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COMBINATION OF CYCLOOXYGENASE-2 INHIBITOR AND DOXORUBICIN INCREASES THE GROWTH INHIBITION AND APOPTOSIS IN HUMAN HEPATOCELLULAR CARCINOMA CELLS

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Inhibition of cyclooxygenase (COX)-2 elicits therapeutic effects in solid tumors that are coupled with the inhibition of cell proliferation and induction of apoptosis in tumor cells. *Aim:* This study was designed to investigate the role of COX-2 inhibitor nimesulide in cell growth and apoptosis of the cultured human hepatocellular carcinoma HepG2 cells. *Methods:* We performed the MTT assay, flow cytometric analysis and cell morphology study to evaluate growth inhibition and cell apoptosis upon the action of nimesulide alone or along with doxorubicin, a common agent for the treatment of human hepatocellular carcinoma. *Results:* Our results showed that the treatment of HepG2 cells with more than 50 μM of nimesulide suppressed COX-2 enzyme activity because of reduced PGE_2 production, and then induced growth inhibition and cell apoptosis despite no alterations of COX-2 protein expression. Importantly, the combination of 50 μM or 100 μM of nimesulide and low concentrations (5 μM to 20 μM) of doxorubicin resulted in enhanced cell growth inhibition, apoptosis induction and reduced VEGF production. *Conclusion:* These data suggest synergistic and/or additive effects of COX-2 inhibitors and chemotherapeutic agents, and may provide the rational for clinical studies of COX-2 inhibitors on the treatment or chemoprevention of human hepatocellular carcinoma.

Key Words: **hepatocellular carcinoma, nimesulide, COX-2, doxorubicin, apoptosis, VEGF.**

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the fourth leading cause of cancer related mortality worldwide, with the highest incidence in Asia, where hepatitis B or C is epidemic [1]. Although the clinical diagnosis and management of early-stage HCC has significantly improved, HCC prognosis remains poor. Only 10–20% of patients qualify for curative surgery [2]. The median survival of patients who have unresectable tumors is only 4 months [3]. Currently, no effective systemic (chemotherapeutic or chemopreventive) treatments are available. Hence, investigating HCC pathogenesis and finding new treatment strategies is an urgent need.

The cyclooxygenase-2 (COX-2) is highly expressed in a variety of human cancers [4, 5]. COX-2 has been associated with tumor growth, angiogenesis, invasion, and metastasis [6, 7]. Overexpression of COX-2 may increase the resistance of cancer cells to apoptosis [8]. Thus, the reduction of the COX-2 enzyme activity or protein expression may inhibit cell growth in cancer cells. Non-steroidal anti-inflammatory drugs (NSAIDs) have been shown to inhibit COX enzymes and may be employed for the chemoprevention of cancer [9, 10]. Some NSAIDs such as nimesulide exhibit high selectivity for COX-2 enzyme, but have little effects on COX-1 enzyme [11]. The expression pattern of COX-2 protein in HCC is well correlated with the differentiation grade, suggesting that abnormal COX-2 expression plays an important role in hepatocarcinogenesis [12, 13]. Inhibition of COX-2 by several inhibitors including celecoxib, NS-398 and nimesulide induces growth inhibition and marked apoptosis in cultured HCC cells by various mechanisms [12, 14–20].

There is also evidence suggesting an important role of angiogenesis in COX-2-mediated hepatocarcinogenesis. COX-2 has been shown to induce angiogenesis via vascular endothelial growth factor (VEGF), a well-studied regulator of pathological angiogenesis [21–23]. Several recent studies show that elevated COX-2 expression correlates with increased VEGF level and microvascular density in human HCCs [16, 24, 25]. In cultured hepatocellular carcinoma cells, VEGF production is increased by overexpression of COX-2 or by treatment with prostaglandin E_2 (PGE_2), and this effect is blocked by inhibition of COX-2 [16]. A separate study also shows a role for PGE_2 in the up-regulation of VEGF in the hepatocellular carcinoma cells [16]. These findings suggest that COX-2 and COX-2-derived PGE_2 signaling may promote hepatocarcinogenesis in part through VEGF-induced angiogenesis. In this regard, targeting COX-2-derived prostaglandin signaling represents a promising strategy to reduce the tumor burden.

Doxorubicin is one of the effective agents for the treatment of patients with unresectable HCCs [26, 27]. Single use of doxorubicin yields response rate of up to 20%, however median survival is not prolonged. There is no convincing evidence from randomized trails that the combination chemotherapy prolongs the survival of patients with unresectable HCC better than single agents. Whether COX-2 inhibitors are beneficial in the treatment of HCC if given in combination with these

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Abbreviation used: COX-2 – cyclooxygenase-2; ELISA – enzyme-linked immunosorbent assay; HCC – hepatocellular carcinoma; MTT – 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; PGE_2 – prostaglandin E_2; PI – propidium iodide; VEGF – vascular endothelial growth factor.

effective agents such as doxorubicin with complementary mechanisms remains unknown.

In this study, we have characterized the effects of COX-2 inhibition on cell growth and survival by treatment with nimesulide, a COX-2 specific inhibitor, in hepatocellular carcinoma cell line HepG2. Our results showed that the treatment with nimesulide enhanced doxorubicin-mediated cytotoxicity and reduced production of PGE_2 and VEGF. Our results that show additive inhibitory effects of COX-2 inhibitor nimesulide and doxorubicin on the growth of hepatocellular carcinoma cells may provide the rationale for clinical studies of COX-2 inhibitors on the treatment or chemoprevention of HCC.

