Growth inhibition and apoptosis induction of Sulindac on Human gastric cancer cells

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Abstract

AIM: To evaluate the effects of sulindac in inducing growth inhibition and apoptosis of human gastric cancer cells in comparison with human hepatocellular carcinoma (HCC) cells.

METHODS: The human gastric cancer cell lines MKN45 and MKN28 and human hepatocellular carcinoma cell lines HepG2 and SMMC7721 were used for the study. Anti-proliferative effect was measured by MTT assay, and apoptosis was determined by Hoechst-33258 staining, electronography and DNA fragmentation. The protein of cyclooxygenase-2 (COX-2) and Bcl-2 were detected by Western dot blotting.

RESULTS: Sulindac could initiate growth inhibition and apoptosis of MKN45, MKN28, HepG2, and SMMC7721 cells in a dose-and time-dependent manner. Growth inhibitory activity and apoptosis were more sensitive in HepG2 cells than in SMMC7721 cells, MKN45 and MKN28 cells. After 24 hours incubation with sulindac at 2 mmol·L⁻¹ and 4 mmol·L⁻¹, the level of COX-2 and Bcl-2 prote in were lowered in MKN45, SMMC7721 and HepG2 cells but not in MKN28 cells.

CONCLUSION: Sulindac could inhibit the growth of gastric cancer cells and HCC cells effectively in vitro by apoptosis induction, which was associated with regression of COX-2 and Bcl-2 expression. The growth inhibition and apoptosis of HCC cells were greater than that of human gastric cancer cells. The different effects of apoptosis in gastric cancer cells may be related to the differentiation of the cells.

Subject headings sulindac; apoptosis; gastric cancer; HCC; COX-2

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INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin and indomethacin were widely used for the treatment of inflammatory joint and muscle pain. The studies of past two decades indicated that NSAID could prevent colorectal cancer[11-13]. As a member of NSAID, sulindac has been shown to induce regression of adenomas in familial adenomatous polyposis (FAP) patients, prevent recurrence of adenomas[14-15] and reduce the risk of colon cancer[16]. Rahman et al[17] demonstrated that sulindac and its irreversible oxidized derivative, sulindac sulfone exhibited a growth inhibitory effect on human hepatocellular carcinoma (HCC) cell lines, indicating that sulindac has chemopreventive effect on colon cancer as well as on other types of cancer in GI tract. Gastric cancer is one of the most common causes of malignancy-related death in China[18-20]. It is imperative to find effective chemopreventive methods to reduce the morbidity and mortality of gastric cancer. Lots of chemical agents have been proved having the chara cet of inducing apoptosis in human gastric cancer cells[12-15]. Recent studies gave the concept that overexpression of cyclooxygenase-2 (COX-2) was the early event in carcinogenesism of gastric cancer[13] and made it the target of chemoprevention against gastric cancer[14]. The present study was undertaken to analyze the effect of sulindac in two gastric cancer cell lines as compared with two HCC cell lines.

MATERIALS AND METHODS

Cell lines and culture

The human gastric cancer cell lines MKN45 and MKN28 were obtained from the Japanese Cancer Research Resources Bank (Tokyo, Japan). HepG2 and SMMC7721 cells were available commercially from the Institute of Biochemistry and Cell Biology, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences. Cells were grown in RPMI 1640 (Gibco) supplemented with 100 mL·L⁻¹ fetal bovine serum (Sijiqing, HangZhou, China), penicilin (100 mg·L⁻¹) and streptomycin (100 mg·L⁻¹) in a humidified atmosphere of 50 mL·L⁻¹ CO₂ at 37°C. Sulindac purchased from Sigma Chemical Co. (St Louis, MO, USA) was freshly prepared in DMSO (less than 5 g·L⁻¹ in water) before use. Vehicle controls of DMSO was included in the studies.

