Effects of celecoxib on the reversal of multidrug resistance in human gastric carcinoma by downregulation of the expression and activity of P-glycoprotein

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We investigated the effects of celecoxib on the cell proliferation and the expression and activity of P-glycoprotein in the human gastric carcinoma multidrug resistance sublines SGC7901/adriamycin and SGC7901/vincristine. The cell proliferation was measured by [^{3}H]thymidine incorporation assay and MTT test. The expression of the multidrug resistant gene (*MDR1*) was detected by real-time quantitative reverse transcription-polymerase chain reaction. P-glycoprotein was measured by Western blot analysis. The intracellular rhodamine 123 accumulation was analyzed by flow cytometry to evaluate the activity of P-glycoprotein. After treatment with celecoxib, the proliferation inhibitions of SGC7901 cell line and the SGC7901/adriamycin and SGC7901/vincristine sublines increased linearly in a positive dose-dependent pattern in both the [^{3}H]thymidine incorporation assay and in the MTT test. The IC_{50} value of the *MDR1/GAPDH* ratio was 5.50×10^{-6} mol/l in SGC7901/adriamycin and 3.89×10^{-6} mol/l in SGC7901/vincristine. P-glycoprotein expression levels in the two multidrug resistance sublines treated with celecoxib were significantly lower than those in control groups, 0.28 vs. 0.71 in the SGC7901/adriamycin subline and 0.21 vs. 0.83 in the SGC7901/vincristine subline, respectively, $P < 0.05$. After treatment with celecoxib, intracellular rhodamine 123 accumulation in the SGC7901/adriamycin and SGC7901/vincristine sublines increased positively in a dose-dependent pattern ($P < 0.05$), and reached more than 50% of that in the SGC7901 cell line at the concentration of 1×10^{-4} mol/l of celecoxib. In conclusion, celecoxib could inhibit proliferation of multidrug resistance in gastric carcinoma sublines. The reversal of multidrug resistance was caused by downregulation of the expression and activity of P-glycoprotein. The results may suggest a new way to reverse P-glycoprotein-dependent multidrug resistance in human gastric carcinoma. *Anti-Cancer Drugs* 18:1075–1080 © 2007 Lippincott Williams & Wilkins.

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Introduction

Reports show that over 95% of patients had already been in an advanced stage of the disease when first diagnosed as having gastric carcinoma; only about 40% had a 5-year survival even with surgical resection [1,2]. Chemotherapy is also an important method in treating advanced gastric carcinoma and an adjuvant postoperation treatment as well. The results, however, are not satisfactory, mainly because the carcinoma cells are resistant to anticancer drugs, a phenomenon which is called multidrug resistance (MDR) [3]. MDR is the protection of a cancer cell population against a variety of drugs with different structures and functions [4].

The accumulated data from in-vitro and xenograft nude mice models *in vivo* have shown that cyclooxygenase-2 (COX-2) selective inhibitors have anticancer effects in gastric carcinoma [5–7]. Interestingly, our primary study showed that celecoxib, one of the COX-2 selective inhibitors, could inhibit the growth of not only human gastric carcinoma parental cell line SGC7901 but also its MDR subline SGC7901/adriamycin (ADR) [8]. The exact mechanisms of its effect have, however, not been revealed. P-glycoprotein (P-gp), a multidrug-resistant pump, is considered to be one of the most important mechanisms of MDR. It is a 170-kDa transmembrane protein and is encoded by the *MDR1* gene [9,10]. Dozens of P-gp transport substrates have been identified, including anticancer agents such as ADR and vincristine (VCR), lipophilic cations such as rhodamine 123 (Rh123) and amphiphiles such as Triton X-100, etc. [11]. P-gp can, therefore, carry a variety of anticancer agents out of the cells and reduce their cytotoxicity [12]. Rh123, one of the P-gp transport substrates, has been used as an indicator for P-gp activity in many experiments [13,14]. To reveal the relationship between COX-2 inhibitor and MDR, we

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investigated the effects of celecoxib on cell proliferation, the expression of P-gp at mRNA and protein levels, and the activity of P-gp in the human gastric carcinoma MDR sublines SGC7901/ADR and SGC7901/VCR.

