

# Uptake characteristics of levofloxacin for the eradication of *Helicobacter pylori* by GES-1 and MGC80-3 cells

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**Abstract. – OBJECTIVE:** The aim of this study was to study the uptake of levofloxacin for the eradication of *Helicobacter pylori* by human gastric epithelial cell lines, GES-1 and MGC80-3.

**MATERIALS AND METHODS:** High performance liquid chromatography (HPLC) and coomassie brilliant blue staining, among other methods, were used to study the uptake of levofloxacin in GES-1 and MGC80-3 cells. The effect of time, concentration, temperature, pH, cyclosporine A, verapamil, and cimetidine on uptake was analyzed.

**RESULTS:** The uptake of levofloxacin by GES-1 and MGC80-3 cells reached a steady state after 15 minutes of incubation, and was enhanced by increasing the extracellular levofloxacin concentration, although not in a linear manner. A maximum uptake was observed at 37°C and pH 7.4. Cyclosporin A and verapamil enhanced uptake in GES-1 cells by 2.07%-13.23% ( $p > 0.05$ ), and 17.5%-35.3% in MGC80-3 cells ( $p < 0.05$ ). The uptake of levofloxacin was not affected by cimetidine.

**CONCLUSIONS:** P-glycoprotein mediates levofloxacin uptake in MGC80-3 cell. Further, P-glycoprotein may be involved in levofloxacin uptake in GES-1 cells. However, organic cation transporters were not involved in levofloxacin uptake in MGC80-3 and GES-1 cells.

*Key Words:*

GES-1 cells, MGC80-3 cells, Levofloxacin, Uptake, HPLC.

## Introduction

Failure to eradicate *Helicobacter pylori* (*H. pylori*) infection has increased over the past several years<sup>1,2</sup>. Clinical treatment has faced enormous challenges due to drug resistance, which has become a focus of research<sup>3,4</sup>. Nevertheless, it is also important to understand the uptake and transport mechanisms of current therapeutics in

the stomach for effective *H. pylori* eradication. However, these mechanisms remain to be fully elucidated.

Levofloxacin (LF) triple therapy, which has been described by the Maastricht IV Consensus<sup>5</sup> and the American College of Gastroenterology Guidelines<sup>6</sup>, promotes *H. pylori* eradication. LF can be taken up in the kidneys<sup>7</sup>, small intestine<sup>8</sup>, and placental barrier through transporter proteins<sup>9</sup>, including P-glycoprotein (P-gp), the organic cation transporter (OCT), the monocarboxylate transporter (MCT), and other transporters. LF may be actively transported in the stomach. However, the mechanisms of LF uptake in human gastric mucosa have not been reported.

Therefore, we evaluated LF uptake in two human gastric epithelial cell lines, the immortalized gastric epithelial cell line, GES-1, and the gastric epithelial cell line, MGC80-3. Further, we explored the influence of time, drug concentration, pH, temperature, and transport inhibitors on the uptake of LF to better understand the mechanism of LF uptake in the human gastric mucosa.

## Materials and Methods

### *GES-1 and MGC80-3 Cell Culture*

GES-1 cells (American Type Culture Collection, ATCT) and MGC80-3 cells (Cell Bank of Chinese Academy of Sciences, Shanghai, China) were cultured in DMEM medium (GIBCO, Grand Island, NY, USA) at 37°C and 5% CO<sub>2</sub>. Cells in the logarithmic growth phase were collected and seeded (2 ml;  $1 \times 10^5$  cells·ml<sup>-1</sup>) in six-well culture plates. Cells were cultured in the presence of LF (Standard Lot: BCBF7004V, purity >99.9%, Sigma, St. Louis, MO, USA) upon reaching confluence.

Cells were pre-treated with the P-gp inhibitors cyclosporine A (10  $\mu\text{mol}\cdot\text{L}^{-1}$ ; standard batch number: 130495-200202; purity 98.8%; NICPBP, Beijing, China) and verapamil (100  $\mu\text{mol}\cdot\text{L}^{-1}$ ; standard batch number: 1001381484; purity  $\geq 99.9\%$ ; Sigma) or the OCT inhibitor cimetidine (100  $\mu\text{mol}\cdot\text{L}^{-1}$ ; standard lot: 101 224 844; Sigma). Uptake was evaluated 5, 7.5, 10, 15, and 30 min after adding HBSS containing the inhibitor and 100  $\mu\text{mol}\cdot\text{L}^{-1}$  LF solution.

