Indomethacin Augmented Antitumor Activity of 5-Fluorouracil in Meth-A Induced Mice

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Background: This study used an ascitic fluid tumor model to assess antitumor effects of indomethacin (INN) and its enhancing effect on the antitumor activity of 5-fluorouracil (5FU).
Methods: Each (monotherapy) or both agents (combined therapy) were administered IP to BALB-c mice on alternate days. Antitumor activity was determined by survival of the experimental animals.
Results: INN (25 (μg/kg IP) improved the survival of the experimental animals significantly ($P < 0.005$) when compared to the nonmedicated controls. The INN was shown to augment the antitumor activity of 5FU significantly at doses of 2.5 ($P < 0.005$) and 10 ($P < 0.005$) mg/kg IP, compared to 5FU monotherapy at each dose. Carcass weight was significantly higher ($P < 0.01$–0.05) in the combination therapy groups than in the controls. This effect was the result of a less marked decrease in food intake, although there was no significant difference in carcass weight between single and combined therapies.
Conclusion: INN was shown to exert its antitumor effect and to augment the antitumor activity of 5FU which led to the improved survival of mice bearing ascitic tumors. This evidence suggests a potential role for INN as an adjunct to standard chemotherapy.

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Key words: ascites tumor, indomethacin, 5-fluorouracil

INTRODUCTION

The nonsteroidal antiinflammatory drug, indomethacin (INN), has been described as a biological response modifier due to its ability to inhibit the increased formation of immunosuppressive prostaglandins (PGs). One of these PGs, prostaglandin E_2 (PGE_2) has been shown to play a role in the regulation of cytokine production[1–4] and in the modulation of cellular immune responses.[5–8] Interestingly, INN apparently acts as an antitumor agent through the stimulation of cellular immune function by the inhibition of immunosuppressive PGE_2 in inflammatory cells and/or tumors. In the 1980s, various papers reported the antitumor effects of INN by in vivo studies.[9–12] Furthermore, INN reduced acetoxymethylmethylnitrosamine-induced-large-bowel carcinogenesis,[13] modulated uterine cervical cancer carcinogenesis induced by methylcholanthrene,[14] and inhibited mammary tumorigenesis induced by 7, 12-dimethylbenz [a] anthracene.[15] Moreover, INN and other nonsteroidal antiinflammatory drugs, e.g., piroxicam, were able to retard the patient's tumor growth in either primary[16] or metastatic lesions.[17] Thus, the antitumor effects of INN have been well defined in solid malignant neoplasms, but very few assessments have been documented in ascitic tumors.

Therefore, this study used IP Meth-A bearing mice as ascitic tumor models to evaluate INN's effectiveness in improving patient survival and the extent of its influence on the antitumor activity of 5-fluorouracil (5FU).

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MATERIALS AND METHODS

Cell Line, Host and Tumor Transplantation

The methylcholanthrene-induced murine fibrosarcoma (Meth-A) intraperitoneal (IP) subculture was donated by Kyowa Hakko Co Ltd, Tokyo, Japan. One-half mL of cell suspension containing 10^6 cells in physiological saline was inoculated into the peritoneal cavity of 6 week old male BALB/c mice (day 0; d_0), which were obtained from Shizuoka Agricultural Laboratory Animals, Hamamatsu, Shizuoka, Japan. These mice were given a standard diet and tap water ad lib.

Drug Preparation and Administration

5FU (Kyowa Hakko Co Ltd, Tokyo, Japan; 250 mg/5

mL/vial) was diluted with physiological saline and adjusted to 1.0, 2.5, 5.0 and 10 mg/kg body weight in 0.5 mL of solution respectively. The INN crystalline was purchased from Sigma (St. Louis, MO, USA) and dissolved in ethanol. The dissolved INN was diluted with physiological saline and adjusted to 2.5, 25 and 250 μg/kg body weight in 0.5 mL of solution. The final concentration of ethanol in solution was less than 0.09%. Physiological saline was used as a control solution.

Experiment 1

This experimental protocol was undertaken in order to determine the dose of each reagent based on survival as a marker for antitumor activity, needed in experiment 2. 5FU was administered IP to the experimental animals at doses of 1.0 (1), 2.5 (2), 5.0 (3) and 10 (4) mg/kg body weight, while INN was given IP to the mice at doses of 2.5 (a), 25 (b) and 250 (c) μg/kg body weight, on d_1, d_3, d_5 and d_7 respectively. The control solution was given IP to the nonmedicated control mice with the same schedule. Each control and test group consisted of 7 randomly assigned mice.