MATERIALS AND METHODS

Reagents. Human HepG2 hepatocellular carcinoma cell line was obtained from The Research Institute on Hepatoma of Shanghai. RMPI 1640, DMEM and MEM culture medium was purchased from Gibco BRL. Fetal bovine serum (FBS) was provided by Sijiqing Biological Engineering Material (Hangzhou, China). Rabbit anti-COX-2 was from Cayman. Sheep anti-rabbit, sheep anti-mouse secondary antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), nimesulide and acridine orange (AO) were purchased from Sigma Chemical Co. (St. Louis, MO). Doxorubicin was provided by Wanle Co. (Shenzhen, China).

Cell culture. HepG2 cells were maintained in DMEM medium supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, and 100 mg/ml streptomycin. HepG2 cells were cultured at 5% CO_2 and 37° C in a humidified incubator (Nacpo-6100, DuPont Company, USA).

MTT assay. The number of viable cells was determined by MTT assay. Each cell sample was plated at a density of 10^4 cells/well in 96-well cluster dishes for 24 h before treatment. Then the medium was changed and nimesulide and doxorubicin were added in various concentrations and analyzed at indicated time points. After addition of MTT solution (5 g/L) in each well, the cells were incubated at 37° for 4 h, and then 150 μL DMSO was added to dissolve the dark blue crystals. The absorbance was measured in an ELISA plate reader (EL301 Strip reader, BIOTEK, USA) with a test wavelength of 570 nm, and the relative percentage of survival was calculated by dividing the absorbance of treated cells by that of the control in each experiment. Percent cytotoxicity was calculated using the formula: percent cytotoxicity = [1-(absorbance of experimental wells/absorbance of control wells)] × 100%.

Cell morphology. After treatement with drugs, cytological morphology changes were observed under the Olympus optical microscope. Cells were subcultured on coverslips in 6-well culture plates. After 24 h, the coverslips were taken out and stained with acridine orange. Cells were observed and photographs were taken under fluorescence microscope.

Flow cytometry. HepG2 cells were seeded in culture flasks. The culture bottles were divided into one medium alone and three drug-treated groups. Each group was in triplicate. When the cells were anchored to the plates, various drugs were added and the cells incubated at 37°, 5% CO_2 for 48 h. Then each group of cells were washed with PBS, trypsinized and then stained with propidium iodide (PI, Sigma, USA). The red fluorescence of DNA-bound PI in each group was measured at 488 nm by FACScan flow cytometry (EPTCS XLL2MCL Beckman Coulter, UK).

Western blot analysis. For immunoblot analysis of COX-2, HepG2 cells were lysed in a buffer consisting of 50 mM Tris-HCl, pH 7.4, 1% Tween 20, 2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 μg/mL aprotinin, and 10 μg/mL leupeptin. Debris was removed by centrifugation (Beckman GPKR machine) at 2,200 g for 20 min at 4 °C. A total of 60 μg of precipitated and denatured protein was separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred electrophoretically to polyvinylidene fuoride membranes (Immun-Blot PVDF membrane, 0.2 μm; Bio-Rad). Membranes were blocked with 5% nonfat milk powder in 0.5 % Tween 20-PBS for 2 h. Primary antibodies to COX-2 were used at a final dilution of 1 : 1000 for overnight, at 4 °C. The blot was washed several times with 0.05% Tween 20-PBS and incubated with the appropriate goat anti-rabbit antibody for 2 h. Immunodetection was carried out using enhanced chemiluminescence reagent following the manufacturer's instructions (Pierce Biotechnology Inc, USA).

Measurement of prostaglandin E2 and VEGF production. HepG2 carcinoma cells were plated at a density of 5×10^5 cells / p60 Petri dish in complete DMEM medium for 12 h. HepG2 cells were treated with 25–100 μM nimesulide for 24 h. After drug treatment, culture supernatant was collected and centrifuged briefly. The amount of PGE_2 in the medium was measured using a commercial RIA kit (Wuhan Boster Co, Wuhan, China) and VEGF level determined with an enzyme-linked immunosorbent assay (ELISA) kit (Promega Corporation) following the manufacturer's instructions.

Statistical analysis. Data from the population of cells treated with different conditions were analyzed using paired Student's t-test or ANOVA test (a comparison of multiple groups), and p value of < 0.05 was considered statistically significant in the experiments.

RESULTS

Nimesulide inhibits the COX-2 activity in HepG2 hepatocellular carcinoma cells. First, we performed the Western blot analysis to examine whether COX-2 protein is expressed in HepG2cells. As shown in Fig. 1, *a*, COX-2 protein was highly expressed in HepG2 cells. This is consistent to a recent immunohitochemistry study that shows high COX-2 protein expression in HepG2 and Huh7 cells [28]. As treatment with 25–100 μM nimesulide for 24 h did not significantly alter expression levels of COX-2 protein in HepG2 cells (Fig. 1, *a*), next

we tested whether treatment with nimesulide inhibits the COX-2 enzyme activity by measuring PGE_2 levels of culture supernatant. The average amount of PGE_2 in the medium alone controls from three independent experiments was 0.65 ng/ml. Compared with the control, the PGE_2 production was reduced by about 80%, 65% and 40% 24 h after the treatment with 25 μM, 50 μM or 100 μM of nimesulide, respectively (Fig. 1, *b*), indicating the dose-dependent inhibition.

