Effect of Aspirin and Indomethacin on Prostaglandin E2 Synthesis in C6 Glioma Cells

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Prostaglandin E2 (PGE2) plays an important role in immunosuppression and tumor growth. PGE2 inhibitors such as aspirin and indomethacin suppress experimental tumor growth. Little is known of the relationship between PGE2 synthesis in brain tumors and the dose of aspirin or indomethacin. The present study was undertaken to evaluate the effect of different doses of aspirin and indomethacin on PGE2 synthesis in C6 glioma cells. C6 glioma cells were incubated with different concentrations (2, 4, and 8 μM) of aspirin and indomethacin for 1, 2, 4, 6, 8, 12, and 24 hours. Intracellular PGE2 concentration was measured by enzyme immunoassay. Each concentration of aspirin and indomethacin effectively inhibited PGE2 synthesis. Concentrations of 2, 4, and 8 μM of aspirin significantly inhibited PGE2 production at 6, 4, and 1 hours, respectively, and the inhibition persisted for more than 24 hours ($p < 0.05$). Concentrations of 2 and 4 μM of indomethacin were effective at 4 and 2 hours ($p < 0.05$), respectively. However, inhibition was not observed beyond 12 hours ($p > 0.05$). Indomethacin 8 μM was effective at 1 hour and the inhibition persisted beyond 24 hours ($p < 0.05$). Our study demonstrates that aspirin and indomethacin inhibit PGE2 synthesis in C6 glioma cells and that low-dose aspirin is as effective as high-dose aspirin. This study may encourage future clinical use of low-dose aspirin in the prevention or treatment of brain tumors.

Key Words: prostaglandin E2, brain tumor, aspirin, indomethacin
(*Kaohsiung J Med Sci* 2004;20:1–5)

Malignant gliomas are the most common primary intracranial tumors. In spite of aggressive surgery, radiation therapy, and chemotherapy, these tumors are uniformly fatal. Immunosuppression has been observed in patients with brain tumors [1–3]. Glioma-mediated immunosuppression has been attributed to the release of soluble factors by the tumor, including transforming growth factor β2 [4–6] and prostaglandin E2 (PGE2) [7–9]. In a previous report, we noted that malignant brain tumors contained a high concentration of PGE2 and that surgical removal reduced PGE2 production [10]. The anti-inflammatory effects of aspirin and indomethacin are mainly due to their ability to inhibit prostaglandin production via cyclooxygenase (COX) [11,12]. Theoretically, inhibition of the synthesis or release of PGE2 by tumors may decrease immunosuppression, inhibiting tumor growth. In experiments, growth of brain tumors is suppressed by administration of PGE2 inhibitors such as aspirin and indomethacin [13,14]. However, little is known of the relationship between PGE2 synthesis in brain tumors and dose of nonsteroidal anti-inflammatory drug (NSAID). In this study, we evaluated the effects of different doses of aspirin and indomethacin on PGE2 synthesis in C6 glioma cells.

Received: October 3, 2003 Accepted: November 3, 2003
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Materials and Methods

C6 glioma cells were cultured in RPMI-1640 culture medium supplemented with 10% fetal bovine serum, 100 U/mL

penicillin, and 100 μg/mL streptomycin. Cultured cells were maintained at 37°C in 5% CO_2. Cells were cultured in standard 96-well microtitre plates (1×10^5 cells/well) at subconfluent conditions and allowed to adhere overnight. They were then incubated with various concentrations of either aspirin (2, 4, or 8 μM) or indomethacin (2, 4, or 8 μM) for 1, 2, 4, 6, 8, 12, or 24 hours. Intracellular PGE2 concentrations were measured using the Biotrak™ PGE2 competitive enzyme immunoassay kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA). All reactions were measured using a micro-ELISA Vmax photometer.

Ten samples of C6 glioma cells were cultured with each concentration of aspirin or indomethacin. Mean values and standard errors of the mean were calculated and were compared using Student's *t* test. A *p* value of 0.05 or less was considered significant.

Results

Each concentration of aspirin and indomethacin effectively inhibited PGE2 synthesis. However, both the onset and duration of inhibition varied with concentration (Table). With higher concentrations, the onset of PGE2 inhibition was earlier and the duration was longer than with lower concentrations. Concentrations of 2 and 4 μM of aspirin inhibited PGE2 production at 6 and 4 hours, respectively, and the inhibition persisted for more than 24 hours ($p < 0.05$). Concentrations of 2 and 4 μM of indomethacin were effective at 4 and 2 hours, respectively ($p < 0.05$). However, inhibition was not observed beyond 12 hours ($p > 0.05$). A concentration of 8 μM of aspirin or indomethacin inhibited PGE2 production at 1 hour and the inhibition persisted for more than 24 hours ($p < 0.05$).

Discussion

Tumor-induced immunosuppression is a fundamental problem in cancer biology and immunotherapy. Increasing evidence has demonstrated that the COX metabolite PGE2 exhibits potent immunosuppressive effects [15–17]. PGE2 has been considered an important modulator of dendritic cell function, altering cytokine production and expression of cell surface markers [18,19]. Recent studies show that COX-2 and PGE2 may play important roles in tumor angiogenesis [15,20,21]. Clinical studies in patients with brain tumors also demonstrate broad suppression of general host immunocompetence [1–3]. PGE2 is associated with glioma-mediated immunosuppression [22–24]. Importantly, the growth of brain tumors can be suppressed by the administration of PGE2 inhibitors such as aspirin and indomethacin [13,14].