MTT assay

Antiproliferative effects were measured by MTT assay. About 10 000 cells per well were plated in 96-well microtitre plates and incubated overnight in 100 µL of culture media. Then cells were treated with various concentrations of sulindac for 24 h and 48 h (0, 0.25, 0.5, 1, 2 and 4 mmol·L⁻¹ for MKN45, MKN28 and SMMC7721 cells, 0, 25, 50, 100, 200 and 400 mmol·L⁻¹ for HepG2 cells); 100 µL MTT (1 g·L⁻¹) was then added to each well, and the cells were further incubated for 4 h. The supernatant was removed and 100 µL DMSO was added to each well, and then incubated for 30 min. The absorbance at wavelength of 570 nm was measured by a micro - ELISA reader. The negative control well has no cells and used as zero point of a bsorbance. Each assay was performed three times in triplicate.

Morphological measurement of apoptosis

The morphological change of apoptosis was assayed under fluorescence microscope following staining with Hoechst33258. The cells were fixed in 200 mL·L⁻¹ ethanol/PBS, stained with 5 mg·L⁻¹ Hoechst33258 (CNI) for 30 min at 37°C, then visualized under UV fluorescence microscope. Apoptosis cells were de fined as cells...
showing nuclear and cytoplasmic shrinkage, chromatin condensation and apoptosis body. At least 300 cells were counted and the percentage of apoptotic cells (Apoptotic Index) was calculated.

**DNA fragmentation analysis**

Following sulindac treatment, cells were washed by PBS, and fixed in ice-cold 700 mL·L⁻¹ ethanol for 24 h. After the ethanol was removed, cells were rinsed in 40 µL 0.2 mol·L⁻¹ Na₂HPO₄/0.1 mol·L⁻¹ citric acid (pH 9.2) for 60 min at room temperature. After being centrifuged at 37°C for 5 min (1000G), the supernatant was collected in Eppendorf tubes, 40 µL 2.5 g·L⁻¹ NP-40 and 40 µL 10 g·L⁻¹ RNase A were added. After being centrifuged for 30 min, protein precipitate was washed with equal amounts of 1.2 M KCl. Equal amount of DNA (15 µL) was electrophoresed in 10 g·L⁻¹ agrose gel impregnated with ethidium bromide (5 mL·L⁻¹) for 2 h at 80 V. DNA fragments were visualized by ultraviolet transillumination.

**Western Dot blot analysis of COX-2 and Bcl-2**

Cells incubated with various concentrations of sulindac for 24 h (0, 2 and 4 mmol·L⁻¹) for MKN28, MKN45 and SMMC7721 cells, 0, 400 and 800 µmol·L⁻¹ for HepG2 cells). These cells were washed by PBS and dissolved in 100 µL extraction buffer (50 mmol·L⁻¹ Tris-HCl (pH 8.0), 150 mmol·L⁻¹ NaCl, 10 g·L⁻¹ Triton-X-100 and 1 mg·L⁻¹ Aprotin). Equal amounts of cell lysates were dotted on nitrocellulose membrane (Amersham). The membrane was incubated first with a primary antibody overnight at 4°C and then with peroxidase-conjugated anti-rabbit or anti-mouse IgG antibody for 2 h. Protein was detected by chemiluminescence (ECL) system. The membranes were washed and added by equal amounts of luminal reagent A and B (Santa Cruz) for 2 min and exposed to film. Rabbit anti-human polyclonal antibody COX-2 (H-62, sc-7951), goat anti-mouse IgG conjugated with HRP (Cat#sc-2005), and Goat anti-rabbit IgG conjugated with HRP (Cat#sc-2004) were purchased from Santa Cruz Co. Mouse anti-human bcl-2 monoclonal antibody (Cat.No.M-0025) was purchased from Antibody Diagnostica Inc(USA).

**Statistical analysis**

Student’s t test was used for results comparison among different groups. The presented data were mean values of at least three different experiments and expressed as X±s. A P value of less than 0.05 is considered statistically significant.