### Drug Extraction

Prior to the experiment, 1 ml Hanks balanced salt solution (HBSS, 37°C) was added to each well, and the cells were washed three times. LF solution was then added to each well, and the cells were incubated at 37°C. The uptake of LF at 25°C and 4°C was evaluated by incubating the cells at room temperature and in the refrigerator. At the predetermined time point, the LF solution was removed, and the cells were washed 4 times with HBSS (4°C). The cell samples were collected in 1.5 ml centrifuge tubes. Samples underwent 3 freeze/thaw cycles (-80°C-37°C) and were sonicated to prepare the cell lysates. The lysate was then cleared by centrifugation at 12000 rotations $\cdot\text{min}^{-1}$  for 10 min and filtered using a 0.22  $\mu\text{m}$  membrane. From the obtained samples, 20  $\mu\text{l}$  was used to determine the intracellular LF content by HPLC. The total cellular protein content was measured<sup>10</sup> with coomassie brilliant blue staining. Intracellular LF uptake was expressed as the ratio of intracellular drug content and the total cellular protein content.

### High Performance Liquid Chromatography (HPLC)

An Agilent 1100 HPLC (Agilent, Santa Clara, CA, USA) was used with a Welch C18 chromatographic column (4.6 mm  $\times$  250 mm, 5  $\mu\text{m}$ ), a mobile phase<sup>11</sup> composed of acetonitrile (chromatographic purity; Tedia Company, Fairfield, OH, USA): citric acid solution (50  $\text{mmol}\cdot\text{L}^{-1}$ ): ammonium acetate (1  $\text{mol}\cdot\text{L}^{-1}$ ) (17:82:1), a flow rate of 1.0  $\text{ml}\cdot\text{min}^{-1}$ , a column temperature of 40°C, a detection wavelength of 295 nm and an injection volume of 20  $\mu\text{l}$ .

### Statistical Analysis

Data were analyzed using a two-tailed Student's *t*-test, and were expressed as the mean  $\pm$  SD. A *p* value less than 0.05 was considered statistically significant.

## Results

### HPLC Method

Under the experimental conditions described above, the retention time of LF was 8.04 min, with no impurity peaks affecting the determination, suggesting good specificity. The linear relationship was acceptable at concentrations ranging from 0.1 to 10  $\mu\text{mol}\cdot\text{L}^{-1}$ . The variation by day was less than 15%, which was in line with the biological sample requirements. The recovery rate of LF in the GES-1 cell standard solution using low, medium and high concentrations (1, 4, and 8  $\mu\text{mol}\cdot\text{L}^{-1}$ ) were 95.6%, 95.1%, and 95.7%, respectively, whereas the recovery rates were 103.4%, 98.1%, and 99.8% in the MGC80-3 cell standard solution.

