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ARTICLE

Anti-Tumor Effect of Non-Steroidal Anti-Inflammatory Drugs on Human Ovarian Cancers

Bing XIN, Yoshihito YOKOYAMA, Tatsuhiko SHIGETO, Hideki MIZUNUMA

Department of Obstetrics and Gynecology, Hirosaki University School of Medicine, Hirosaki, Aomori, 036-8562, Japan

Many reports have demonstrated that non-steroidal anti-inflammatory drugs (NSAIDs) suppress malignant transformation and tumor growth, and some NSAIDs are expected to be new anti-cancer agents. In this study, we examined the anti-tumor effects of the non-specific cyclooxygenase (COX) inhibitors aspirin and piroxicam, and the selective COX-2 inhibitor meloxicam on xenotransplanted ovarian cancer. Tumor growth and survival were compared in female nu/nu mice, xenografted with subcutaneous OVCAR-3 tumors or with intraperitoneal DISS tumors and treated with aspirin (200 ppm in diet, everyday), piroxicam (150 ppm in diet, everyday) or meloxicam (162 ppm in diet, everyday). Al, of the agents tested significantly suppressed the growth of OVCAR-3 tumors xenotransplanted subcutaneously as compared to the control. There was a significant difference in inhibition of OVCAR-3 tumor growth between meloxicam and aspirin treatment. Meloxicam and piroxicam treatment significantly prolonged survival of mice with malignant ascites derived from DISS cells as compared to control and aspirin treatment. Mice treated with meloxicam survived significantly longer than those treated with piroxicam. There was no significant difference in survival between control and aspirin treatment. Necropsy revealed that one of the 6 cancer-bearing mice treated with piroxicam suffered from stomach perforation. These results indicate that a selective COX-2 inhibitor produces greater anti-tumor effect against ovarian cancer than a nonselective COX inhibitor and that meloxicam may have a potential of leading to a novel therapeutic strategy against ovarian cancer. (Pathology Oncology Research Vol 13, No 4, 365–369)

Key words: non-steroidal anti-inflammatory drugs, selective COX-2 inhibitor, non-specific COX inhibitor, meloxicam, ovarian cancer

Introduction

Ovarian cancer has the highest mortality rate among gynecological malignancies. Patients with this disease at stage b have a 5-year survival rate of only 28%, and unfortunately 60% of patients are diagnosed with already advanced disease.[13] Survival rates for patients with this disease have shown modest improvement in the past decade, but remain unsatisfactory.[39] With the discovery that aspirin reduces the risk of human colon cancer,[3] non-steroidal anti-inflammatory drugs (NSAIDs) have been intensively studied as modulators of tumor growth.

NSAIDs that inhibit cyclooxygenase (COX) and suppress prostaglandin (PG) synthesis have been widely used as anti-inflammatory, anti-pyretic and analgesic agents. There are at least two isoforms of COX. COX-1 is expressed constitutively in many tissues and PGs produced by COX-1 mediate the housekeeping function such as cytoprotection of gastric mucosa, regulation of renal blood flow and platelet aggregation, whereas COX-2 cannot be detected in the majority of normal tissues but can be induced by a variety of cytokines and mitogens.[33] Epidemiological studies revealed a 40-50% reduction in mortality from colorectal cancer in individuals taking NSAIDs, and there is also evidence that they decrease the incidence and progression of other types of cancer, suggesting a possible role of COX in tumor formation.[32] It has been demonstrated that NSAIDs may lead to anti-tumor effect via inhibition of COX-2.[34] Case-control studies

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Correspondence: Yoshihito YOKOYAMA, Department of Obstetrics and Gynecology, Hirosaki University School of Medicine, 5-Zaifu-cho, Hirosaki, 036-8562, Japan. Fax: +81-172-37-6842, E-mail: yokoyama@cc.hirosaki-u.ac.jp

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demonstrated evidence for time- and dose-dependent decreases in the risk of developing ovarian cancers with the consumption of several NSAIDs.[2,5,21] Shigemasa et al[31] reported that COX-2 expression might play an important role in ovarian cancer development. Ferrandina et al[12] described that increased COX-2 expression was associated with chemotherapy resistance and outcome in ovarian cancer patients and Denkert et al[7] reported that COX-2 expression was an independent prognostic factor in ovarian cancer. Pharmacological studies suggest that COX-2 could be a useful therapeutic target.[29,38]