Materials and methods

Reagents

Celecoxib was obtained from Pfizer Pharmaceuticals; (Pfizer Pharmaceuticals Ltd; La Jolla, California, USA) MTT and Rh123 were purchased from Sigma; (Sigma; St. Louis, Missouri, USA) goat-anti-human P-gp polyclonal antibody was purchased from Santa Cruz Biotechnology; (Santa Cruz, California, USA) and [methyl-^{3}H] thymidine was purchased from Amersham Pharmacia Biotec (Piscataway, New Jersey, USA). The sequences of oligonucleotides in this study are as follows: *MDR1*-F: 5′-CATCGAGTCACTGCCTAATAAATA-3′; *MDR1*-R: 5′-GCTTCTTGGACAACCTTTTCACT-3′; TaqMan probe: 5′-GCCACCAGAGAGCTGAGTTCC-3′; glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as internal control, *GAPDH*-F: 5′-TGGGTGTGAACCAC GAGAA-3′; *GAPDH*-R: 5′-GGCATGGACTGTGGTCAT GA-3′; TaqMan probe: 5′-CTGCACCACCAACTGC TTAGC-3′. These primers and probes were synthesized by Shanghai Biology Engineering Company (Shanghai P.R. China).

Cell culture

The human gastric carcinoma cell line SGC7901 and its MDR sublines SGC7901/ADR and SGC7901/VCR were gifts from the academician and Professor Daiming Fan (Institute of Digestive Diseases, Xijing Hospital, Fourth Military Medical University, Xi'an, China). The resistant indices of the SGC7901/VCR subline to VCR, methotrexate, ADR and 5-fluorouracil are 65, 24, 1.6 and 363, respectively. The resistant indices of the SGC7901/ADR subline to ADR, VCR, methotrexate and 5-fluorouracil are 101, 25, 9.1 and 1583, respectively [15]. The SGC7901 cell line was cultured with RPMI 1640 containing 5% newborn bovine serum. The MDR sublines were cultured with RPMI 1640 containing 5% newborn bovine serum, 0.3 μg/ml ADR or 1 μg/ml VCR to maintain the characteristics of MDR. All of them were cultured at 37°C and in 5% CO_2 in a humidified atmosphere in a CO_2-controlled incubator. The cells were maintained when the medium was changed every 1–2 days and the cells were subcultured every 3–5 days by treatment with 0.25% trypsin. The MDR sublines were cultured in the medium free of ADR or VCR for 2 weeks before treatment with celecoxib.

[^{3}H]-thymidine Incorporation assay

The effects of celecoxib on the growth of the SGC7901 cell line and the SGC7901/ADR and SGC7901/VCR sublines were determined by [^{3}H]thymidine ribotide ([^{3}H]TdR) incorporation into DNA. First, 5×10^4 cells per well were incubated for 24 h with or without the presence of celecoxib. The concentrations of celecoxib ranged from 1×10^{-8} to 1×10^{-4} mol/l. Then, each group was exposed to 1 ml of 37 kBq/ml of methyl-[^{3}H]thymidine for 2 h. For cell harvest, the plates were washed five times with phosphate-buffered solution (PBS), air dried and 200 μl of 1% Triton X-100 was added per well. After 2 h, 100 μl of Triton X-100 was harvested from each well and mixed with 10 ml of scintillation liquid. After dark adaptation for 2 h, the cell-associated radioactivity was determined by liquid-scintillation counting. The ratio of cell proliferation inhibition = (1 – [^{3}H]thymidine incorporation of treatment group/[^{3}H]thymidine incorporation of control group) × 100%.

MTT assay

The cells of the SGC7901 cell line and the SGC7901/ADR and SGC7901/VCR sublines were seeded into triplicate wells of 96-well plates at a density of 1×10^4 cells/ml and cultured for 24 h. Then the cells were treated with celecoxib at concentrations ranging from 1×10^{-8} to 1×10^{-4} mol/l. Then, 20 μl MTT (5 mg/ml) was added to each well after 24 h treatment with celecoxib. After 4 h, the cell supernatants were discarded, MTT crystals were dissolved with dimethylsulfoxide and the absorbance was measured at 570 nm (A_{570nm}). The ratio of cell proliferation inhibition = (1 – average A_{570nm} value of treatment group/average A_{570nm} value of control group) × 100%.

