection, RV39 mRNA was detected in 16HBE14o- cells at a multiplicity of

infection (MOI) of 1 (Fig 1). In the presence of the SR extract (10 mg/mL), baicalin (10 mM), or wogonin (10 mM), RV39 mRNA expression increased significantly ( $P < 0.05$ ,  $n = 4-6$ ), compared with control. These doses were selected because at these concentrations the SR extract or its major flavonoids

would not stimulate any increase in short-circuit current (ie chloride ion secretion) in epithelial cells (unpublished data).

Our previous study demonstrated that the inflamed bronchial epithelia mainly secreted two pro-inflammatory cytokines (IL-6 and IL-8) into the supernatant.<sup>4</sup> To measure the secretion of IL-6 and IL-8 more quantitatively, the supernatant samples obtained from normal (non-infected) 16HBE14o-cells stimulated by the SR extract (10 mg/mL), baicalin, baicalin or wogonin (10 mM each) for 72 hours were analysed by ELISA. Exposure to the three major flavonoids significantly increased the release of IL-6 and IL-8, compared to the time-matched control ( $P < 0.05$ ,  $n = 312$ , Fig 2). The SR extract significantly increased the release of IL-8 only ( $P < 0.05$ ,  $n = 3-16$ ).

Whether the SR extract and its major flavonoids had any effect on IL-6 and IL-8 release when the cells were exposed to a low concentration of poly-L-arginine (0.3 mM) and RV39 (MOI of 0.1) was determined. Poly-L-arginine and RV39 did not cause any significant increase in IL-6 and IL-8 levels ( $P > 0.05$ ,  $n = 7-16$ , Fig 3). In addition, RV39 and poly-L-arginine did not have any additive effect on promoting IL-6 and IL-8 release (data not shown), compared with data obtained from RV39- or poly-L-arginine-treated epithelia. There was a large variability in the release of IL-6 and IL-8. In general, the SR extract or its flavonoids did not show any significant difference in the IL-6 and IL-8 release, compared with cells treated with a combination of poly-L-arginine and RV39 ( $P > 0.05$ ,  $n = 3-16$ ), except for the effect of baicalin or wogonin on IL-6 release.

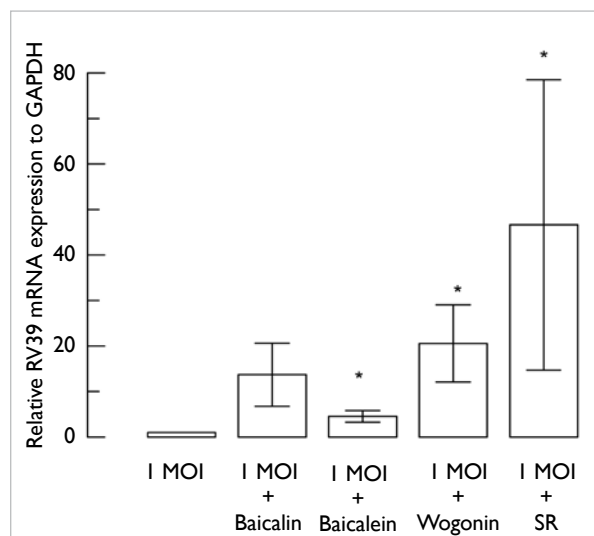


FIG 1. Analysis of rhinovirus (RV39) mRNA expression using quantitative real-time polymerase chain reaction: the epithelia are infected with RV39 at a multiplicity of infection (MOI) of 1 with or without baicalin, baicalein, wogonin, or *Scutellariae radix* (SR) extract. The mean±SE relative expression of RV39 mRNA is normalised to GAPDH and is shown as fold-changes relative to the untreated controls \*  $P < 0.05$  compared with a MOI of 1