Experiment 2

From experiment 1, the dose of each reagent was determined, i.e., 2.5 and 10 mg/kg body weight for 5FU and 25 μg/kg body weight for INN. Either 5FU or INN was administered IP to the mice on d_1, d_3, d_5 and d_7 and combined treatment of 5FU with INN was carried out in the same manner. Physiological saline was given IP to the untreated control mice. Therefore, in this experimental protocol, 6 groups were prepared: A, nonmedicated control; B, 5FU (2.5 mg/kg); C, 5FU (10 mg/kg); D, INN (25 μg/kg); E, 5FU (2.5 mg/kg) plus INN (25 μg/kg); and F, 5FU (10 mg/kg) plus INN (25 μg/kg). Each control and test group consisted of 7 randomly assigned mice. Antitumor activity was evaluated by survival, which was expressed as mean days ± SD (n = 7).

Body Weight and Food Intake

In experiment 2, the carcass weight and food intake were assessed. At the time of death, the abdominal wall was incised and the peritoneal fluid was completely removed. The body weight was measured, and this value equaled the true carcass weight. Results were expressed as mean grams (g) ± SD (n = 7). The food intake was measured at 2 to 3 day intervals and averaged for each mouse. This value was expressed as mean g/mouse/day ± SD. Statistical analysis looked at differences between monotherapy and combined treatments.

Statistical Analysis

Statistical analysis was performed using the two-way ANOVA and two-tailed tests. A statistically significant difference was present when the P value was less than 0.05.

RESULTS

Experiment 1

Antitumor activity was determined by survival. Survival in each group is shown in Table 1. With 5FU monotherapy, survival was significantly improved ($P < 0.005$) in mice 2, 3 and 4, when compared to the nonmedicated controls. There was no significant difference between mouse 2 and 3. Survival was significantly ($P < 0.005$) prolonged in mouse 4 when compared with 2 or 3. Based on these data, the 5FU was used at 2 effective doses in experiment 2, i.e., 2.5 and 10 mg/kg. Although the data are not shown, 5FU at a dose of 20 mg/kg was shown to be toxic during preliminary study. With INN monotherapy, at lower doses, a remarkable antitumor effect against the nonmedicated controls was demonstrated ($P < 0.005$), but there were no significant differences among the 3 INN monotherapy groups in experiment 1. Considering that the maximal peritoneal fluid volume ranged from 4.0 to 5.0 mL, and the mean body weight of the mice was about 20 g, and that one-tenth of the maximal plasma concentration of INN in human subjects is approximately 0.1 μg/mL at a usual dose (2.0 mg/kg), the INN at a dose of 25 μg/kg was determined to be appropriate.

Experiment 2

As shown in Table 2, the survival of the experimental animals in the medicated groups was significantly ($P < 0.005$) improved when compared to the nonmedicated controls. With regard to combination treatment, INN was shown to significantly improve the survival of the mice at doses of 2.5 (group E, $P < 0.005$) and 10 (group F, $P < 0.005$) mg/kg as compared with those on 5FU monotherapy at respective doses. Interestingly, INN monotherapy (group D) produced significantly ($P < 0.05$) improved survival rates as compared to 5FU

Table 1. Survival (experiment 1).

	Survival (days; mean ± SD, n = 7)
Nonmedicated controls	11.0 ± 0.7
1. 5Fu (1.0 mg/kg bw*)	10.8 ± 1.2
2. 5Fu (2.5 mg/kg bw)	14.3 ± 0.9
3. 5Fu (5.0 mg/kg bw)	14.7 ± 0.9
4. 5Fu (10 mg/kg bw)	16.4 ± 0.9
a. INN (2.5 μg/kg bw)	15.7 ± 1.2
b. INN (25 μg/kg bw)	15.9 ± 1.2
c. INN (250 μg/kg bw)	16.4 ± 1.8

* body weight. Survival in each group (experiment 1) is expressed in days. Control vs. 1; no significant difference. Control vs. 2, 3 and 4; $P < 0.005$. 2 vs. 3; no significant difference. 4 vs. 2 and 3; $P < 0.005$. Control vs. a, b and c; $P < 0.005$. There were no significant differences among a, b and c.

Table 2. Survival (experiment 2).

	Survival (days; mean ± SD, n = 7)
A. Nonmedicated controls	10.7 ± 0.5
B. 5Fu (2.5 mg/kg bw*)	13.6 ± 0.5
C. 5Fu (10 mg/kg bw)	15.4 ± 0.5
D. INN (25 μg/kg bw)	14.6 ± 0.7
E. 5Fu (2.5 mg/kg bw) + INN (25 μg/kg bw)	15.3 ± 0.7
F. 5Fu (10 mg/kg bw) + INN (25 μg/kg bw)	17.6 ± 0.7

* body weight. Survival in each group (experiment 2) is expressed in days. A vs. B, C, D, E and F; $P < 0.005$. B vs. E; $P < 0.005$. C vs. F; $P < 0.005$. Overall data for survival are summarized in the order of A << B << D < E < C << F (< = slightly improved and << = significantly improved).