Expression of *MDR1* by real-time fluorescence-quantitative reverse transcription-polymerase chain reaction

The cells of SGC7901 cell line and the SGC7901/ADR and SGC7901/VCR sublines (4.0×10^5 cells/well) were seeded in six-well plates and cultured for 24 h. Celecoxib was then added in the same medium at concentrations ranging from 1×10^{-8} to 1×10^{-4} mol/l. After culturing for another 24 h, the medium was aspirated and the cells were washed twice with PBS. Total RNA was isolated by adding 1 ml of Trizol into each well and cDNA was synthesized by routine reverse transcription methods. Using TaqMan techniques and *GAPDH* gene as internal control, fluorescence signal was examined in real time. Polymerase chain reaction (PCR) was performed in a reaction system as follows: 10 × buffer 3.6 μl; 25 mmol/l $MgCl_2$ 3 μl; 10 mmol/l dNTP 0.9 μl; upstream and downstream primer 1 μl for *MDR1* or *GAPDH*; TaqMan primer 1 μl; heat-resistant TaqDNA polymerase 0.3 μl; dH_2O 14.8 μl and cDNA 2.5 μl. No cDNA template was present in the control. PCR was started after denaturation for 2 min at 94°C. One cycle consisted of a denaturation step for 20 s at 94°C, an annealing step for 30 s at 53°C (gathering fluorescence) and an extension step for 60 s at 60°C. After 45 cycles, the values of C_T for *MDR1* and *GAPDH* of all samples were measured. C_T represents the PCR cycle required to cross an arbitrarily placed threshold. The relative expression of *MDR1* (*MDR1*/*GAPDH* ratio) was reported as follows: $2^{-\Delta C_T}$, $\Delta C_T = C_{TMDR1} - C_{TGAPDH}$ [16].

Expression of P-glycoprotein by Western blot analysis

The cells of SGC7901 cell line and the SGC7901/ADR and SGC7901/VCR sublines (1.0×10^6 cells/well) were seeded in 50-ml flasks and cultured for 24 h. Celecoxib was then added in the same medium at a concentration of 1×10^{-4} mol/l and the cells were cultured for another 24 h. The cells were dissolved for 5 min on ice. Proteins were measured by Lowry analysis and stored at -70°C. Then 40 μg protein per lane was separated by 5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, transferred to nitrocellulose membrane and probed with goat antihuman P-gp polyclonal antibody for 2 h at 37°C. The membrane was washed and then incubated in horse antigoat IgG for 1.5 h at 37°C. After washes, the color of protein bands on the membrane was developed by 3,3′-diaminobenzidine liquids for 5 min and then scanned by a scanner. β-Actin was used as an internal control. The optica density (OD) of the protein band was analyzed by Bandscan figure software (Glyko; Novato, California, USA) and then the ratio of OD for P-gp to OD for β-actin was calculated.

Intracellular rhodamine 123 accumulation by flow cytometry

The cells of the SGC7901 cell line and the SGC7901/ADR and the SGC7901/VCR sublines (3×10^5 cells/well) were seeded in six-well plates and cultured for 24 h. Celecoxib was then added at concentrations ranging from 1×10^{-8} to 1×10^{-4} mol/l. After incubation for another 24 h, the cells were digested and washed twice with PBS. Then the cells were incubated with 1 ml medium containing 5 μg Rh123 labeled with fluorescence for 30 min at 37°C. After two washes with PBS, the cells were suspended with 1 ml PBS. The fluorescence as an indicator for intracellular Rh123 accumulation was measured by flow cytometry.