Aspirin, piroxicam and meloxicam belong to NSAIDs and are all used worldwide. Aspirin and piroxicam are non-specific COX inhibitors while meloxicam is categorized as a selective COX-2 inhibitor. Antiproliferative and antitumor effects of NSAIDs on experimental tumors have been widely studied. Many reports revealed that aspirin has chemopreventive and inhibitory effects on drug-induced colon and lung carcinogenesis.[6,11] Aspirin also inhibited cell proliferation in *in vitro*.[1,26] Piroxicam inhibited rat drug-induced small intestinal and colon tumor growth[18,23] and its inhibitory effect was involved in induction of apoptosis and repression of angiogenesis.[20] Some reports demonstrated that meloxicam inhibits the growth of non-small cell lung cancer and colorectal cancer.[14,35] Antitumor effect of other various NSAIDs was also tested in experimental animal models of stomach and breast cancers.[27,30] In spite of a significant interest in the role of NSAIDs in gastrointestinal, lung and breast malignancies, only a limited number of studies were reported on NSAIDs in human ovarian cancers.[10] In this study, we compared three types of NSAIDs showing different capacities to inhibit COX, in regard to their inhibitory effect on growth of human ovarian cancers.

Materials and methods

Cell lines and cell culture

OVCAR-3 was obtained from the American Type Culture Collection (Rockville, MD) and DISS was kindly provided from Dr. Saga (Jichi Medical School, Tochigi, Japan). Both cell lines derived from human ovarian cancers, and were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 mg/ml streptomycin at 37°C in a water-saturated atmosphere with 5% CO_2/95% air.

Animal experiments

The animal experiments were conducted in accordance with the Guidelines for Animal Experimentation, Hirosaki University. Eight-week-old female BALB/c nu/nu mice were used in this study. All mice were group-housed in plastic cages with stainless-steel grid tops in an air-conditioned room maintaining 12-h light-dark cycles in the Institute for Animal Experiments of Hirosaki University and fed with water and food *ad libitum*.

Cancer-bearing mouse model

OVCAR-3 cells (5×10^6) were inoculated subcutaneously in 500 μl of RPMI-1640 medium in the back region of nude mice. All the mice were numbered, housed separately and examined twice weekly for tumor development. The tumor was grown until the longer diameter became 2 mm before starting treatment. Then, the experimental mice were divided into four groups containing 6 mice each (day 0). Control group received basal diet alone. Aspirin group was given 200 ppm aspirin[25] (Sigma-Aldrich, St Louis, MO), piroxicam group was given 150 ppm piroxicam[22] (Pfizer, Tokyo, Japan) and meloxicam group was given 162 ppm meloxicam[16] (Boehringer Ingelheim, Germany) in the diet every day until the end of the study. Tumor dimensions were measured twice weekly using a caliper and tumor volume was calculated using the equation $V\ (mm^3) = A \times B^2/2$, where A is the largest diameter and B is the smallest diameter.[36] Mice were sacrificed on day 21 to remove the tumor for measuring its weight.

Peritoneal carcinomatosa mouse model

DISS cells (0.5×10^6) were inoculated into the peritoneal cavity of nude mice in 500 μl of sterile PBS. It has been reported that the average survival of DISS cell-transplanted mice is about 30 days. The experimental mice were divided into four groups of 6 mice each. After confirming ascites to be produced on day 4, the mice were treated in the same way as in the cancer-bearing mouse model. Survival time for each group was evaluated.

Statistical analysis

Differences in tumor volume between the groups were analyzed using non-parametric Mann-Whitney test. Survival curves were calculated by the Kaplan-Meier method, and the statistical significance of differences in the survival curves between the groups was evaluated by log-rank test. Other statistical analyses were carried out by Student's t-test. A result was deemed significant at $P < 0.05$.

