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Effect on liver tumor growth in rats of allopurinol and 5-fluorouracil in combination with hepatic artery ligation*

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Summary. Rats with an experimental solitary liver tumor of a nitrosoguanidine-induced colonic adenocarcinoma were subjected to hepatic artery ligation (HAL) alone or in combination with 5-fluorouracil (5-FU) in three different doses, with or without the addition of allopurinol. The drugs were injected i.p. on 3 consecutive days before or after the HAL procedure. HAL alone significantly reduced the tumor growth compared with the control procedure ($P < 0.001$). This observation was correlated with a significantly prolonged survival for the ligated animals ($P < 0.01$). The administration of a low dose of 5-FU (15 mg/kg per day) in combination with allopurinol (100 mg/kg per day) enhanced tumor growth compared with that in animals treated with 5-FU only ($P < 0.01$) or nontreated animals ($P < 0.05$). A significant increase in survival was observed in animals given a high dose of 5-FU (60 mg/kg per day) after HAL compared with nontreated animals ($P < 0.001$) as well as animals subjected to HAL alone ($P < 0.02$). All animals receiving more than 15 mg/kg per day 5-FU before HAL succumbed within 10 days. The addition of allopurinol did not protect the animals against this mortality. These observations indicate that the effect of HAL followed by 5-FU is dose-dependent and that, at least in this treatment modality, allopurinol does not modulate the toxicity of 5-FU.

Introduction

The most common source of secondary, malignant liver tumor growth is metastasis from primary tumors within the gastrointestinal tract, particularly colorectal cancer. If this is not treated, the prognosis for these patients is very dismal [5]. In patients with resectable liver tumors, i.e., localized tumors without extrahepatic tumor growth, liver resection can be a curative procedure [1]. In patients with unresectable liver tumor growth, there are at present only palliative treatment modalities available. Based on findings of a predominantly arterial blood supply to liver tumors, devascularization of the liver has been tested [4, 6]. Tumor necrosis and tumor regression have been observed after devascularization of the liver, but no prolongation of survival has yet been reported [2, 26].

The single cytotoxic drug of choice in patients with advanced gastrointestinal cancer is 5-fluorouracil (5-FU), which has been given systemically, i.a., intraportally, or i.p. to achieve palliation [11, 17, 19, 28, 29]. Although there have been observations of up to 88% response to regional administration to the liver, no improvement in survival has been reported compared with i.v. chemotherapy [3, 14]. Intraportal or i.a. infusion of 5-FU has also been used after devascularization of the liver. Apart from one study by Taylor [31], there have been no observations of an increased response rate or survival with this combination compared with single treatment.

A new approach to treatment with 5-FU is by biochemical modulation of its effect, since 5-FU has to be enzymatically converted to be incorporated into RNA or interfere with thymidylate synthase (TS), which is necessary for the synthesis of DNA [10, 23, 24]. The cytotoxic effect of 5-FU is believed to be due to the formation of a tenary complex of FdUMP 5,10-methylenepteroylmonoglutamic acid (CH_2FH_4) and TS [9].

Allopurinol is a potent inhibitor of xanthine oxidase used clinically in the treatment of gout. Another action of allopurinol is the inhibition of the last enzyme, orotidylate decarboxylase (ODCase), in the de novo pyrimidine synthesis caused by the metabolite oxipurinol. The inhibition of ODCase caused by oxipurinol increases the intracellular concentration of the enzyme substrate orotidine monophosphate (OMP). OMP competes with uracil or 5-FU for the orotate phosphoribosyltransferase (OPRTase), which transfers phosphoribosyl phosphate from phosphoribosyl pyrophosphate (PRPP), producing uridine or 5-fluorouridine monophosphate from uracil and 5-FU, respectively [12, 27]. Besides being activated by OPRTase, as indicated by a high PRPP concentration, 5-FU can be activated by thymidine phosphorylase or uridine phosphorylase and kinase.

The rationale for using allopurinol as a biochemical modulator to protect normal cells is based on two assumptions: (1) normal cells depend on OPRTase and malignant cells, on other pathways, to activate 5-FU; and (2) the intracellular concentration of OPRTase in malignant cells exceeds that in normal cells.

Allopurinol has been used in combination with 5-FU to protect normal cells, thereby increasing the maximum

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tolerated dose of 5-FU [13, 16, 18, 33]. However, clinical experience with 5-FU in combination with allopurinol in patients with colorectal cancer shows no improvement in the response rate in most cases, except in one study, which was also the first in which the response rate was 50% in patients receiving more than 2.0 g/m^2 5-FU [13].

The aim of this study was to investigate the influence of 5-fluorouracil on hepatic tumor growth and on survival, alone or in combination with allopurinol, before or after ligation of the hepatic artery (HAL).