Data analysis

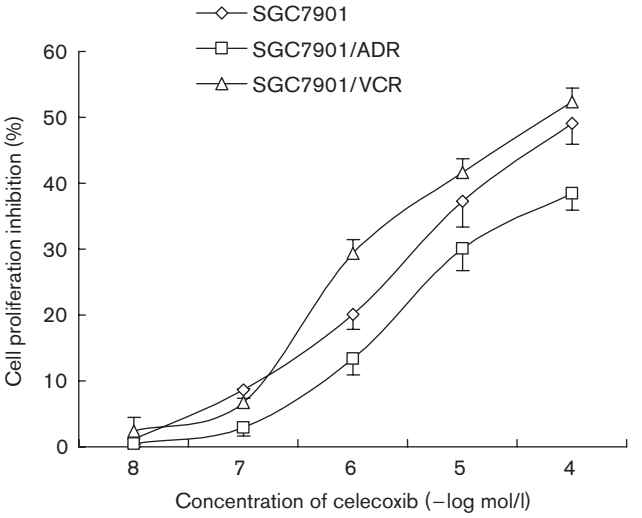
The data resulting from the ratio of cell proliferation inhibition, the *MDR1*/*GAPDH* ratio and intracellular accumulation of Rh123 were presented as mean ± SD, and were analyzed with one-way analysis of variance using SPSS 10.0 software. P value < 0.05 was considered to be significant.

Results

Proliferation inhibition by [^{3}H]thymidine incorporation assay and MTT test

After treatment with celecoxib at concentrations ranging from 1×10^{-6} to 1×10^{-4} mol/l for 24 h, the [^{3}H]thymidine incorporations in the SGC7901 cell line and the SGC7901/ADR and SGC7901/VCR sublines were significantly lower than those in untreated corresponding control groups, $P < 0.05$. The ratios of cell proliferation inhibition in the SGC7901 cell line and the SGC7901/ADR and SGC7901/VCR sublines were all lineally positive dose-dependent (Fig. 1). Similar results were also shown in MTT test (Fig. 2). At the concentration of 1×10^{-4} mol/l of celecoxib, the ratios were the highest (about 35–55%).

Fig. 1



Effects of celecoxib on [^{3}H]-thymidine incorporations in the SGC7901 cell line and the SGC7901/ADR and SGC7901/VCR sublines. Ordinate: the ratio of cell proliferation inhibition = (1 − [^{3}H]thymidine incorporation of treatment group/[^{3}H]thymidine incorporation of control group) × 100%. Each value is the mean ± SD of three separate experiments in which duplicate determinations were made. ADR, adriamycin; VCR, vincristine.

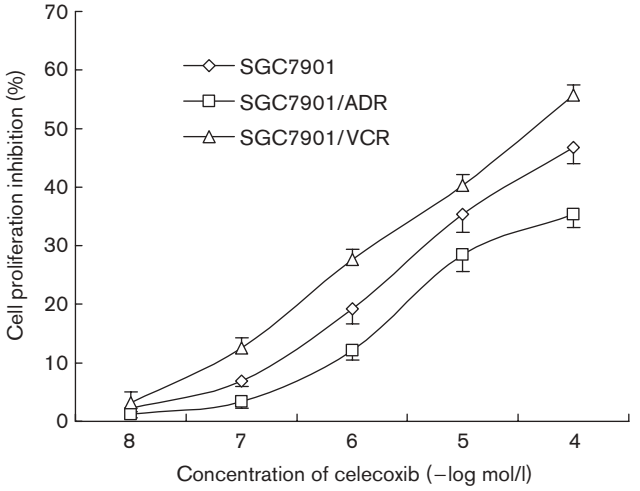
Decreased expression of *MDR1* messenger RNA in multidrug resistant sublines treated with celecoxib

No *MDR1* gene expression was detected in the SGC7901 cell line. The control *MDR1*/*GAPDH* ratio in the SGC7901/ADR subline was 37.87 ± 3.8. After treatment with celecoxib at concentrations ranging from 1×10^{-8} to 1×10^{-4} mol/l, *MDR1*/*GAPDH* ratios in SGC7901/ADR were significantly decreased in a linearly negative dose-dependent pattern, $P < 0.05$ (Fig. 3). Similar results were also obtained in the SGC7901/VCR subline (Fig. 3). The IC_{50} value of *MDR1*/*GAPDH* ratio was 5.50×10^{-6} mol/l in SGC7901/ADR and 3.89×10^{-6} mol/l in SGC7901/VCR.