Results

Anti-tumor effect of aspirin, piroxicam and meloxicam in the cancer-bearing mouse model

Tumor volume curves at days 9-21 in the aspirin and meloxicam groups were significantly lower compared with the control group (*Fig. 1a*, $P < 0.05$). Tumor volume curve at day 14-21 in the piroxicam group was significantly

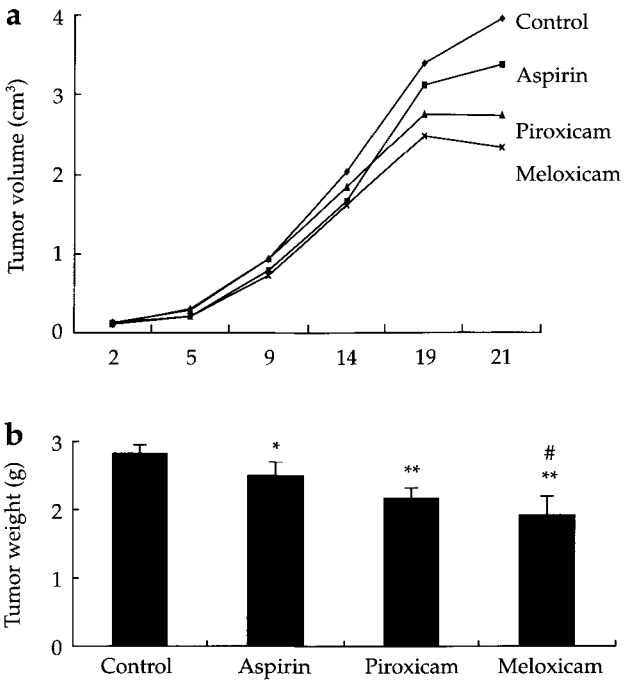

*Figure 1. Anti-tumor effect of aspirin, piroxicam and meloxicam on OVCAR-3 tumor. (a) Comparison of tumor growth in cancer-bearing mice. Differences in tumor volume curves between the groups were analyzed using non-parametric Mann-Whitney test. (b) Comparison of final tumor weight in cancer-bearing mice. Final tumor weight was significantly smaller in every treatment group than in controls. There was also significant difference in final tumor weight between meloxicam and aspirin. Data presented are mean ± SD. *P < 0.01, **P < 0.001 versus controls; #P < 0.01 versus aspirin.*

lower than that in the control group (*Fig. 1a*, $P < 0.05$). There was significant difference in tumor growth from day 14 to 21 between meloxicam and aspirin treatment (*Fig 1a*, $P < 0.05$). The final tumor weights were 2.8 ± 0.13 g for the control, 2.5 ± 0.2 g for the aspirin group, 2.2 ± 0.15 g for the piroxicam group and 1.9 ± 0.28 g for the meloxicam group (expressed as mean ± SD). Tumor inhibition rate was 11.5% for aspirin, 23% for piroxicam and 32% for meloxicam, tumors being significantly smaller in every treatment group than in the controls (*Fig. 1b*, $P < 0.01$, $P < 0.001$ and $P < 0.001$, respectively).

Anti-tumor effect of NSAIDs in the peritoneal carcinomatosa mouse model

In the peritoneal carcinomatosa model, mean survival times were 25.5 ± 3.0, 25.8 ± 2.9, 30.4 ± 3.2, 34.3 ± 3.9 days (expressed as mean ± SD) in the control, aspirin, piroxicam and meloxicam groups, respectively. Meloxicam and piroxicam treatment significantly prolonged survival of mice as compared to control and aspirin treatment (*Fig. 2*, $P < 0.005$, meloxicam versus control and aspirin, respectively; $P < 0.05$, piroxicam versus control and aspirin, respectively). Mice treated with meloxicam survived significantly longer than those treated with piroxicam (*Fig. 2*, $P < 0.05$). There was no significant difference in survival between control and aspirin treatment (*Fig. 2*).

Discussion

In this study, it emerged that each of NSAIDs tested here had a potential of inhibiting the growth of ovarian cancer and meloxicam produced the greatest anti-tumor effect in solid and intraperitoneal ovarian malignancies. The epidemiological study suggested the biological effect of NSAIDs in reducing ovarian cancer incidence.[5] The present study provides direct evidence of an inhibitory effect of NSAIDs, especially meloxicam, on ovarian cancer growth.