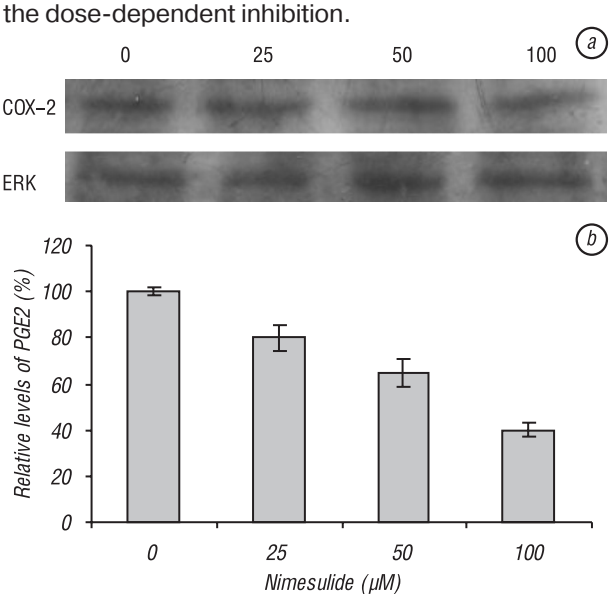

Fig. 1. Effects of nimesulide on the COX-2 protein expression and PGE_2 production in human HepG2 cells. (*a*) HepG2 cells were treated with 25–100 μM nimesulide for 24 h as indicated. The total protein extracts were subjected to Western blot analysis using anti-COX-2 antibody. Anti-ERK-2 antibody was used as a loading control. (*b*) The HepG2 cells were treated with 25–100 μM nimesulide. After incubation for 24 h, the PGE_2 level in the culture medium was measured by a PGE_2 EIA kit following the manufacturer's protocol. The means ± S.E of three independent experiments in each treatment are shown

Nimesulide inhibits cell growth and enhances doxorubicin-mediated apoptosis of cultured HepG2 cells. To determine whether COX-2 inhibition by nimesulide contributes to growth inhibition of HepG2 cells we measured the number of apoptotic cells after treatment with various doses of nimesulide by MTT assay. As shown in the left panel of Fig. 2, *a*, the number of viable cells started to decline by treatment with a low concentration of nimesulide (25 μM) and continued to drop by up to 40% at 400 μM of nimesulide. As expected, cell apoptosis induced by doxorubicin is more evident than that by nimesulide as treatment with 80 μM of doxorubicin induced death of almost all cells in the culture (Fig. 2, *a*, right panel). The apoptosis triggered by nimesulide or doxorubicin was shown in a concentration-dependent manner.

Because nimesulide-induced cell death is through COX-2 inhibition and doxorubicin acts as cell cycle inhibitor on triggering cell apoptosis, we hypothesize that the inhibition of COX-2 by nimesulide may enhance the doxorubicin-mediated cytotoxity. To test this possibility, we performed the MTT assay by co-treatment of HepG2 cells with doxorubicin and nimesulide. As shown in Fig. 2, *b*, compared to cell treated with 5 μM of doxorubicin alone, addition of 25, 50 or 100 μM nimesulide yielded a 2 to 3.5 fold increase in cell death, respectively. This synergistic effect of nimesulide persisted even at higher concentrations of doxorubicin, 10 μM or 20 μM. Since treatment with more than 40 μM of doxorubicin alone already induced apoptosis of most cells, an increase in doxorubicin-mediated cytotoxity of HepG2 cells by addition of nimesulide was limited (Fig. 2, *b*, right two panels).