### Effect of Time and Extracellular LF Concentrations

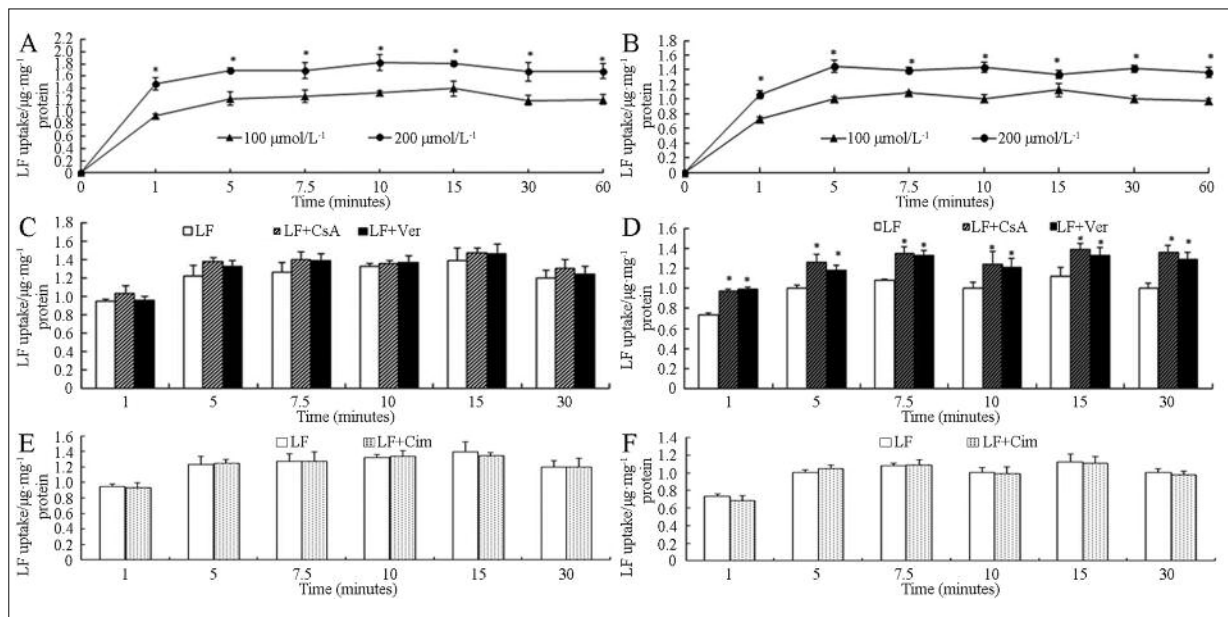
The uptake of LF was affected by incubation time and concentration. Samples were collected at 1, 5, 7.5, 10, 15, 30, and 60 min after adding HBSS containing 100 or 200  $\mu\text{mol}\cdot\text{L}^{-1}$  LF to each well. Drug uptake by GES-1 and MGC80-3 cells increased significantly from 1 to 5 min, slowly increased from 7.5 to 15 min, and reached a steady state thereafter. The uptake of 200  $\mu\text{mol}\cdot\text{L}^{-1}$  LF was significantly greater than the uptake of 100  $\mu\text{mol}\cdot\text{L}^{-1}$  ( $p < 0.05$ ; Figure 1A, B).

### Effect of Temperature

Cells were treated with HBSS containing 100  $\mu\text{mol}\cdot\text{L}^{-1}$  LF, and were incubated for 30 min at 37°C, 25°C, or 4°C. The LF uptake by GES-1 cells at 37°C, 25°C, and 4°C was  $1.17 \pm 0.06$ ,  $1.02 \pm 0.08$ , and  $0.64 \pm 0.10$   $\mu\text{g mg}^{-1}$  protein, respectively. In contrast, the uptake in MGC80-3 cells was  $1.00 \pm 0.07$ ,  $0.86 \pm 0.06$ , and  $0.60 \pm 0.05$   $\mu\text{g mg}^{-1}$  protein at 37°C, 25°C, and 4°C, respectively. These data indicate that LF uptake in the two cell lines reached a maximum at 37°C, and that uptake at 37°C was greater than that at 25°C ( $p > 0.05$ ) and significantly greater than that at 4°C ( $p < 0.01$ ).

### Effect of Solution pH

The effect of pH on LF uptake was evaluated 30 min after the addition 100  $\mu\text{mol}\cdot\text{L}^{-1}$  LF in the presence of varying pH values (5.4, 6.4, 7.4 and 8.4). The peak drug uptake was observed at pH 7.4. Uptake was significantly lower at pH 5.5 ( $p < 0.01$ ) and lower at pH 6.4 and pH 8.4 ( $p > 0.05$ ). The drug uptake by GES-1 cells at pH 5.4,



**Figure 1.** **A, B**, Effects of concentration and time on the levofloxacin uptake by GES-1 and MGC80-3 cells.  $n=6$ , mean  $\pm$  SD; 200  $\mu\text{mol}\cdot\text{L}^{-1}$  group compared with the 100  $\mu\text{mol}\cdot\text{L}^{-1}$  group,  $*p < 0.05$ . **C, D**, Effects of P-gp inhibitor on the transport of levofloxacin by GES-1 and MGC80-3 cells.  $n = 6$ , mean  $\pm$  SD; LF: levofloxacin; CsA: Cyclosporin A; Ver: Verapamil. P-gp inhibitor group compared with the control group,  $*p < 0.05$ . **E, F**, Effects of OCTs inhibitor on the transport of levofloxacin by GES-1 and MGC80-3 cells.  $n = 6$ , mean  $\pm$  SD; LF: levofloxacin; Cim: cimetidine.

pH 6.4, pH 7.4, and pH 8.4 was  $0.82 \pm 0.05$ ,  $1.08 \pm 0.08$ ,  $1.21 \pm 0.05$ , and  $1.09 \pm 0.07 \mu\text{g mg}^{-1}$  protein, respectively, and  $0.72 \pm 0.04$ ,  $0.91 \pm 0.06$ ,  $1.04 \pm 0.09$ , and  $0.90 \pm 0.09 \mu\text{g mg}^{-1}$  protein in MGC80-3 cells.