Downregulation of P-glycoprotein expression in multidrug resistant sublines treated with celecoxib

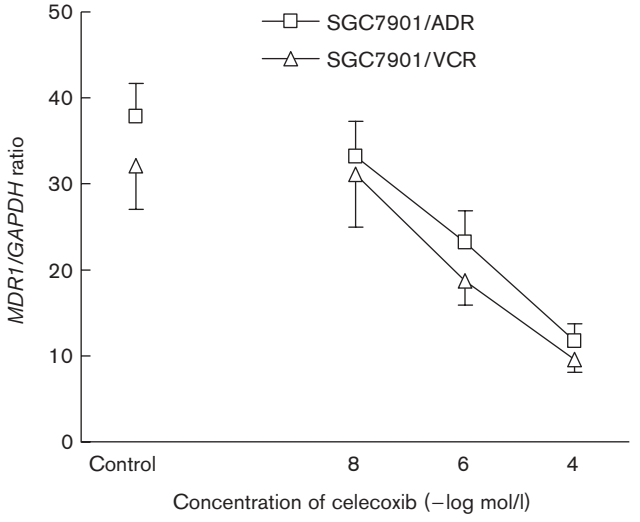
The expression of P-gp was negative in the SGC7901 cell line, and obviously positive in the SGC7901/ADR and SGC7901/VCR sublines (Fig. 4). Celecoxib at a concentration of 1×10^{-4} mol/l downregulated the expression of P-gp in both MDR sublines. The ratios of P-gp/β-actin in treatment groups were significantly lower than those in control groups, 0.28 vs. 0.71 in the SGC7901/ADR subline and 0.21 vs. 0.83 in the SGC7901/VCR subline, $P < 0.05$ (Fig. 4).

Fig. 2



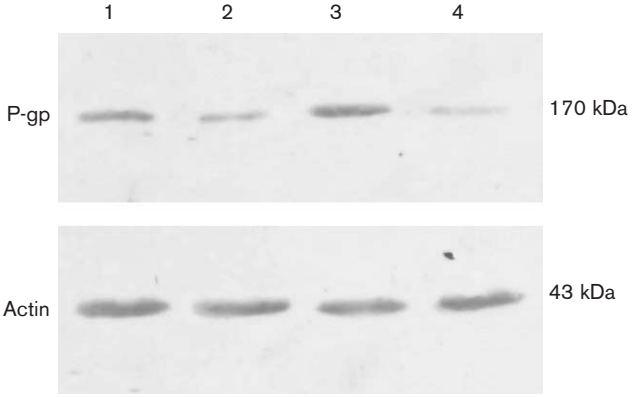
Effects of celecoxib on A_{570nm} values in the SGC7901 cell line, and the SGC7901/ADR and SGC7901/VCR sublines. Ordinate: the ratio of cell proliferation inhibition = (1 − average A_{570nm} value of treatment group/average A_{570nm} value of control group) × 100%. Each value is the mean ± SD of three separate experiments in which duplicate determinations were made. ADR, adriamycin; VCR, vincristine.

Fig. 3



Effects of celecoxib on *MDR1/GAPDH* ratios in SGC7901/ADR and SGC7901/VCR sublines. Each value is the mean ± SD of three separate experiments in which duplicate determinations were made by reverse transcription-polymerase chain reaction. ADR, adriamycin; VCR, vincristine.

Fig. 4



Expression of P-glycoprotein (P-gp) in MDR sublines (Western blot): 1, SGC7901/ADR subline (control); 2, SGC7901/ADR subline (treated with celecoxib); 3, SGC7901/VCR subline (control); 4, SGC7901/VCR subline (treated with celecoxib). The concentration of celecoxib was 1×10^{-4} mol/l. ADR, adriamycin; MDR, multidrug resistance; VCR, vincristine.

Increased intracellular rhodamine 123 accumulations in multidrug resistant sublines treated with celecoxib