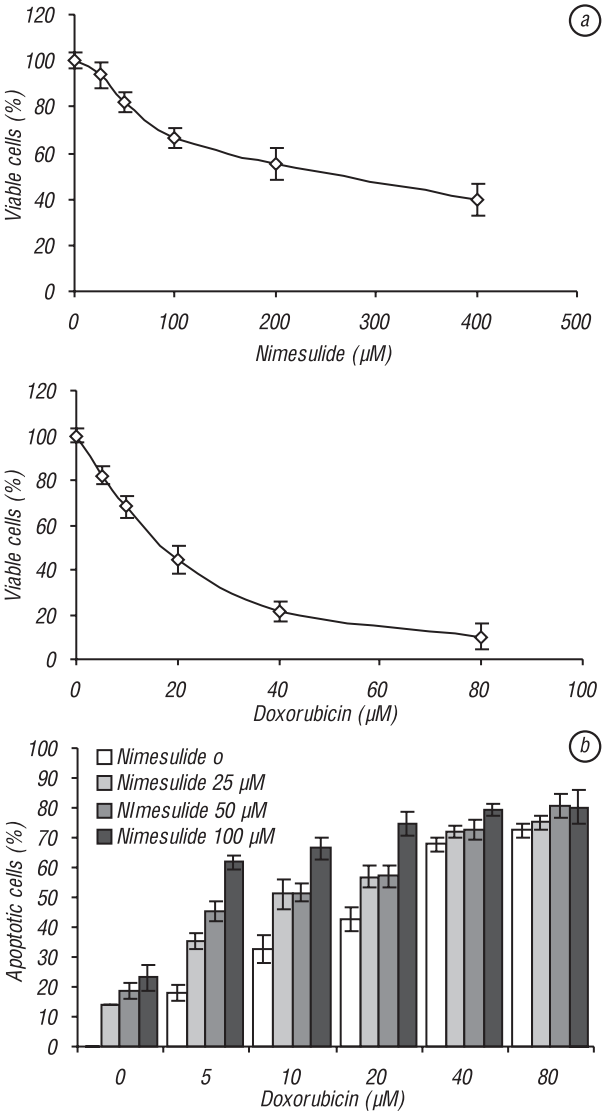

Fig. 2. Co-treatment of nimesulide and doxorubicin additively inhibited the growth of HepG2 cells. (*a*) The cells were treated with indicated 25–400 μM nimesulide or indicated 5–80 μM of doxorubicin for 24 h. The cell survival was measured by MTT assay. Results were obtained from 3 independent experiments and the bar represents S.E. Significant changes between untreated and treated samples are marked by asterisks: $*p < 0.05$ and $**p < 0.01$. (*b*) The cells were co-treated with 5–80 μM doxorubicin alone or together with 25–100 μM nimesulide for 24 h. Then the cell survival was measured by MTT assay. Results were obtained from 4 independent experiments and the bar represents the S.E. Significant changes between doxorubicin alone and co-treatment with nimesulide samples are marked by asterisks: $*p < 0.05$

To further confirm the cytotoxic synergy between nimesulide and doxorubicin we performed morphology study by staining cells with acridine orange. We observed the normal morphology of drug-untreated HepG2 cells: shape of tri- or multi-angles with abundant

cytoplasma, and large oval nuclei with dispersed chromatin (Fig. 3, *a*, left panel). 24 h after treatment with 100 μM of nimesulide and 10 μM of doxorubicin, HepG2 cells exhibited the typical morphology of apoptotic cells, including cell shrinkage, deep-dyed pyknotic nuclei, margination of nuclear chromatin, cytoplasmic blebbing and clusters of apoptotic bodies (Fig. 3, *a*, right panel). Also, we measured the DNA content by propidium iodide (PI) staining, and cell apoptosis was reflected by the appearance of a cell population with subdiploid and pre-G1phase through flow cytometric analysis. Consistent with MTT assay, addition of 100 μM of nimesulide significantly increased a portion of subdiploid and pre-G1 phase (21.7%) compared to that of doxorubicin alone group (10.6%) (Fig. 3, *b*, bottom two panels). By contrast, a much smaller portion of pre-G1 phase was shown in the cells treated with nimesulide alone (Fig. 3, *b*, right top panel).

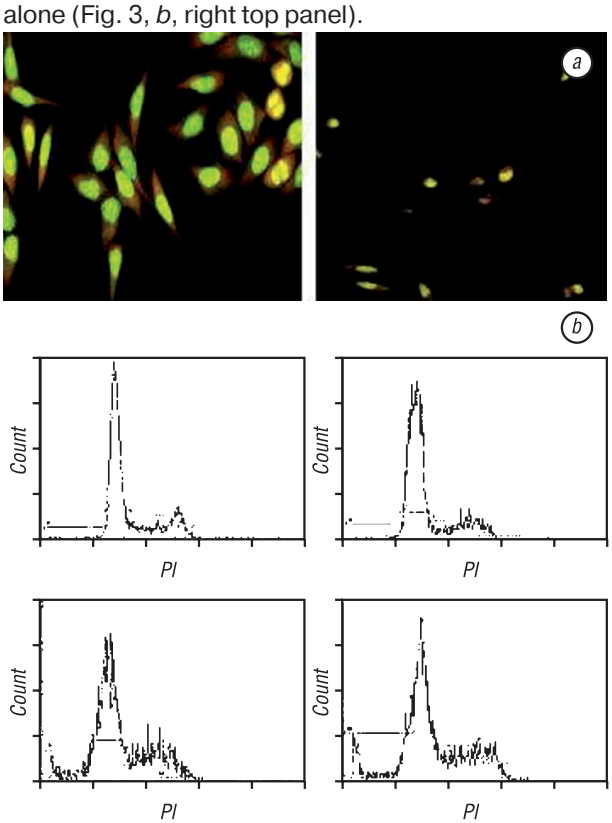

Fig. 3. Co-treatment of nimesulide and doxorubicin enhanced apoptosis induction of HepG2 cells. (*a*) The HepG2 cells were treated with or without nimesulide 100 μM and doxorubicin 10 μM for 24 h and stained with acridine orange. The cell morphology was observed under fluorescent microscope. (*b*) Flow cytometry analysis of DNA content of HepG2 cells after treated with 100 μM of nimesulide and/or 20 μM of doxorubicin for 24 h. Representative DNA histograms were from 3 independent experiments. The percentage of pre-G1 phase at various culture conditions is indicated