**RESULTS**

**Effects of sulindac on cell growth**

Various concentrations of sulindac were incubated with cells for 24 h and 48 h. Cell growth was determined by MTT assay. As shown in Figure 1, sulindac could inhibit the growth of gastric cancer cells and HCC cells in a dose- and time-dependent manner. Sulindac showed a more potent effect in reducing HepG2 cells’ growth as compared with SMMC7721, MKN45 and MKN28 cells. The cell death rate was more obvious in MKN45 cells than in MKN28 cells (Figure 1).

**Apoptosis of cells induced by sulindac**

To evaluate the apoptosis of cells, Hoechst-33258 staining and agarose gel electrophoresis of genomic DNA were used. The Hoechst-33258 staining showed apoptosis in all four types of cells, which was characterized by cytoplasmic and nuclear shrinkage, chromatin condensation and apoptosis body (Figure 2). The apoptosis was more evident in HepG2 cells than in SMMC7721 and gastric cancer cells and the AI of MKN45 cells were higher than that of MKN28 cells (Figure 3). DNA fragmentation was shown as a ladder pattern on agarose gel.
Differential expression of COX-2 and Bcl-2 protein in sulindac-treated cells

The protein levels of COX-2 and Bcl-2 were determined by Western dot blotting. After treatment with 2 mmol·L⁻¹ and 4 mmol·L⁻¹ of sulindac for 24 h, the protein level of COX-2 and Bcl-2 showed marked decrease in MKN45, HepG2 and SMMC7721 cells, whereas the protein level remained unchanged in MKN28 cells (Figure 4).

DISCUSSION

Since Adolphie et al reported that certain NSAIDs were capable of inhibiting proliferation of Hela cells in 1972, the chemopreventive effect of NSAIDs has been widely studied in vivo and in vitro in recent years[15-16]. Most results indicated that the mechanism related to this capability was by the inhibition of cyclooxygenase-2 (COX-2) which was not found in most normal tissues and could be induced by cytokines and growth factors[17-19]. Elevated level of COX-2 suggested the existence of inflammation or carcinoma[20-25]. Lim et al found that all 104 gastric cancer tissues showed positive expression of COX-2 but not in normal gastric mucosa. Ratnasinge et al[26] found that gastric cancer tissues could produce more prostagland in than normal gastric mucosa. COX-2 was also found to be related to tumor angiogenesis[27-28] and metastasis[29-31]. In this study, we confirmed the results of other studies that COX-2 was positive in MKN45, MKN28 and HepG2 cell lines as well as in SMMC7721 cells and the level of COX-2 protein was much lower in MKN28 cells. The results also confirmed that COX-2 was correlative with carcinogenesis in GI tract.

One of the strongest evidence that NSAID has the capability of chemoprevention and treatment of colorectal cancer was the obvious effect of sulindac in treatment of FAP[32-33]. Pasricha et al reported that the number and size of polyps were reduced in 24 FAP patients after treatment with sulindac and induction of apoptosis was regarded...
as the main mechanism. However, there we re no studies about growth inhibition and apoptosis induction of sulindac in gastric cancer. In this study, two gastric cancer cell lines with different status of differentiation were used. The growth inhibition and apoptosis of HepG2 cells were more obvious as compared with MNK45 and MNK28 cells. Since sulindac is a pro-drug and is metabolized to sulindac sulfide and sulfone by the gut flora and in the liver [34], HCC cells might be able to convert sulindac to its metabolic derivative and increase its capability of growth inhibition and apoptosis induction. We suggested that the effects of growth inhibition of sulindac on gastric cancer might be increased in vitro in vivo. We also concluded that sulindac could induce apoptosis in gastric cancer cells and HCC cells, which may account for its growth inhibitory effects. Bcl-2 is one of the most important factors in apoptosis process [38]. The elevated expression of COX-2 could increase the level of Bcl-2 in the epithelial cells of rat colon and decrease the apoptotic rate of colonic cells [36]. Prostaglandin E2, mediated by COX-2 from arachidonic acid, could inhibit apoptosis of human colon cancer cells in vitro and increase expression of Bcl-2 in cancer cells [31]. Liu et al. [30] also confirmed the relationship between COX-2 and Bcl-2 in prostate cancer. We found that the levels of COX-2 in MNK45, SMMC7721 and HepG2 2 cells as well as Bcl-2 were decreased after treatment with sulindac, whereas both COX-2 and Bcl-2 were unchanged in MNK28 cells. Apoptosis was also more evident in MNK45, SMMC7721 and HepG2 than in MNK28 cells. It was suggested that COX-2 and Bcl-2 were involved in apoptosis of gastric cancer cells and HCC cells induced by sulindac.

