

## Effects of *Lactobacillus* spp. on improving the efficiency of *Scutellaria baicalensis* treatment in *Helicobacter pylori* infection

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*Helicobacter pylori* infection is associated with chronic gastritis, peptic ulcers, and gastric cancer. About 50% of the population in the world is infected by *H. pylori*. Furthermore, 70% to 95% of *H. pylori*-infected patients are suffering from peptic ulcer. Huang Qin usually refers to the dried root of *Scutellaria baicalensis* Georgi. It is been used as an herbal medicine to treat inflammation, cardiovascular diseases, respiratory and gastrointestinal infections in Chinese medicine for many years. Its active compounds include baicalin, baicalein, wogonin derivatives and  $\beta$ -sitosterol. Baicalein, a primary metabolite of baicalin, differs from its mother compound merely by the 7-substituent (i.e. it possesses a phenolic hydroxyl (7-OH) instead of a glucuronic acid). It has been showed antibacterial, lipid-lowering, anti-lipid peroxidative, and anti-arthritis activities. In our study, minimum bactericidal concentration of baicalein, its obviously has higher bactericidal concentration as least 4-fold higher than that of baicalin. Therefore, the biotransformed baicalein is highly possible to treat or prevent *H. pylori* infection. Microbial enzymes play important roles in biotransformation.  $\beta$ -glucosidase were noted in association with the pharmacological actions of herbal medicines. In this study, five *Lactobacillus* spp., JB-3, L21, P2, LS and LAPS, were screened for their  $\beta$ -glucosidase activities. Strain JB-3 showed the best  $\beta$ -glucosidase activity, the enzyme activity was 2 fold higher than rest of the strains. By monitoring the concentration of baicalein, strain JB-3 could efficiently convert baicalin into baicalein. The conversion ability of  $9.6 \times 10^9$  CFU/mL of JB-3 was doubling the amount of baicalein in an hour. In infected AGS cells, 31.5  $\mu$ M of baicalein significantly decreased *H. pylori*-induced IL-8 by 20 % after 6 hrs treatment than baicalin. In this study, we determined that bioconversion of *Lactobacillus* spp. could improve the biological activity of *Scutellaria baicalensis* extract for treating *H. pylori* infection.

Keywords: *Scutellaria baicalensis*, baicalein, *Lactobacillus* spp., *Helicobacter pylori*

## 呋喃喹啉衍生類藥物抑制日本腦炎病毒之機制研究

### Identification of furoquinolone derivatives against the infection of Japanese Encephalitis Virus

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日本腦炎病毒 (Japanese encephalitis virus, JEV)，經由蚊蟲為媒介叮咬感染，感染症狀為發燒、頭痛、高燒，嚴重者急性腦膜炎、痙攣、產生神經性後遺症，甚或死亡。除預防疫苗接種外，現今對日本腦炎並無特殊有效治療方法。JEV 為一單股正向 RNA 套膜病毒，可轉譯出一條多聚蛋白，並藉由其本身的結構蛋白及非結構蛋白，進行病毒複製組裝及干擾宿主發炎反應路徑。本實驗利用倉鼠腎細胞 (Baby hamster syrian kidney, BHK-21) 及人類橫紋肌細胞瘤 (Rhabdomyosarcoma, TE761)，分別以細胞存活試驗 (Cell cytotoxicity assay) 觀察 furoquinolone 對細胞的半毒殺濃度 (Concentration of 50% cytotoxicity)，分別為  $>500\mu\text{M}$  及  $180.5\mu\text{M}$ 。以 BHK-21 感染 JEV 後給予不同濃度的 furoquinolone 藥物治療，觀察細胞病變 (Cytopathic effect, CPE)、病毒斑抑制實驗 (Plaque assay) 以及 Real-Time PCR 確定病毒 RNA 量。結果發現 CPE 程度、病毒斑數量與 JEV 病毒 RNA 量隨著 furoquinolone 藥物濃度上升而下降。在 Plaque assay 中 Pre-treatment、Simultaneous-treatment、Post-treatment 等不同給藥治療方式下，發現 furoquinolone 抑制 JEV 病毒斑產生的最佳作用時機為 Simultaneous-treatment 及 Post-treatment 治療模式，並發現  $110\mu\text{M}$  即能達到半抑制濃度 (Concentration of 50% inhibition, IC<sub>50</sub>)。經由 Virus attachment 及 Virucidal activity assay 確定 furoquinolone 抗 JEV 之機轉並非為抑制病毒貼附細胞或影響病毒顆粒活性，再以流式細胞儀發現 furoquinolone 抗 JEV 機轉為抑制 JEV 所誘發的細胞凋亡路徑，搭配螢光顯微鏡觀察，粒線體損害因加入 furoquinolone 治療後而降低，接下來以西方墨點法 (Western blotting) 確認細胞凋亡相關路徑蛋白質的表現。本研究希望藉由鑑定 furoquinolone 藥物抑制 JEV 病毒感染的相關路徑機轉，發展出可應用在治療日本腦炎病人之方向，以抑制體內病毒的持續感染並降低死亡率。

Key words: Japanese encephalitis virus, furoquinolone, cytopathic effect, CC<sub>50</sub>, IC<sub>50</sub>