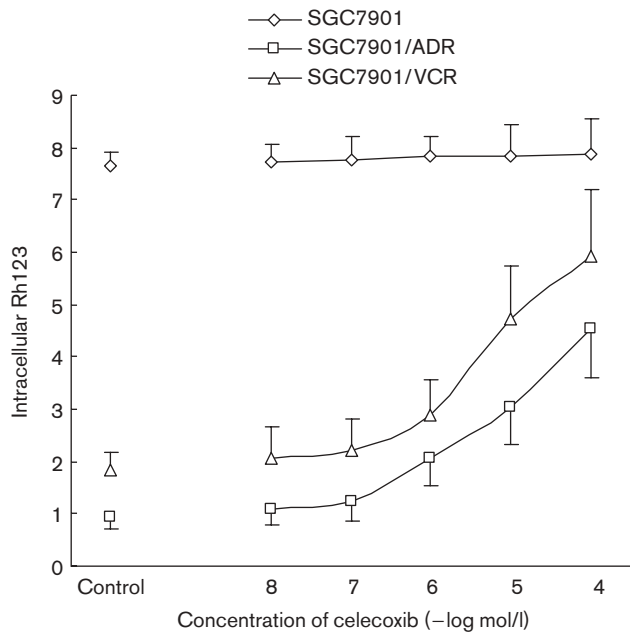
Intracellular Rh123 accumulations in the SGC7901 cell line were relatively high. Rh123 accumulations in the SGC7901/ADR and SGC7901/VCR sublines were, however, only about 25% of those in the SGC7901 cell line before treatment with celecoxib. After treatment with celecoxib, Rh123 accumulations in both MDR sublines increased positively in a dose-dependent pattern, $P < 0.05$. With 1×10^{-4} mol/l of celecoxib, Rh123 accumulations in MDR sublines were more than 50% of those in the SGC7901 cell line (Fig. 5).

Discussion

COX is a key enzyme required for the conversion of arachidonic acid to prostaglandin. Two isoforms of COX have been identified, i.e. COX-1 and COX-2. Overexpression of COX-2 in gastric carcinoma has been shown [17–19] and COX-2 selective inhibitors could inhibit proliferation of gastric carcinoma [6,20]. Both [^{3}H]thymidine incorporation assay and MTT test in this study demonstrated that celecoxib was able to inhibit proliferation of the SGC7901 cell line and the SGC7901/ADR and SGC7901/VCR sublines in a positive dose-dependent pattern. The higher inhibition in P-gp-free SGC7901 cell lines, compared with P-gp-expressing SGC7901/ADR sublines, might reflect the biological variety of these cells. This finding indicates a new way for treatment of gastric MDR sublines and can be beneficial for improving the prognosis of gastric carcinoma, as MDR is still a major obstacle to successful chemotherapy.

An important mechanism of MDR involves overexpression of P-gp, which consists of two homologous halves. Each half has an N-terminal hydrophobic domain containing six transmembrane domains, followed by a hydrophobic domain containing a nucleotide-binding site

Fig. 5



Effects of celecoxib on intracellular Rh123 concentration in the SGC7901 cell line, and the SGC7901/ADR and SGC7901/VCR sublines. Each value is the mean ± SD of three separate experiments in which duplicate determinations were made by flow cytometry. ADR, adriamycin; Rh123, rhodamine 123; VCR, vincristine.

for binding and hydrolyzing ATP. P-gp has therefore been proposed to be an active transporter for anticancer agents using the energy released by ATP hydrolysis [10]. Human hepatocellular carcinoma MDR subline induced by ADR showed higher expression of *MDR1* and COX-2 than its parent cell line [21]. By using adenovirus-mediated transfer of rat COX-2 cDNA into renal rat mesangial cells, an increase of *MDR1* and P-gp activity in COX-2 overexpressing cells was detected [22]. Similarly, in our study, we observed overexpression of P-gp in both the SGC7901/ADR and SGC7901/VCR sublines, but no expression of P-gp in the SGC7901 cell line. Furthermore, our study confirmed that celecoxib downregulated P-gp expression at mRNA and protein levels in both MDR sublines. The noticeably increased intracellular Rh123 accumulations in MDR sublines treated with celecoxib suggest that fewer anticancer agents would be pumped out of the cells because of the decreased activity of P-gp. We considered, therefore, that celecoxib, besides inhibiting proliferation, was also able to restore the chemosensitivity of the two MDR sublines by downregulating the expression and activity of P-gp. It could be of potential therapeutic interest.