Several mechanisms have been proposed affecting the pathways regulating cellular proliferation and apoptosis by NSAI ds. Although parts of the mechanisms were related to COX-2 inhibition, most of them were COX independent [39-44]. Sulindac and its derivatives have different mechanisms in inhibiting apoptosis of cancer cells. Sulfone neither inhibits COX nor has anti-inflammatory properties, but can produce chemopreventive effect similar to that of sulindac [45-46]. The death rate of MNK28 cells was higher than its apoptotic rate in our study, which might be caused by the COX-2 in dependent way. As COX-2 specific inhibitors, Celecoxib and Rofecoxib have been used clinically with few side effects [47]. The chemopreventive effect of COX-2 specific inhibitor has not yet been well studied as compared with COX-2 nonspecific inhibitors [48]. As one of COX-2 nonspecific inhibitors, sulindac had little effect in renal prostanoid synthesis and provides additional advantage for its use in clinical trials [49]. The concentration and time course of sulindac in inhibiting growth and inducing apoptosis of gastric cancer cells in vivo need further investigations. Other possible mechanisms of action of sulindac need to be further studied.

REFERENCES

1. Binder HY. Prevention of colorectal cancer: tumor progression chemoprevention, and COX-2 inhibition. Gastroenterology, 2000; 119:267-272
2. Yuan CJ, Mandal AK, Zhang ZJ, Mukherjee AB. Transcriptional regulation of cyclooxygenase-2 gene expression: novel effects of non-steroidal anti-inflammatory drugs. Cancer Res, 2000;60:1048-1091
3. Wu YL, et al. Effect of sulindac on gastric cancer cells. Cancer Res, 2000; 60:2085-2089
4. Wang CD, Chen YL, Wu T, Liu YR. Association between low expression of somatostatin receptor II gene and lymphoid metastasis in patients with gastric cancer. World Clin J Digest, 1999;8:864-866
5. Wang DX, Fang DC, Liu WW. Study on alteration of multiple genes in intestinal metastasis of gastric hyperplasia and gastric. Shijie Huaren Xiaohua Zazhi, 2000;8:855-859
6. Gu HP, Ni CR, Zhan RZ. Relationship between CD15 mRNA and its protein expression and gastric carcinoma invasion. World J Gastroenterol, 2000;6:851-854
7. Wang H, Zhang FX, Liang JR. Apoptosis induction and treatment for gastric cancer. Biochem Pharmacol, 1999;53:315-318
8. Lim HY, Joo HJ, Choi HY, Yi JW, Yang MS, Cho DY, Kim H S, Nam DK, Lee KB, Kim HC. Increased expression of cyclooxygenase-2 protein in human gastric carcinoma. Clin Cancer Res, 2000;6:519-525
9. Lim JT, Piazza GA, Han EK, Delohery TM, Li H, Finn TS, Buttany R, Yamamoto H, Sperl GJ, Bendel K, Gross PH, Pumakucu R, Weinstein IB. Sulindac derivatives inhibit growth and induce apoptosis in human prostate cancer cell lines. Biochem Pharmacol, 1999;58:1097-1107
10. Rahman MA, Dit-Amsat RH, Lemoine MG, Frazier ML, Sinicrope FA, et al. COX-2 specific inhibitors, Celecoxib and Rofecoxib have been used clinically with few side effects [47]. The chemopreventive effect of COX-2 specific inhibitor has not yet been well studied as compared with COX-2 nonspecific inhibitors [48]. As one of COX-2 nonspecific inhibitors, sulindac had little effect in renal prostanoid synthesis and provides additional advantage for its use in clinical trials [49]. The concentration and time course of sulindac in inhibiting growth and inducing apoptosis of gastric cancer cells in vivo need further investigations. Other possible mechanisms of action of sulindac need to be further studied.