Material and methods

In all, 80 inbred Wistar rats of both sexes, weighing 200–220 g, were inoculated with a transplantable *N*-methyl-*N*-nitroso-guanidine-induced colonic adenocarcinoma [30]. The rats were maintained on a standard rat pellet diet and water ad libitum. A cell suspension of the adenocarcinoma containing 1.0×10^6 viable tumor cells was inoculated just under the liver capsule in the central liver lobe [8]. Access to the liver was achieved using ether anesthesia by a midline incision.

The rats were relaparotomized 12 days later (day 0), and the tumor was measured in the greatest (a) and smallest (b) superficial diameters. The tumor volume was calculated [8] according to the formula

$$V = \frac{a \times b^2}{2}.$$

The rats were randomly divided into six groups:

Group A (10 animals). Controls were subjected to tumor measurement only.

Group B (10 animals). HAL was carried out just distal to the ramification of the gastroduodenal artery [7].

Group C (15 animals). HAL was carried out on day 0, followed by i.p. bolus injection of 5-FU on days 1, 2, and 3. 5-FU was given at three different doses: 15 mg/kg per day (low dose), 30 mg/kg per day (intermediate dose), and 60 mg/kg per day (high dose), with five animals in each subgroup.

Group D (15 animals). This group was treated identically to group C except that these animals also received allopurinol (100 mg/kg per day) in two equal doses. One dose was given 8 h before the administration of 5-FU and the second dose was given simultaneously with 5-FU.

Group E (15 animals). On days 1–3, this group received the same doses of 5-FU on the same schedule as group C. HAL was carried out on day 4.

Group F (15 animals). This group was treated identically to group E, with the addition of allopurinol as in group D.

5-FU alone or in combination with allopurinol was diluted with 0.9 *M* NaCl to a total volume of 2.0 ml and injected i.p. as a bolus. Animals subjected to the control procedure or HAL only were injected with 2.0 ml 0.9 *M* NaCl. Laparotomy and tumor size measurements were done on day 4 in all animals. The rats were kept in their cages and their survival was recorded. No autopsy was done when the animals succumbed.

The results are presented as the mean ± SEM. The changes in tumor volume between day 0 and day 4 and differences in survival between the groups were analyzed using Student's *t*-test.

Results

There were no differences in tumor volume between the six groups or subgroups on day 0. In animals subjected to HAL (group B), tumor growth was significantly retarded compared with that in nontreated animals (group A) ($P < 0.001$) (Fig. 1). Significantly retarded tumor growth compared with controls (group A) was also observed in all animals subjected to the HAL procedure on day 0 followed by the administration of 5-FU, with or without concomitant allopurinol (groups C and D) ($P < 0.01$).

Animals exposed to the intermediate dose of 5-FU alone (group E) showed a significantly retarded tumor growth rate compared with nontreated animals (group A) ($P < 0.01$), but, regardless of the dose of 5-FU, tumor growth was significantly faster in these animals than in those subjected to HAL (group B) ($P < 0.02$).

Additional treatment with allopurinol (group F) significantly increased the tumor growth rate in animals given the low dose of 5-FU compared with controls ($P < 0.05$), but the intermediate dose of 5-FU significantly retarded tumor growth compared with the control procedure (group A) ($P < 0.05$).

Allopurinol in combination with 5-FU, regardless of the dose of 5-FU, induced significantly faster tumor growth compared with that in animals subjected to the HAL procedure (group B) ($P < 0.001$).

Animals subjected to the HAL procedure (group B) survived significantly longer than nontreated animals (group A) ($P < 0.01$) (Fig. 2). The survival of animals treated with HAL followed by the low or intermediate dose of 5-FU (group C) was comparable with that of nontreated animals, whereas the survival of animals given the high dose of 5-FU after HAL was double that of nontreated animals ($P < 0.001$) and significantly longer than that of animals subjected to HAL only (group B) ($P < 0.02$).

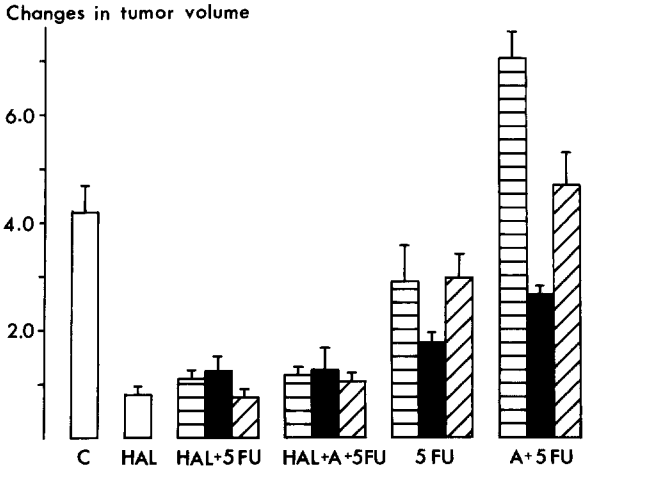

Fig. 1. Changes in tumor volume from day 0 to day 4 (mean ± SEM). ▤ 15 mg/kg per day; ■ 30 mg/kg per day; ▨ 60 mg/kg per day; *A*, allopurinol