Studies have shown that verapamil is able to inhibit the efflux pump and elevate the intracellular concentration of the anticancer agents by competitively blocking P-gp. It can reverse MDR *in vitro*, but might be toxic or lethal *in vivo* with the same dosage [23], and thus unsuitable for clinical application. The recommended oral dosage of celecoxib in adult patients with rheumatoid arthritis is 100–200 mg twice daily, and a mean peak plasma celecoxib concentration (C_{max}) of 705 g/l (1.85×10^{-6} mol/l) was reached 2.8 h after a single 200-mg dose in volunteers under fasting conditions [24]. In this study, celecoxib at concentrations ranging from 1×10^{-6} to 1×10^{-4} mol/l significantly downregulated the expression and activity of P-gp. It means that 1×10^{-6} mol/l of celecoxib might be safe and effective in the reversal of gastric carcinoma MDR, although the optional dosages for clinical use will need to be further investigated in humans.

The mechanism of downregulation of P-gp by celecoxib is still not clear. The efficient concentrations (1×10^{-6} to 1×10^{-4} mol/l) of celecoxib used in this study were high compared with the IC_{50} value (4×10^{-8} mol/l for COX-2 and 1.5×10^{-5} mol/l for COX-1) reported by Penning *et al.* [25], but they are not really comparable. The IC_{50} value of celecoxib from Penning's study indicated a direct reaction between celecoxib and monosubstituted 5-aryl analogs, and the concentration of celecoxib in this study was an extracellular one. A higher extracellular concentration of celecoxib might be necessary for it to enter the cells. It was difficult, therefore, to question the selection of celecoxib over either COX-2 or COX-1 in this study. Although it was demonstrated that anticancer effects of COX-2 inhibitors might involve either COX-2 or non-COX-2 pathways [26–28], the exact mechanism of downregulation of P-gp by celecoxib needs to be investigated later on.

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References

1 Schwarz RE, Zagala NK. Recurrence patterns after radical gastrectomy for gastric cancer: prognostic factors and implications for postoperative adjuvant therapy. *Ann Surg Oncol* 2002; **9**:394–400.
2 Sanchez-Bueno F, Garcia-Marcilla JA, Perez-Flores D, Perez-Abad JM, Vicente R, Aranda F, *et al.* Prognostic factors in a series of 297 patients with gastric adenocarcinoma undergoing surgical resection. *Br J Surg* 1998; **85**:255–260.
3 Choi JH, Lim HY, Joo HJ, Kim HS, Yi JW, Kim HC, *et al.* Expression of multidrug resistance-associated protein1, P-glycoprotein, and thymidylate synthase in gastric cancer patients treated with 5-fluorouracil and doxorubicin-based adjuvant chemotherapy after curative resection. *Br J Cancer* 2002; **86**:1578–1585.
4 Wilkoff W, Dulmadge EA. Resistant and cross-resistance of cultured leukemia P388 cells to vincristine, adriamycin, adriamycin analogs, and actinomycin D. *J Natl Cancer Inst* 1978; **61**:1521–1524.
5 Wong BC, Jiang X, Fan XM, Lin MC, Jiang SH, Lam SK, *et al.* Suppression of RelA/p65 nuclear translocation independent of IkappaB-alpha degradation by cyclooxygenase-2 inhibitor in gastric cancer. *Oncogene* 2003; **22**:1189–1197.

6 Liu C, Tang C, Wan X, Wang C, Zhou X. The effects of selective cyclooxygenase-2 inhibitors on the growth of gastric adenocarcinoma. *Sichuan Da Xue Xue Bao Yi Xue Ban* 2003; **34**:480–483.

7 Tang C, Liu C, Zhou X, Wang C. Enhanced inhibitive effects of combination of rofecoxib and octreotide on the growth of human gastric cancer. *Int J Cancer* 2004; **112**:470–474.

8 Zheng WB, Wang CH, Qiang O, Tang CW. Inhibitory effects of celecobix combined with octreotide on growth of multidrug resistant human gastric cancer cell line SGC7901/ADR. *Chin J Cancer* 2004; **23**:1628–1632.

9 Warmann S, Hunger M, Teichmann B, Flemming P, Gratz KF, Fuchs J. The role of the MDR1 gene in the development of multidrug resistance in human hepatoblastoma: clinical course and *in vivo* model. *Cancer* 2002; **95**:1795–1801.

10 Tsuruo T. Molecular cancer therapeutics: recent progress and targets in drug resistance. *Intern Med* 2003; **42**:237–243.

11 Ferte J. Analysis of the tangled relationships between P-glycoprotein-mediated multidrug resistance and the lipid phase of the cell membrane. *Eur J Biochem* 2000; **267**:277–294.