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4. Wang CD, Chen YL, Wu T, Liu YR. Association between low expression of somatostatin receptor II gene and lymphoid metastasis in patients with gastric cancer. World Clin J Digest, 1999;8:864-866
5. Wang DX, Fang DC, Liu WW. Study on alteration of multiple genes in intestinal metastasis of gastric hyperplasia and gastric. Shijie Huaren Xiaohua Zazhi, 2000;8:855-859
6. Gu HP, Ni CR, Zhan RZ. Relationship between CD15 mRNA and its protein expression and gastric carcinoma invasion. World J Gastroenterol, 2000;6:851-854
7. Wang H, Zhang FX, Liang JR. Apoptosis induction and treatment for gastric cancer. Biochem Pharmacol, 1999;53:315-318
8. Lim HY, Joo HJ, Choi HY, Yi JW, Yang MS, Cho DY, Kim H S, Nam DK, Lee KB, Kim HC. Increased expression of cyclooxygenase-2 protein in human gastric carcinoma. Clin Cancer Res, 2000;6:519-525
9. Lim JT, Piazza GA, Han EK, Delohery TM, Li H, Finn TS, Buttany R, Yamamoto H, Sperl GJ, Bendel K, Gross PH, Pumakucu R, Weinstein IB. Sulindac derivatives inhibit growth and induce apoptosis in human prostate cancer cell lines. Biochem Pharmacol, 1999;58:1097-1107
10. Rahman MA, Dit-Amsat RH, Lemoine MG, Frazier ML, Sinicrope FA, et al. COX-2 specific inhibitors, Celecoxib and Rofecoxib have been used clinically with few side effects [47]. The chemopreventive effect of COX-2 specific inhibitor has not yet been well studied as compared with COX-2 nonspecific inhibitors [48]. As one of COX-2 nonspecific inhibitors, sulindac had little effect in renal prostanoid synthesis and provides additional advantage for its use in clinical trials [49]. The concentration and time course of sulindac in inhibiting growth and inducing apoptosis of gastric cancer cells in vivo need further investigations. Other possible mechanisms of action of sulindac need to be further studied.
carcinoma. Cancer Res, 1999;59:198-204

32 Masferrer JL, Leahy KM, Koki AT, Zweifel BS, Settle SL, Woerner BM, Edwards DA, Flickinger AG, Moore RJ, Seibert K. Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. Cancer Res, 2000;60:1306-1311

33 Yang ZB, Ouyang Q, yuquan WEI, Liu XQ, Lei S. Sulindac inhibits proliferation of HT-29 colon adenocarcinoma cells: effects on cell cycle and apoptosis. ZhongHua xiaohua Zazhi, 2000;20:102-104

34 Wang YK, Ji XL, Ma NX. Expression of p53, bcl-2 and c-erbB-2 genes in precarcinomatous gastric mucosa. Shijie Huaren Xiaohua Zazhi, 1999;7:114-116

35 Yuan RW, Ding Q, Jiang HY, Qin XF, Zou SQ, Xia SS. Bcl-2, P53 protein expression and apoptosis in pancreatic cancer. World Chin J Digestol, 1999;7:851-854