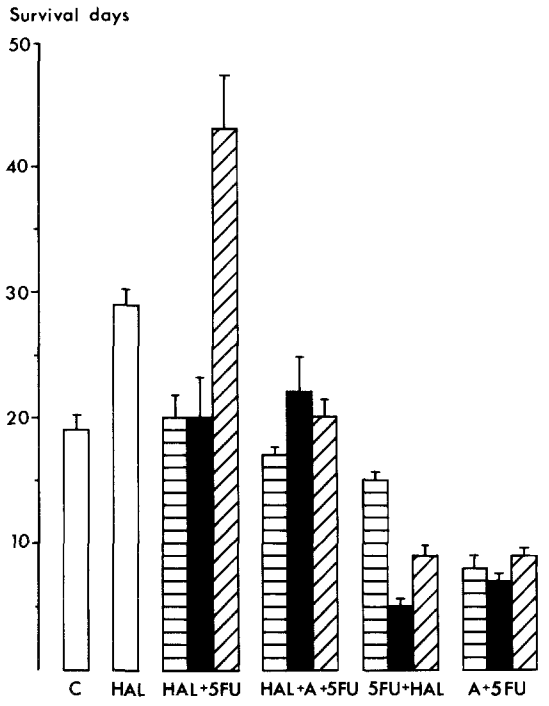

Fig. 2. Survival from day 0 (mean ± SEM). ▤ 15 mg/kg per day; ■ 30 mg/kg per day; ▨ 60 mg/kg per day; *A*, allopurinol

The concomitant administration of allopurinol to animals treated with HAL followed by 5-FU, (group D) regardless of the dose, resulted in survival similar to that in controls (group A).

In all animals given 5-FU with or without allopurinol before HAL (groups E and F), except those given the low dose of 5-FU without allopurinol, survival was significantly shorter than in controls (group A) ($P < 0.001$).

Discussion

The experimental colonic adenocarcinoma used in this study was sensitive to the intermediate dose of 5-FU, as indicated by the retarded tumor growth rate compared with that in nontreated animals, possibly indicating that the present experimental tumor uses the OPRTase pathway to a great extent in activating 5-FU. Further support for this was observed when the OPRTase pathway for the activation of 5-FU was blocked by the administration of allopurinol; tumor growth was significantly enhanced at the low dose of 5-FU.

In animals receiving the low or high dose of 5-FU, no significant retardation of tumor growth was observed. The reason for this may be that the low dose was insufficient and the high dose induced an expansion of the dUMP pool, thereby causing decreased inhibition of TS.

In animals receiving HAL alone, tumor growth was retarded and survival prolonged compared with nontreated animals. This observation supports similar previous findings [15, 25]. Additional treatment with 5-FU, regardless of the dose, with or without allopurinol, induced no further retardation of tumor growth compared with HAL alone.

The survival observed in rats given 60 mg/kg per day 5-FU after HAL was longer than that in both nontreated animals and those subjected to HAL only, whereas lower doses resulted in survival similar to that in nontreated animals, possibly indicating that the benefit of adding 5-FU after HAL is dose-dependent. The addition of allopurinol in animals receiving 5-FU after HAL did not improve their survival compared with that of nontreated animals, regardless of the dose.

Although no autopsy or analyses of hematologic and gastrointestinal toxicity were carried out in this study, the short survival observed in animals receiving 5-FU before HAL, regardless of the dose, probably indicates death due to toxicity. The administration of allopurinol induced no protection against this assumed toxicity. 5-FU and allopurinol were both given regionally, which may explain the lack of protection against toxicity, since the systemic administration of allopurinol has been found to induce protection against systemic toxicity following the intravesical instillation of high doses of 5-FU [20].

The experimental tumor and tumor models used seem to be appropriate for further investigations into the biochemical modulation of 5-FU. This study indicates that allopurinol has no beneficial effects and may be dangerous, in view of the enhanced tumor growth observed. Another way to modulate the effect of 5-FU is to increase the inhibition of TS by leucovorin [32]. There have been encouraging clinical reports of an increased response rate in patients with gastrointestinal cancer with this mode of modulation compared with 5-FU alone [21, 22].

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