Co-treatment with doxorubicin and nimesulide reduces production of VEGF in HepG2 cells. VEGF, one of the most potent angiogenic factors, has been shown to play a pivotal role in tumor angiogenesis, including HCC. A line of evidence reveals that the elevated COX-2 expression correlates with increased VEGF level and microvascular density in human HCCs [16, 24, 25]. However, it is not known whether the inhibition of COX-2 reduces VEGF production in hepatocellular carcinoma cells. To test this possibility, we treated HepG2 cells with 25 μM or 50 μM of nimesulide and measured VEGF levels in the culture supernatants by an ELISA. As shown in Fig. 4, treatment with 50 μM of nimesulide alone led to an about 2 fold reduction in VEGF levels and this effect was largely augmented by addition of 5 μM or 10 μM of doxorubicin. VEGF production was also significantly reduced if the cells were co-treated with doxorubicin and a lower concentration (25 μM) of nimesulide, suggesting the synergistic inhibition of VEGF production in HepG2 cells by two agents.

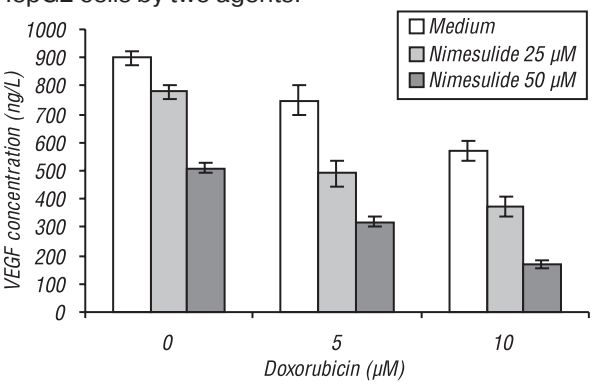

Fig. 4. Co-treatment with nimesulide and doxorubicin reduced VEGF production of cultured HepG2 cells. The cells were co-treated with 5–10 μM doxorubicin and 25–50 μM nimesulide for 48 h. The VEGF levels were determined in culture supernatants by ELISA following the manufacturer's protocol. VEGF secretion was significantly reduced in the co-treatment group compared with the single nimesulide or doxorubicin treated group (*$p < 0.05$)

DISCUSSION

Doxorubicin is one of the most often used drugs for treatment of HCC and single use of doxorubicin yields response rate of about 20% for unresectable tumors. However, there is no convincing evidence that the use of doxorubicin improves the prognosis of patients with HCC, therefore the effect of this drug on HCC is still limited [29–32]. NSAIDs, such as aspirin, indomethacin and sulindac, may play a role in the inhibition of proliferation and induction of apoptosis in tumor cells through the inhibition of COX-2 activity [33–35]. The anti-inflammatory and anti-angiogenic effects of NSAIDs have been explored for the cancer therapy and some of these agents are currently under clinical trials [36, 37]. Nimesulide, a specific COX-2 inhibitor, can bind specifically to the large catalytic moiety of COX-2, with much less adverse effects on the gastrointestinal tract compared to the non-specific NSAIDs. A selective COX-2 inhibitor JTE-522 has been reported to enhance cytotoxity in bladder cancer with 5-fluorouracil [38]. A combination of common antitumor drugs and COX-2 inhibitors may enhance chemotherapeutic efficacy, reduce drug dose and adverse side effects. It has been shown that nimesulide inhibits the growth of human hepatoma cell line SMMC-7721 *in vitro* [17]. However, studies on the combination of selective COX-2 inhibitors including nimesulide and common chemotherapy agents against human hepatocellular carcinoma are not documented. In this study, we investigated the effects of a combination of nimesulide and doxorubicin on the growth inhibition in human hepatocellular

carcinoma HepG2 cells. Our results showed that treatment of nimesulide significantly inhibited cell growth in HepG2 cells. Moreover, the combination of doxorubicin and nimesulide additively increased the cytotoxicity and growth inhibition in HepG2 cells. Therefore, our results suggest that the use of COX-2 inhibitors may be beneficial when combined with doxorubicin for the treatment of patients with HCC.

Prostaglandins, including PGE_2, synthesized by COX enzymes, are reported to increase cell growth and induce proliferation in the cultured rat hepatocytes [39, 40]. In human hepatocellular carcinoma cells, treatment with prostaglandin E_2 (PGE_2) increases VEGF production and this effect is blocked by inhibition of COX-2, suggesting a link of COX-2 with VEGF signaling in hepatocarcinogenesis [16]. In support of these findings, we have observed the high expression of COX-2 protein in HepG2 cells and inhibition of COX-2 by nimesulide led to reduced production of PGE_2 and VEGF, and decreased viable cells in the culture. Therefore, the interplay between COX-2-derived prostaglandin signaling and other growth-regulatory pathways such as VEGF is expected to provide important therapeutic implications.

Apoptosis is an important physiological process that prevents the formation of tumor clone and the failures of apoptosis lead to the development of many tumors including hepatocellular carcinoma [41]. The recent reports have demonstrated that NSAIDs induce apoptosis in different tumor cells [12, 42]. It is also conceivable that apoptosis might occur in the human hepatocellular carcinoma cells in response to nimesulide. To evaluate the role of nimesulide in cell apoptosis, we cultured HepG2 cells and treated cells with various concentrations of nimesulide or together with a low concentration of doxorubicin. Flow cytometry analysis of sub-diploid peak of DNA content revealed the ability of nimesulide to trigger apoptosis in HepG2 cells at these concentrations that were sufficient to inhibit cell proliferation. In addition, the nimesulide-induced apoptosis is significantly increased by addition of a low concentration of doxorubicin. Thus, apoptosis may be one of the mechanisms for nimesulide to inhibit cell growth in HepG2 cells, especially when nimesulide is combined with doxorubicin.