Table 3. Carcass weight (experiment 2).

	Survival (g; mean ± SD, n = 7)
A. Nonmedicated controls	19.4 ± 1.8
B. 5Fu (2.5 mg/kg bw*)	20.6 ± 2.6
C. 5Fu (10 mg/kg bw)	21.8 ± 0.9
D. INN (25 μg/kg bw)	20.1 ± 1.3
E. 5Fu (2.5 mg/kg bw) + INN (25 μg/kg bw)	21.0 ± 1.1
F. 5Fu (10 mg/kg bw) + INN (25 μg/kg bw)	22.2 ± 1.6

* body weight. Carcass weight in each group (experiment 2) is expressed in grams (g). A vs. B and D, no significant difference. A vs. C and F; $P < 0.01$. A vs. E; $P < 0.05$. B vs. E and C vs. F, no significant difference.

monotherapy at a dose of 2.5 mg/kg (group B). Because there was no significant difference between group D and E, this seemed to indicate that the antitumor effect was predominantly due to INN in combination with 5FU (2.5 mg/kg). The overall data for survival can be summarized as A << B << D < E < C << F (< = slightly improved and << = significantly improved).

As shown in Table 3, the carcass weight was significantly higher in groups C ($P < 0.01$), E ($P < 0.05$) and F ($P < 0.01$), but there was no significant difference in group B and D when compared to the controls. As long as the ascites tumor was used as the experimental model, 5FU and INN combination therapy was not shown to produce any significant changes in carcass weight, compared to 5FU monotherapy.

Food intake during the experimental course is shown in Fig. 1. In both INN or 5FU monotherapy and 5FU with INN combined treatments, a decrease in food intake seemed less remarkable than in the nonmedicated control. It is worthwhile to note that the addition of INN to 5FU prohibited the marked decrease in food intake in contrast to that of the controls, and the addition of INN to 5FU moderately or significantly ($P < 0.005$–0.025) raised the amount of food intake in combination therapy

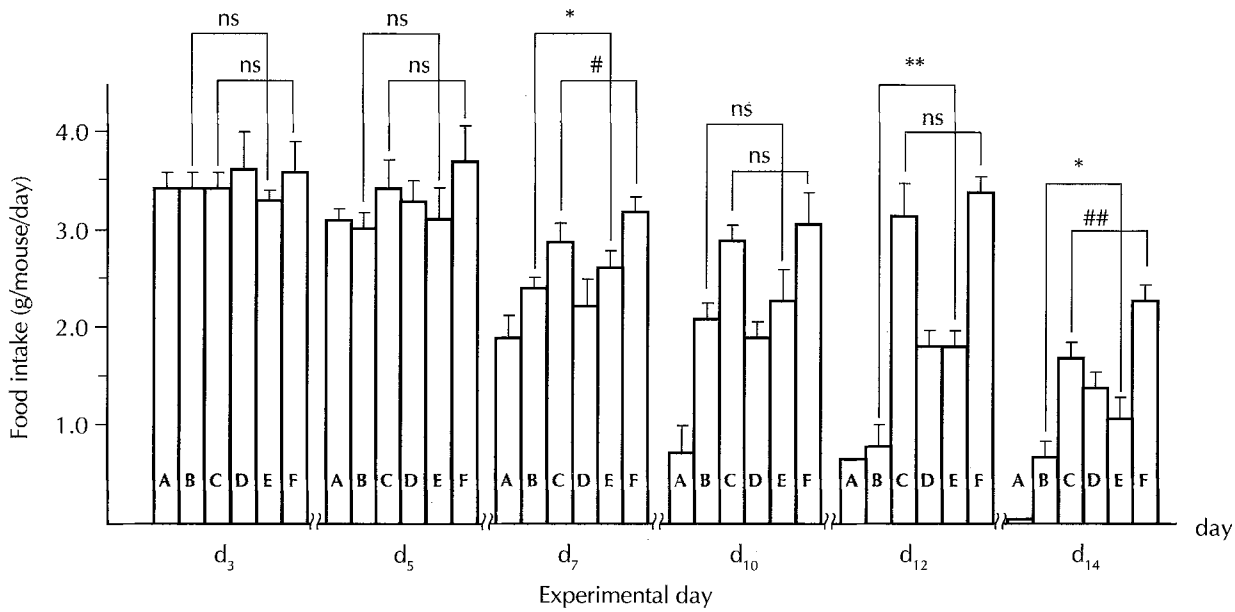

Fig. 1. Food intake on each experimental day is expressed in g/mouse/day (Only mean values are shown when the number of mice is ≤ 2). The letters in each column indicate each experimental group. B vs. E and C vs. F; ns = no significant difference; * and # = $P < 0.025$; ** and ## $P < 0.005$. On d_7 A << D < B << E << C << F; on d_{10} A << D < B < E << C < F; on d_{12} B << D ≅ E << C < F and on d_{14} B << D ≅ E << C << F (≅ = nearly the same, < = moderately increased, << = significantly increased: P values are not indicated to avoid confusion).