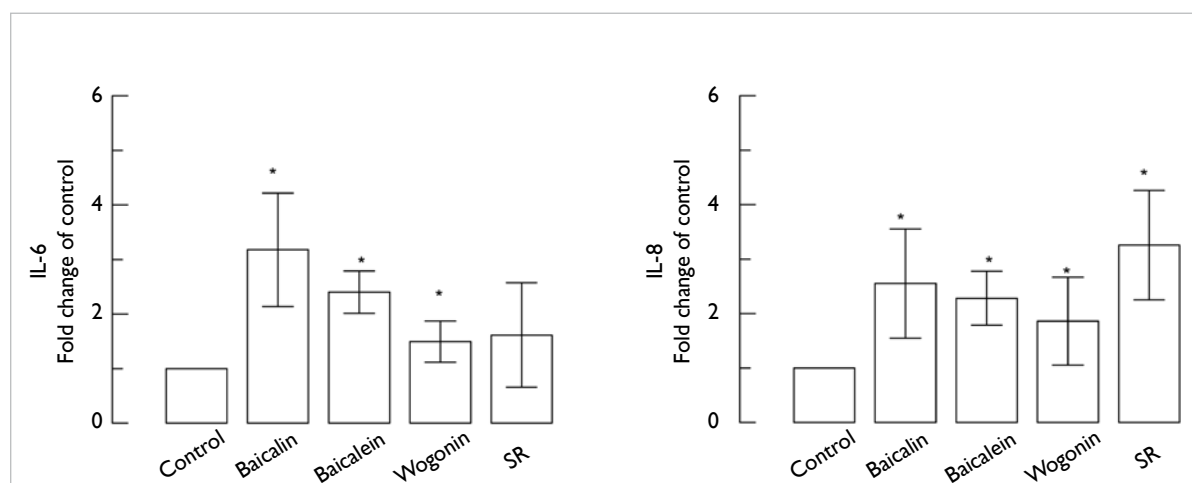
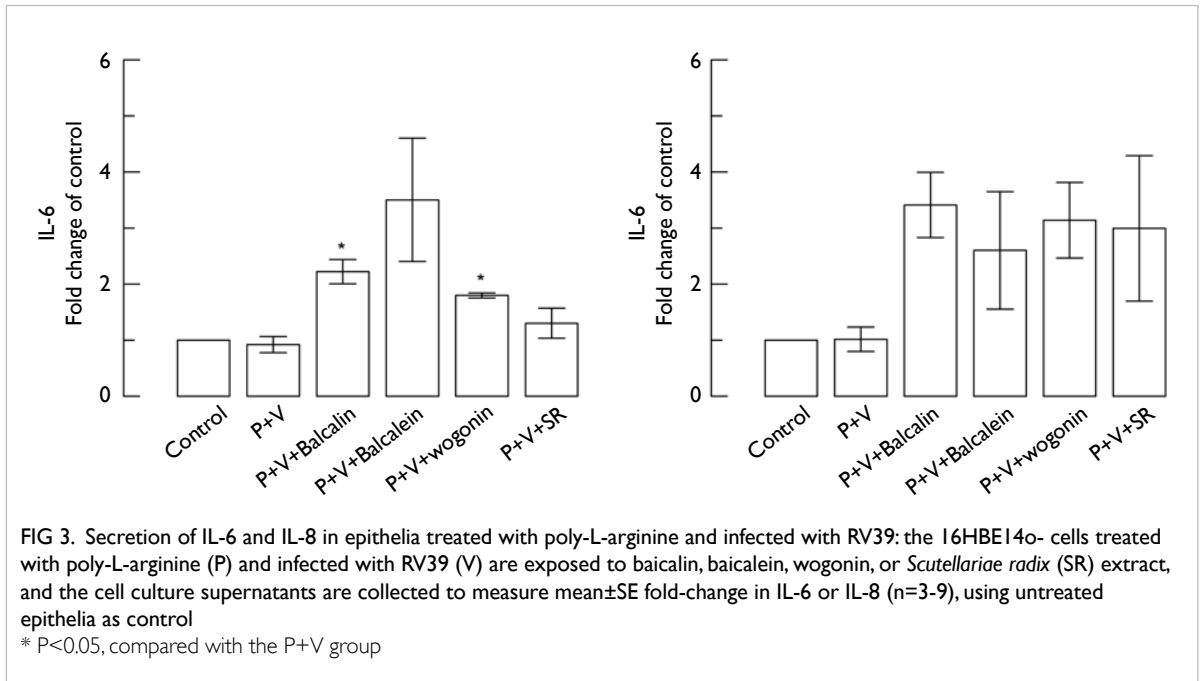


FIG 2. Secretion of IL-6 and IL-8: the 16HBE14o-cells are exposed to baicalin, baicalein, wogonin, or *Scutellariae radix* (SR) extract, and the cell culture supernatants are collected to measure mean±SE fold-change in IL-6 or IL-8 ( $n = 3-9$ ), using untreated epithelia as a control \*  $P < 0.05$ , compared with control



## Discussion

*Huangqin* has been shown to possess anti-inflammatory, antipyretic, anti-allergic, anticonvulsant, antiviral, and antitumor properties.<sup>5</sup> It has been used to treat inflammation-related disorders such as gastroenteritis in China and Japan. Many biological activities of SR are related to its flavonoids (eg baicalein, baicalin, and wogonin).<sup>5</sup> However, our results suggested that the SR extract and its major flavonoids showed no significant inhibition of RV39 replication (antiviral effect), but the relative RV39 mRNA expression was increased. The underlying molecular mechanism of these findings remains unknown. Future experiments should be carried out to measure the virus release to determine whether the enhanced viral mRNA expression is a result of increased replication or altered kinetics of virus release. Although the SR extract and its flavonoids are anti-inflammatory in nature,<sup>5</sup> our cytokine release data showed that they stimulated significant IL-6 and IL-8 release from the 16HBE14o- epithelial cells. To mimic the damage seen in asthma inflammation, the bronchial epithelium was challenged with highly charged cationic polypeptides such as poly-L-arginine, which is similar in structure and function to the biologically active moiety of MBP. At a low concentration of poly-L-arginine, RV39 infection did not promote any increase in IL-6 and IL-8. However, when the epithelia were infected with RV39 in the presence of poly-L-arginine, two flavonoids (baicalin and wogonin) promoted the release of IL-6. This may be due to increased RV39 mRNA expression. Further

experiments are needed to quantify the infectivity of RV39 in 16HBE14o- cells treated with SR extract and its major flavonoids. We cannot exclude the possibility that this is a species- and tissue-specific effect. Nonetheless, our data challenged the traditional belief that the SR extract and its major flavonoids are anti-inflammatory, and also question their therapeutic use as antiviral (anti-rhinovirus) agents, because these agents may affect airway inflammation in asthma.

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