In patients with hepatocellular carcinoma, hypervascularity correlated with the over-expression of VEGF and significantly associated with the tumor extension and shorter median survival time [25, 43, 44]. Prostaglandin E_2 increases the expression and the secretion of VEGF in hepatocellular carcinoma cells [16, 45]. We found that the inhibition of COX-2 by nimesulide reduced VEGF production in HepG2 cells. Interestedly, treatment of doxorubicin also inhibited VEGF secretion by HepG2 cells, and co-treatment of doxorubicin and nimesulide markedly decreased the levels of VEGF in cultured supernatants. Together, our data suggest that co-treatment with doxorubicin and nimesulide induces growth inhibition and cell death in part through the inhibition of VEGF production in HepG2 cells. However, the precise mechanism of the inhibition of VEGF production in this situation needs further investigation.

This study for the first time presents evidence that a combination of COX-2 inhibitor nimesulide and doxorubicin additively inhibits growth of the human hepatocellular carcinoma cells. This effect was mainly observed owing to increased apoptosis and reduced VEGF production. The study provides a new strategy that the combination of COX-2 inhibitors and doxorubicin may be effective in the treatment of human hepatocellular carcinoma.

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REFERENCES

1. **Bosch FX, Ribes J, Borras J.** Epidemiology of primary liver cancer. Semin Liver Dis 1999; **19:** 271–85.

2. **Lai EC, Fan ST, Lo CM, Chu KM, Liu CL, Wong J.** Hepatic resection for hepatocellular carcinoma. An audit of 343 patients. Ann Surg 1995; **221:** 291–8.

3. **Pawarode A, Voravud N, Sriuranpong V, Kullavanijaya P, Patt YZ.** Natural history of untreated primary hepatocellular carcinoma: a retrospective study of 157 patients. Am J Clin Oncol 1998; **21:** 386–91.

4. **Shariat SF, Kim JH, Ayala GE, Kho K, Wheeler TM, Lerner SP.** Cyclooxygenase-2 is highly expressed in carcinoma in situ and T1 transitional cell carcinoma of the bladder. J Urol 2003; **169:** 938–42.

5. **Chen WS, Liu JH, Wei SJ, Liu JM, Hong CY, Yang WK.** Colon cancer cells with high invasive potential are susceptible to induction of apoptosis by a selective COX-2 inhibitor. Cancer Sci 2003; **94:** 253–8.

6. **Chen WS, Wei SJ, Liu JM, Hsiao M, Kou-Lin J, Yang WK.** Tumor invasiveness and liver metastasis of colon cancer cells correlated with cyclooxygenase-2 (COX-2) expression and inhibited by a COX-2-selective inhibitor, etodolac. Int J Cancer 2001; **91:** 894–9.

7. **Han C, Leng J, Demetris AJ, Wu T.** Cyclooxygenase-2 promotes human cholangiocarcinoma growth: evidence for cyclooxygenase-2-independent mechanism in celecoxib-mediated induction of p21waf1/cip1 and p27kip1 and cell cycle arrest. Cancer Res 2004; **64:** 1369–76.

8. **Krysan K, Merchant FH, Zhu L, Dohadwala M, Luo J, Lin Y, Heuze-Vourc'h N, Pold M, Seligson D, Chia D, Goodglick L, Wang H, Strieter R, Sharma S, Dubinett S.** COX-2-dependent stabilization of survivin in non-small cell lung cancer. Faseb J 2004; **18:** 206–8.

9. **Sinicrope FA, Gill S.** Role of cyclooxygenase-2 in colorectal cancer. Cancer Metastasis Rev 2004; **23:** 63–75.

10. **Kismet K, Akay MT, Abbasoglu O, Ercan A.** Celecoxib: a potent cyclooxygenase-2 inhibitor in cancer prevention. Cancer Detect Prev 2004; **28:** 127–42.

11. **Hawkey CJ.** COX-2 inhibitors. Lancet 1999; **353:** 307–14.

12. **Leng J, Han C, Demetris AJ, Michalopoulos GK, Wu T.** Cyclooxygenase-2 promotes hepatocellular carcinoma cell growth through Akt activation: evidence for Akt inhibition in celecoxib-induced apoptosis. Hepatology 2003; **38:** 756–68.

13. **Cervello M, Foderaa D, Florena AM, Soresi M, Tripodo C, D'Alessandro N, Montalto G.** Correlation between expression of cyclooxygenase-2 and the presence of inflammatory cells in human primary hepatocellular carcinoma:

possible role in tumor promotion and angiogenesis. World J Gastroenterol 2005; **11:** 4638–43.

14. **Kern MA, Schubert D, Sahi D, Schoneweiss MM, Moll I, Haugg AM, Dienes HP, Breuhahn K, Schirmacher P.** Proapoptotic and antiproliferative potential of selective cyclooxygenase-2 inhibitors in human liver tumor cells. Hepatology 2002; **36:** 885–94.