Moreover, meloxicam significantly suppressed the growth of ovarian cancer as compared to aspirin and showed more potent anti-tumor effect on this disease than piroxicam. Many lines of evidence suggest that COX-2 might be involved in various aspects of carcinogenesis and tumor progression.[19,24,35] The inhibition of COX-2 activity by NSAIDs blocks these activities and thus may account for the antitumor effect.[34] Shigemasa et al[31] reported that COX-2 expression might play an important role in ovarian cancer development. We previously demonstrated that overexpression of COX-2 in ovarian cancer cells might be involved in ovarian carcinogenesis.[28] Taking these results, it is suggested that COX-2 could be a useful therapeutic target for ovarian cancer. Meloxicam is a selective COX-2 inhibitor, whereas aspirin and piroxicam are nonselective COX inhibitors that block the activity of both of COX-1 and COX-2. IC50 of meloxicam, piroxicam and aspirin was 4.7 mM, 35.3 mM and 29.3 mM for COX-2, respectively and 36.6 mM, 4.4 mM and 3.57 mM for COX-1, respectively. While aspirin and piroxicam were about 8 times more active

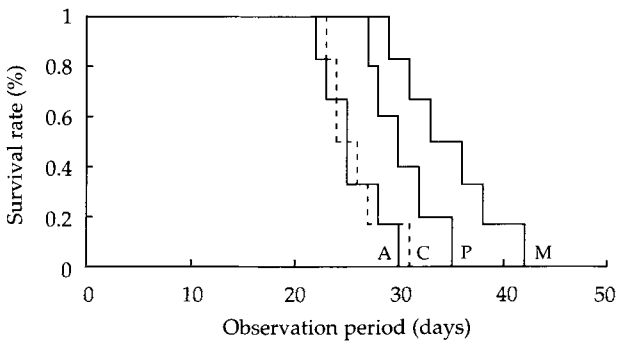

Figure 2. Anti-tumor effect of aspirin, piroxicam and meloxicam in DISS tumor in the peritoneal carcinomatosa model. The statistical significance of differences in the survival curves between the groups was evaluated by log-rank test. (A: aspirin, C: control, M: meloxicam, P: piroxicam)

against COX-1 than against COX-2, meloxicam showed the greatest selectivity for COX-2 with 0.12 of COX-2/COX-1 ratio.[4] One of the important NSAID-associated adverse effects is gastrointestinal toxicity. COX-1 mediates gastroprotective prostaglandin production and excessive inhibition of COX-1 is closely associated with the occurrence of severe gastrointestinal side effects.[17] Actually, about 20% of human patients taking long-term therapeutic doses of piroxicam experience significant adverse gastrointestinal side effects.[15] In this study, necropsy revealed that one of the 6 cancer-bearing mice treated with piroxicam suffered from stomach perforation. Although we found in this study that piroxicam has an antitumor effect that is comparable to that of meloxicam, we feel apprehensive for its toxicity.

Epidemiological studies suggested a decreased incidence of ovarian adenocarcinoma in patients regularly taking aspirin,[5] and an in vitro study showed that aspirin inhibited proliferation of OVCAR-3 cells dose-dependently.[10] On the other hand, aspirin reduced the outcome of anticancer therapy in Meth A sarcoma-bearing mice.[8] Din et al[9] reported that aspirin often shows cell type-specific action and sometimes produces opposite effects. In this study, while aspirin significantly suppressed the growth of solid OVCAR-3 tumor, it did not show antitumor effect on intraperitoneal DISS tumor at all. This is the first report describing the inhibitory effect of NSAIDs on the growth of human ovarian cancer in *in vivo*. However, further evaluation of aspirin and piroxicam as anti-tumor agents will be needed concerning histological specificity and gastrointestinal toxicity.

In conclusion, the present study showed that meloxicam, a selective COX-2 inhibitor, produced the greatest antitumor effect on human ovarian cancer without gastrointestinal adverse effects. Meloxicam may have a potential of safely leading to a novel therapeutic strategy against ovarian cancer.

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