Recent reviews have summarized the accumulating evidence that NSAIDs may be used clinically to prevent or treat cancer [25–27]. NSAIDs suppress malignant transformation and tumor growth by stimulating apoptosis and inhibiting angiogenesis. Numerous experimental, epidemiologic, and clinical studies have found that long-term use of aspirin or other NSAIDs results in a lower risk of colorectal cancer, adenomatous polyps, and, to some extent, other tumors such as breast, lung, esophageal, and gastric cancer [28]. Despite enthusiasm about the potential usefulness of NSAIDs as anticancer agents, little has been reported about the effects of aspirin or other NSAIDs on brain tumors.

Table. Alteration in intracellular prostaglandin (PG) E2 concentration (pg/μL) after treatment with aspirin or indomethacin

Drug and dose	1 hr	2 hr	4 hr	6 hr	8 hr	12 hr	24 hr
Control	62.9 ± 12.4	47.3 ± 5.8	43.3 ± 5.6	46.8 ± 6.9	52.3 ± 7.7	52.4 ± 7.5	50.4 ± 6.8
Aspirin							
2 μM	53.5 ± 8.6	40.6 ± 8.5	51.5 ± 10.6	12.5 ± 2.6*	24.5 ± 3.9*	28.3 ± 4.0*	29.3 ± 3.5*
4 μM	55.4 ± 6.9	51.5 ± 8.4	33.4 ± 6.4*	21.0 ± 4.9*	19.4 ± 3.4*	22.4 ± 5.1*	30.5 ± 4.9*
8 μM	38.4 ± 3.0*	19.4 ± 3.9*	19.5 ± 3.3*	20.4 ± 4.5*	19.5 ± 5.0*	24.3 ± 5.6*	28.5 ± 3.8*
Indomethacin							
2 μM	69.5 ± 15.9	52.5 ± 9.8	32.2 ± 5.6*	12.3 ± 2.4*	32.5 ± 4.0*	62.5 ± 7.3	50.3 ± 5.5
4 μM	62.4 ± 4.5	31.5 ± 5.8*	42.4 ± 7.5	9.3 ± 2.0*	23.4 ± 3.3*	58.4 ± 7.1	56.5 ± 4.1
8 μM	40.3 ± 3.2*	16.4 ± 3.4*	16.4 ± 3.2*	39.6 ± 4.1*	32.7 ± 4.4*	28.4 ± 5.2*	33.4 ± 3.4*

*Significant decrease in intracellular PGE2 concentration when compared with control, Student's *t* test, $p < 0.05$.

Two distinct isoforms of the COX enzyme, COX-1 and COX-2, have been identified, and these are encoded by different genes [29,30]. COX-1 is constitutively expressed in most tissues and plays an important role in platelet aggregation and gastric cytoprotection [31,32]. Although COX-2 is expressed constitutively in the human kidney and brain, its expression is induced rapidly in response to growth factors, oncogenes, tumor promoters, and carcinogens, as well as to physiologic stress stimuli [33]. NSAIDs vary in their ability to inhibit COX-1 or COX-2 at different concentrations and in different tissues. Traditional NSAIDs (such as aspirin, indomethacin, sulindac, and ibuprofen) are non-selective and inhibit COX-1 and COX-2. Traditional NSAIDs and selective COX-2 inhibitors (e.g. celecoxib) inhibit chemically-induced carcinogenesis in rats and mice [34,35]. The highest tolerated dose of non-selective NSAIDs typically reduced the number and size of tumors by 40% to 60%, but did not completely eliminate the growth of chemically-induced adenomatous polyps and cancers. However, high doses of celecoxib inhibit 90% of tumors in rats and are better tolerated than comparable doses of non-selective NSAIDs [34,35]. COX-1 activity, perhaps through the induction of COX-2, may also be essential in the development of colorectal cancer [36,37]. A role for COX-1 in the induction of COX-2 might explain why, in epidemiologic studies, aspirin use is associated with a reduced risk of colorectal cancer even at doses and dosing intervals that could not sustain COX-2 inhibition in nucleated cells [38,39].

Despite enthusiasm about the potential usefulness of NSAIDs, particularly the selective COX-2 inhibitors, as anticancer drugs, unresolved questions about their safety, efficacy, mechanisms of action, optimal treatment regimens, and contraindications limit their clinical application in chemoprevention and therapy. Aspirin prophylaxis has become standard in the prevention of cardiovascular disease and its balance of risks and benefits is clearer than for other NSAIDs. Therefore, we evaluated the effectiveness of aspirin and indomethacin on the inhibition of PGE2 synthesis in C6 glioma cells. The results showed that low-dose aspirin (2 and 4 μM) was as effective as aspirin at a higher dose (8 μM) in inhibiting PGE2 synthesis in C6 glioma cells, although low-dose aspirin acted later. The onset of PGE2 inhibition with low-dose indomethacin (2 and 4 μM) was later and the duration was shorter than with high-dose indomethacin (8 μM). In this study, we demonstrated that aspirin and indomethacin can inhibit PGE2 production, and that low-dose aspirin is as effective as high-dose aspirin. Clinically, low-dose aspirin has been widely used to prevent myocardial infarction and thrombotic stroke while minimizing gastrointestinal toxicity. This study may encourage future clinical use of low-dose aspirin in the prevention and treatment of brain tumors.

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