15. **Fodera D, D'Alessandro N, Cusimano A, Poma P, Notarbartolo M, Lampiasi N, Montalto G, Cervello M.** Induction of apoptosis and inhibition of cell growth in human hepatocellular carcinoma cells by COX-2 inhibitors. Ann N Y Acad Sci 2004; **1028:** 440–9.

16. **Cheng AS, Chan HL, To KF, Leung WK, Chan KK, Liew CT, Sung JJ.** Cyclooxygenase-2 pathway correlates with vascular endothelial growth factor expression and tumor angiogenesis in hepatitis B virus-associated hepatocellular carcinoma. Int J Oncol 2004; **24:** 853–60.

17. **Tian G, Yu JP, Luo HS, Yu BP, Yue H, Li JY, Mei Q.** Effect of nimesulide on proliferation and apoptosis of human hepatoma SMMC-7721 cells. World J Gastroenterol 2002; 8: 483–7.

18. **Park MK, Hwang SY, Kim JO, Kwack MH, Kim JC, Kim MK, Sung YK.** NS398 inhibits the growth of Hep3B human hepatocellular carcinoma cells via caspase-independent apoptosis. Mol Cells 2004; **17:** 45–50.

19. **Huang DS, Shen KZ, Wei JF, Liang TB, Zheng SS, Xie HY.** Specific COX-2 inhibitor NS398 induces apoptosis in human liver cancer cell line HepG2 through BCL-2. World J Gastroenterol 2005; **11:** 204–7.

20. **Hu KQ, Yu CH, Mineyama Y, McCracken JD, Hillebrand DJ, Hasan M.** Inhibited proliferation of cyclooxygenase-2 expressing human hepatoma cells by NS-398, a selective COX-2 inhibitor. Int J Oncol 2003; **22:** 757–63.

21. **Ding YB, Shi RH, Tong JD, Li XY, Zhang GX, Xiao WM, Yang JG, Bao Y, Wu J, Yan ZG, Wang XH.** PGE2 up-regulates vascular endothelial growth factor expression in MKN28 gastric cancer cells via epidermal growth factor receptor signaling system. Exp Oncol 2005; **27:** 108–13.

22. **Tsujii M, Kawano S, Tsuji S, Sawaoka H, Hori M, DuBois RN.** Cyclooxygenase regulates angiogenesis induced by colon cancer cells. Cell 1998; **93:** 705–16.

23. **Cianchi F, Cortesini C, Bechi P, Fantappie O, Messerini L, Vannacci A, Sardi I, Baroni G, Boddi V, Mazzanti R, Masini E.** Up-regulation of cyclooxygenase 2 gene expression correlates with tumor angiogenesis in human colorectal cancer. Gastroenterology 2001; **121:** 1339–47.

24. **Tang TC, Poon RT, Lau CP, Xie D, Fan ST.** Tumor cyclooxygenase-2 levels correlate with tumor invasiveness in human hepatocellular carcinoma. World J Gastroenterol 2005; **11**: 1896–902.

25. **Imura S, Miyake H, Izumi K, Tashiro S, Uehara H.** Correlation of vascular endothelial cell proliferation with microvessel density and expression of vascular endothelial growth factor and basic fibroblast growth factor in hepatocellular carcinoma. J Med Invest 2004; **51**: 202–9.

26. **Hochster HS, Green MD, Speyer J, Fazzini E, Blum R, Muggia FM.** 4′Epidoxorubicin (epirubicin): activity in hepatocellular carcinoma. J Clin Oncol 1985; **3**: 1535–40.

27. **Nerenstone SR, Ihde DC, Friedman MA.** Clinical trials in primary hepatocellular carcinoma: current status and future directions. Cancer Treat Rev 1988; **15:** 1–31.

28. **Kern MA, Haugg AM, Koch AF, Schilling T, Breuhahn K, Walczak H, Fleischer B, Trautwein C, Michalski C, Schulze-Bergkamen H, Friess H, Stremmel W, Krammer PH, Schirmacher P, Muller M.** Cyclooxygenase-2 inhibition induces apoptosis signaling via death receptors and mitochondria in hepatocellular carcinoma. Cancer Res 2006; **66**: 7059–66.

29. **Burroughs A, Hochhauser D, Meyer T.** Systemic treatment and liver transplantation for hepatocellular carcinoma: two ends of the therapeutic spectrum. Lancet Oncol 2004; **5:** 409–18.

30. **Llovet JM.** Updated treatment approach to hepatocellular carcinoma. J Gastroenterol 2005; **40:** 225–35.

31. **Carr BI.** Hepatocellular carcinoma: current management and future trends. Gastroenterology 2004; **127:** S218–24.

32. **Yeo W, Mok TS, Zee B, Leung TW, Lai PB, Lau WY, Koh J, Mo FK, Yu SC, Chan AT, Hui P, Ma B, Lam KC, Ho WM, Wong HT, Tang A, Johnson PJ.** A randomized phase III study of doxorubicin versus cisplatin/interferon alpha-2b/doxorubicin/fluorouracil (PIAF) combination chemotherapy for unresectable hepatocellular carcinoma. J Natl Cancer Inst 2005; **97**: 1532–8.