36 Zhang CS, Wang WL, Peng WD, Hu PZ, Chai YB, Ma FC. Promotion of apoptosis of SMMC7721 cells by Bcl-2 ribozyme. Shijie Huaren Xiaohua Zazhi, 2000;8:417-419

37 Sheng H, Shao J, Morrow JD, Beauchamp RD, DuBois RN. Modulation of apoptosis and Bcl-2 expression by prostaglandin E2 in human colon cancer cells. Cancer Res, 1998;58:362-366

38 Liu XH, Yao S, Itoh F, Fukushima H, Hinda Y, Imai K. Overexpression of cyclooxygenase-2 protein is less frequent in gastric cancers with microsatellite instability. Int J Cancer, 1999;84:400-403

39 Yamamoto H, Itoh F, Fukushima H, Hinda Y, Imai K. Overexpression of cyclooxygenase-2 protein is less frequent in gastric cancers with microsatellite instability. Int J Cancer, 1999;84:400-403

40 Yamamoto H, Itoh F, Fukushima H, Hinda Y, Imai K. Overexpression of cyclooxygenase-2 protein is less frequent in gastric cancers with microsatellite instability. Int J Cancer, 1999;84:400-403

41 Majima M, Hayashi I, Muramatsu M, Katada J, Yamashina S, Katoh M. Cyclooxygenase-2 enhances basic fibroblast growth factor-induced angiogenesis through induction of vascular endothelial growth factor in rat sponge implants. Br J Pharmacology, 2000;130:641-649

42 Herrmann C, Block C, Geison C, Haas K, Weber C, Winde G, Morov T, Muller O. Sulindac sulfide inhibits ras signaling. Oncogene, 1998;17:1769-1776

43 Gilhooly EM, Rose DP. The association between a mutated ras gene and cyclooxygenase-2 expression in human breast cancer cell lines. Int J Oncol, 1999;15:267-270

44 Shureiisi I, Chen D, Lotan R, Yang P, Newman RA, Fischer SM, Lippman SM. 15-Lipox ygenase-1 mediates nonsteroidal anti-inflammatory drug-induced apoptosis independently of cyclooxygenase-2 in colon cancer cells. Cancer Res, 2000;60:6846-6850

45 DeJong TA, Skinner SA, Malcontenti WC, Yogiagis D, Bailey M, VanDriell IR, O’Brien PE. Inhibition of rat colon tumors by sulindac and sulindac sulfone is independent of K-ras (codon 12) mutation. Am J Physiol Gastrointest Liver Physiol, 2000;278:G226-G272

46 Rahman MA, Dharm RK, Masunage R, Yamanoi A, Kohno H, Nagasue N. Sulindac and exisulind exhibit a significant antiproliferative effect and induce a poptosis in human hepatocellular carcinoma cell lines. Cancer Res, 2000;60:20:85-2089

47 Goldstein JL, Silverstein FE, Agrawal NM, Hubbard RC, Kaiser J, Maua RJ, Verburg KM, Steven Geis G. Reduced risk of upper gastrointestinal ulcer complications with celecoxib, a novel COX-2 inhibitor. Am J Gastroenterol, 2000;95:1681-1690

48 Reddy BS, Yoshinobu Y, Lubet R, Steele V, Kelloff G, Paulson S, Seibert K, Rao CV. Chemoprevention of colon cancer by specific cyclooxygenase-2 inhibitor or, celecoxib, administered during different stages of carcinogenesis. Cancer Res, 2000;60:293-297

49 Hsueh CT, Chiu CF, Kelsen DP, Schwartz GK. Selective inhibition of cyclooxygenase-2 enhances mitoxantrone-C-induced apoptosis. Cancer Chemother Pharmacol, 2000;45:389-396

50 Sawaoka H, Tsuiji S, Tsui M, Gunawan BS, Sasaki Y, Kawan S, Horii M. Cyclooxygenase inhibitors suppress angiogenesis and reduce tumor growth in vivo. Laboratory Investigation, 1999;12:1469-1477

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