12 Warmann S, Hunger M, Teichmann B, Flemming P, Gratz KF, Fuchs J. The role of the MDR1 gene in the development of multidrug resistance in human hepatoblastoma: clinical course and in vivo model. *Cancer* 2002; **95**:1795–1801.

13 Petriz J, Garcia LJ. Flow cytometric analysis of P-glycoprotein function using rhodamine123. *Leukemia* 1997; **11**:1124–1130.

14 Limtrakul P, Khantamat O, Pintha K. Inhibition of P-glycoprotein function and expression by kaempferol and quercetin. *J Chemother* 2005; **17**:86–95.

15 Cai XJ, Zhang XY, Fan DM. *In vitro* study of anti-cancer agents resistant patterns of resistant human gastric cancer cell lines. *J Fourth Milit Med Univ* 1994; **15**:86–88.

16 Schmittgen TD, Teske S, Vessella RL, True LD, Zakrajsek BA. Expression of prostate specific membrane antigen and three alternatively spliced variants of PSMA in prostate cancer patients. *Int J Cancer* 2003; **107**:323–329.

17 Lim HY, Joo HJ, Choi JH, Yi JW, Yang MS, Cho DY, *et al*. Increased expression of cyclooxygenase-2 protein in human gastric carcinoma. *Clin Cancer Res* 2000; **6**:519–525.

18 Koga T, Shibahara K, Kabashima A, Sumiyoshi Y, Kimura Y, Takahashi I, *et al*. Overexpression of cyclooxygenase-2 and tumor angiogenesis in human gastric cancer. *Hepatogastroenterology* 2004; **51**: 1626–1630.

19 Sun WH, Sun YL, Fang RN, Shao Y, Xu HC, Xue OP, *et al*. Expression of cyclooxygenase-2 and matrix metalloproteinase-9 in gastric carcinoma and its correlation with angiogenesis. *Jpn J Clin Oncol* 2005; **35**: 707–713.

20 Hu PJ, Yu J, Zeng ZR, Leung WK, Lin HL, Tang BD, *et al*. Chemoprevention of gastric cancer by celecoxib in rats. *Gut* 2004; **53**:195–200.

21 Fantappie O, Masini E, Sardi I, Raimondi L, Bani D, Solazzo M, *et al*. The MDR phenotype is associated with the expression of COX-2 and iNOS in a human hepatocellular carcinoma cell line. *Hepatology* 2002; **35**: 843–852.

22 Patel VA, Dunn MJ, Sorokin A. Regulation of MDR1 (P-glycoprotein) by cyclooxygenase-2. *J Biol Chem* 2002; **277**:38915–38920.

23 Sonneveld P, Wiemer E. Inhibitors of mutidrug resistance. *Curr Opin Oncol* 1997; **9**:543–548.

24 Clemett D, Goa KL. Celecoxib: a review of its use in osteoarthritis, rheumatoid arthritis and acute pain. *Drugs* 2000; **59**:957–980.

25 Penning TD, Talley JJ, Bertenshaw SR, Carter JS, Collins PW, Docter S, *et al*. Synthesis and biological evaluation of the 1,5-diarylpyrazole class of cyclooxygenase-2 inhibitors: identification of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benze nesulfonamide (SC-58635, celecoxib). *J Med Chem* 1997; **40**:1347–1365.

26 Wong BC, Jiang XH, Lin MC, Tu SP, Cui JT, Jiang SH, *et al*. Cyclooxygenase-2 inhibitor (SC-236) suppresses activator protein-1 through c-Jun NH_2-terminal kinase. *Gastroenterology* 2004; **126**:136–147.

27 Honjo S, Osaki M, Ardyanto TD, Hiramatsu T, Maeta N, Ito H. COX-2 inhibitor, NS398, enhances Fas-mediated apoptosis via modulation of the PTEN-Akt pathway in human gastric carcinoma cell lines. *DNA Cell Biol* 2005; **24**:141–147.

28 Fan XM, Jiang XH, Gu Q, Ching YP, He H, Xia HH, *et al*. Inhibition of Akt/PKB by a COX-2 inhibitor induces apoptosis in gastric cancer cells. *Digestion* 2006; **73**:75–83.