groups when compared to that obtained by monotherapy alone (B vs. E and C vs. F). Up to and including d_5, there were no statistically significant differences among the groups (A–F) with regard to food intake. From d_7 onward, there appeared to be obvious differences in food intake among the 6 groups. Results obtained by statistical analysis are described in detail in the legend for Fig. 1. With advanced tumor proliferation, food intake in groups C and F became prominently and significantly ($P < 0.005$–0.025) greater than in the other groups. It is interesting to note that the amount of food intake in group D was significantly ($P < 0.005$–0.01) greater than in group B, and nearly the same as group E in advanced stages, particularly on d_{12} and d_{14}.

DISCUSSION

The antitumor activity of INN still remains controversial, however more studies support the highly suppressive action of PGE_2 to cellular immune function.[11] The cyclo-oxygenase inhibitor, INN, has the potential ability to exert antitumor effects by inhibiting the exaggerated formation of immunosuppressive PGE_2 in inflammatory cells and/or tumors, though there are some reports contrary to this with regard to the antitumor effects of INN.[18–20] In fact, exogenously added PGE_2 analogue has been shown in vivo to act as a general immunosuppressant.[11] Furthermore, INN has also been reported to modulate chemically induced tumorigenesis and retard tumor growth. Moreover, in cancer patients as well as in animals with transplantable tumors, INN has been shown to exert an antitumor effect.

This preliminary study used Meth-A treated mice to assess INN's effectiveness in the improvement of survival and INN's influence on the antitumor activity of 5FU. Tumor cells produce high levels of PGs, among which PGE_2 is a major product that exerts a suppressive effect on immune cells.[21,22] As previously demonstrated in IP Meth-A bearing animals, the PGE_2 level in ascites has been shown to originate mainly from peritoneal macrophages[23] and to impair immunocompetence.[24] The Meth-A employed in the present study, which infrequently causes cachexia, was shown to reduce food intake and to induce weight loss. This phenomenon is probably caused by a loss of appetite which progresses in accordance with advancing tumor proliferation and/or increasing accumulation of ascites. During monotherapy with INN, food intake was consistently higher and survival was significantly better than that of the control. Furthermore, with 5FU and INN combination therapy, carcass weight and food intake were demonstrated to be significantly greater than that obtained by 5FU monotherapy. This evidence suggests that INN may also act as an antianorexic agent and has been well documented elsewhere.[25–27] Moreover, a better nutritional state has been reported to improve survival and chemotherapy tumor response.[28–30] These reports together with the present findings, show that the antitumor effects of INN, as evaluated by survival criteria, are caused by the inhibition of PGE_2 formation. In fact, INN treatment at a dose of 25 μg/kg IP strikingly inhibited PGE_2 production in ascites, where the PGE_2 level in the ascitic fluid was 1270.0 ± 87.2 pg/mL in the nonmedicated control, and 31.0 ± 6.6 pg/mL in the INN treated group, (mean ± SD, n = 5). This effect was continually monitored for 24 hours following INN administration. However, this effect disappeared over the next 24 hours, which suggested the necessity for daily administration of INN rather than alternate days. Furthermore, the addition of INN to 5FU appears to augment the antitumor activity of 5FU, probably by restoring nutritional state as shown by less appetite reduction and body weight losses.

INN has been reported to augment the cytotoxicity of methotrexate both in vivo and in vitro by accelerating the intracellular uptake of antitumor agents and/or altering tissue fatty acid composition.[31,32] Moreover, INN has also been shown to enhance the radiosensitivity of the tumors.[33,34] The effectiveness of INN in combination with interleukin-2 as immunotherapy agents has been demonstrated.[35] Previous studies have been undertaken using a solid tumor model, but very few assessments have been documented using the ascitic tumor model, with regard to the antitumor effects of INN and its enhancing effect on the cytotoxicity of antitumor agents.

In summary, INN can regulate the exaggerated production of immunosuppressive PGE_2 in ascites, thus leading to the restoration of immunoinflammatory reactions against tumors. Although there is evidence indicating that INN has no direct antitumor activity,[36,37] INN should be considered as an adjunct in the standard chemotherapy and/or immunotherapy of tumors.

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