33. **Lin J, Hsiao PW, Chiu TH, Chao JI.** Combination of cyclooxygenase-2 inhibitors and oxaliplatin increases the growth inhibition and death in human colon cancer cells. Biochem Pharmacol 2005; **70**: 65867.

34. **Buttar NS, Wang KK, Leontovich O, Westcott JY, Pacifico RJ, Anderson MA, Krishnadath KK, Lutzke LS, Burgart LJ.** Chemoprevention of esophageal adenocarcinoma by COX-2 inhibitors in an animal model of Barrett's esophagus. Gastroenterology 2002; **122:** 1101–12.

35. **Wight NJ, Gottesdiener K, Garlick NM, Atherton CT, Novak S, Gertz BJ, Calder NA, Cote J, Wong P, Dallob A, Hawkey CJ.** Rofecoxib, a COX-2 inhibitor, does not inhibit human gastric mucosal prostaglandin production. Gastroenterology 2001; **120:** 867–73.

36. **Spano JP, Chouahnia K, Morere JF.** Cyclooxygenase 2 inhibitors and lung carcinoma. Bull Cancer 2004; **91:** S109–12 (in French).

37. **Guastalla JP, Bachelot T, Ray-Coquard I.** Cyclooxygenase 2 and breast cancer. From biological concepts to clinical trials. Bull Cancer 2004; **91:** S99–108 (in French).

38. **Mizutani Y, Kamoi K, Ukimura O, Kawauchi A, Miki T.** Synergistic cytotoxicity and apoptosis of JTE-522, a selective cyclooxygenase-2 inhibitor, and 5-fluorouracil against bladder cancer. J Urol 2002; **168:** 2650–4.

39. **Kimura M, Osumi S, Ogihara M.** Stimulation of DNA synthesis and proliferation by prostaglandins in primary cultures of adult rat hepatocytes. Eur J Pharmacol 2000; **404:** 259–71.

40. **Hashimoto N, Watanabe T, Ikeda Y, Yamada H, Taniguchi S, Mitsui H, Kurokawa K.** Prostaglandins induce proliferation of rat hepatocytes through a prostaglandin E2 receptor EP3 subtype. Am J Physiol 1997; **272:** G597–604.

41. **Philchenkov A, Zavelevich M, Kroczak TJ, Los M.** Caspases and cancer: mechanisms of inactivation and new treatment modalities. Exp Oncol 2004; **26:** 82–97.

42. **Cui W, Yu CH, Hu KQ.** In vitro and in vivo effects and mechanisms of celecoxib-induced growth inhibition of human hepatocellular carcinoma cells. Clin Cancer Res 2005; **11:** 8213–21.

43. **Qin LX, Tang ZY.** Recent progress in predictive biomarkers for metastatic recurrence of human hepatocellular carcinoma: a review of the literature. J Cancer Res Clin Oncol 2004;**130:** 497–513.

44. **Uematsu S, Higashi T, Nouso K, Kariyama K, Nakamura S, Suzuki M, Nakatsukasa H, Kobayashi Y, Hanafusa T, Tsuji T, Shiratori Y.** Altered expression of vascular endothelial growth factor, fibroblast growth factor-2 and endostatin in patients with hepatocellular carcinoma. J Gastroenterol Hepatol 2005; **20:** 583–8.

45. **Li YQ, Tao KS, Ren N, Wang YH.** Effect of c-fos antisense probe on prostaglandin E2-induced upregulation of vascular endothelial growth factor mRNA in human liver cancer cells. World J Gastroenterol 2005;**11:** 4427–30.

КОМБИНИРОВАННОЕ ПРИМЕНЕНИЕ ИНГИБИТОРА ЦИКЛООКСИГЕНАЗЫ-2 И ДОКСОРУБИЦИНА ПРИВОДИТ К УГНЕТЕНИЮ РОСТА И АПОПТОЗУ КЛЕТОК ГЕПАТОКАРЦИНОМЫ ЧЕЛОВЕКА

Угнетение циклооксигеназы-2 (ЦОГ-2) оказывает терапевтический эффект при лечении больных с солидными опухолями и сопровождается снижением пролиферации опухолевых клеток и индукцией апоптоза. *Цель:* изучение роли ингибитора ЦОГ-2 — нимесулида в процессах роста и апоптоза культивированных клеток гепатокарциномы человека HepG2. *Методы:* для оценки апоптоза и угнетения роста клеток при применении нимесулида самостоятельно и в сочетании с доксорубицином применяли МТТ-анализ, проточную цитометрию и стандартные морфологические методы. *Результаты:* установлено, что обработка клеток HepG2 cells нимесулидом в концентрации > 50 μM приводила к угнетению активности ЦОГ-2 за счет снижения продукции PGE_2, после чего отмечали подавление роста и апоптоз клеток при неизмененном уровне экспрессии ЦОГ-2. Комбинированное применение 50 μM или 100 μM нимесулида и доксорубицина в концентрации 5–20 μM обусловило усиленное угнетение роста клеток, индукции апоптоза и снижение продукции VEGF. *Выводы:* полученные данные свидетельствуют о синергическом и/или аддитивном эффекте при применении ингибиторов ЦОГ-2 и химиотерапевтических препаратов.

Ключевые слова: **гепатокарцинома, нимесулид, ЦОГ-2, доксорубицин, апоптоз, VEGF.**