### Effect of P-gp Inhibitors

The drug uptake in cyclosporine A- and verapamil-treated GES-1 and MGC80-3 cells at varying time points was increased compared to that of the untreated control group. Uptake increased 2.07%-13.23% ( $p > 0.05$ ) and 17.5%-35.3% ( $p < 0.05$ ), in the GES-1 and MGC80-3 cells, respectively (Figure 1C, D).

### Effect of OCT Inhibitors

No significant difference was observed in drug uptake in GES-1 and MGC80-3 cells treated with cimetidine compared to untreated control cells ( $p > 0.05$ ; Figure 1E, F).

## Discussion

The study of gastric drug distribution is important for effective *H. pylori* eradication; however, studies evaluating gastric drug uptake are rare,

particularly when evaluating drug transporters. Studies in the kidneys<sup>7</sup>, small intestine<sup>8</sup>, placental barrier<sup>9</sup>, and other cell types have shown that drug transporters, including P-gp, OCTs, and MCTs, are involved in LF uptake. Thus, one or several transporters may promote LF uptake in the gastric mucosa. This study sought to clarify the uptake mechanisms of LF in human gastric cells to better understand gastric mucosal drug transporters.

Based on our studies using varying LF concentrations, temperatures, and pH, we found that GES-1 and MGC80-3 cells quickly take up LF, and that the uptake increases with increasing drug concentration. Nevertheless, these two parameters did not show a positive correlation, and did not display characteristics of passive diffusion. This suggests that the absorption of LF in MGC80-3 and GES-1 cells was mediated by transport proteins<sup>12</sup>. It was also found that the uptake of levofloxacin in these two kinds of cells were associated with temperature and pH values. Under normal circumstances, the transport activity of cell membrane transport protein was usually the maximum at 37°C and neutral pH value. This study also showed a maximum intake volume of levofloxacin at 37°C and pH 7.4, which showed

that the uptake of levofloxacin in GES-1 and MGC80-3 cells involved in the role of transport proteins from another angle.

P-gp is a common drug efflux transporter that has been extensively studied. Studies using Caco-2<sup>13,14</sup>, BeWo<sup>9,15</sup>, and other cell lines have shown that the uptake and transport of LF was mediated by P-gp. Further, P-gp is known to express in GES-1 and MGC80-3 cells<sup>16</sup>. We, therefore, evaluated the effect of the P-gp inhibitors, cyclosporine A and verapamil, on LF uptake<sup>17</sup>. Our data indicate that drug uptake was increased following P-gp inhibitor treatment, particularly in MGC80-3 cells, where the uptake was significantly higher (17.5%-35.3%,  $p < 0.05$ ). P-gp expression is higher in gastric cancer cells than in GES-1 cells<sup>16</sup>, which may explain the different proportion of drug uptake observed between the two cell lines. These findings were also similar to observations in BeWo<sup>9</sup> and Caco-2<sup>14</sup> cells. Therefore, our results indicate that P-gp may regulate LF uptake in GES-1 and MGC80-3 cells.

OCTs participate in the distribution, excretion, and absorption of drugs *in vivo*. Cimetidine is a specific inhibitor of OCTs<sup>18,19</sup>. LF is a zwitterion, which can be transported via OCTs<sup>14,20</sup>. Our data revealed that cimetidine had no significant effect on LF uptake, indicating that OCTs either did not participate in LF uptake in GES-1 and MGC80-3 cells, or that OCTs are not expressed in these cells. These results were consistent with the uptake characteristics of LF in Caco-2 cells<sup>13</sup>; however, OCTs did contribute to LF uptake in the LLC-PK1 kidney epithelial cell line<sup>21</sup>.

## Conclusions

This paper shows that LF uptake in human gastric cells is mediated by transport proteins, specifically P-gp. This study was carried out in human gastric mucosal cells. Evaluation of LF uptake, transport, and transporter expression level in corresponding human tissues and organs during *H. pylori* infection will have clinical value, and will be the main direction of our future research. This study offers a novel perspective in approaching *H. pylori* therapy. More importantly, these data provide in-depth information regarding the gastric mucosa transporter system, which provides an experimental basis for the development of targeted drug delivery systems and novel dosage types, thereby promoting future studies on drug uptake and transport in the stomach.

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## Conflict of Interest

The Authors declare that there are no conflicts of interest.

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