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Primary study in experimental antiangiogenic therapy of nasopharyngeal carcinoma with AGM-1470 (TNP-470)

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Abstract

Objective: To evaluate the efficacy of the angiogenesis inhibitor AGM-1470 for the experimental treatment of nasopharyngeal carcinoma (NPC).

Methods: A NPC human tumour model was built by tumour-bearing nude mice using the NPC cell line CNE-2. Twenty-one BALB/c nude mice bearing CNE-2 xenografts were randomized into a treatment group and a control group. In the treatment group, AGM-1470 was injected 30 mg/kg subcutaneously every other day; while the vehicle (three per cent ethanol solution in 0.9 per cent saline) was given to the mice in control group. Tumour volumes and animal weights were measured every third day. Autopsy was performed after 18 days of treatment. The tumour tissue as well as the murine tissues of heart, kidney, and liver in each mouse were removed for formalin fixation and routine HE staining. Pathological evaluation was performed in these tissues.

Results: There was a significant difference in tumour volume between the two groups at day 9 of treatment and this increased thereafter. At day 15 of treatment, the tumour volume was 4251 ± 559 mm^3 ($n = 10$) in the control group versus 3122 ± 967 mm^3 ($n = 11$) in the AGM-1470 treated group ($p = 0.004$); and T:C ratio (mean tumour volume of treated/mean tumour volume of control) was 0.73, resulting in a 27 per cent decrease in tumour growth. Central necrosis and consequential shrinkage of tumours occurred in both groups at the end of experiment. Physical toxicity and histological toxicity of heart, liver, and kidney did not result from AGM-1470 therapy.

Conclusions: AGM-1470 suppresses the growth of the human NPC cell line CNE-2. Treatment by AGM-1470 has no physical nor histological toxicity. Angiogenesis inhibitors may be effective in the treatment of the local lesion of NPC.

Key words: Nasopharyngeal neoplasms; Carcinoma; Neovascularization inhibition, treatment

Introduction

Nasopharyngeal carcinoma (NPC) occurs worldwide, but the highest incidence is in south China (Hwang, 1983; Li *et al.*, 1983; Fandi *et al.*, 1994). Cantonese are the most frequently affected (Li *et al.*, 1983; Fandi *et al.*, 1994). Although NPC is markedly radiosensitive, the best five-year survival reported for Stage IV NPC is 30 per cent (Al-Sarraf and McLaughlin, 1995). This poor survival is caused by the high incidence of local, regional, and systemic recurrences (Fandi *et al.*, 1994; Al-Sarraf and McLaughlin, 1995). The failure of loco-regional control is also a significant risk factor for development of distant metastases (Kwong *et al.*, 1994). So, further exploration of multidisciplinary therapeutic possibilities to improve loco-regional control and eradicate micrometastases is crucial for improvement of survival (Lee *et al.*, 1992; Fandi *et al.*, 1994; Kwong *et al.*, 1994).

Tumour angiogenesis is an essential step in tumour growth and metastasis (Scott and Harris, 1994; Fox *et al.*, 1996). Although it is a continuous process, tumour angiogenesis has been subdivided into the following six discrete steps (Fox *et al.*, 1996): the release of angiogenic factors; alteration in endothelial cell morphology; release of proteolytic enzymes; endothelial cell migration and capillary morphogenesis; endothelial cell proliferation; and microvessel differentiation. Tumour angiogenesis is reported to be a significant prognostic indicator in many malignancies (Fox *et al.*, 1996; Tao and Lin, 1996), including NPC (Roychowdhury *et al.*, 1996; Qian *et al.*, 1997), in which the tumour microvessel density is correlated with distant metastasis.

Folkman (1972) first suggested antiangiogenesis as an antineoplastic modality a quarter of a century ago, but only in the present decade has it become a practical reality (Editorial, 1991). Antiangiogenesis

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could have a role in preventing the switch of small tumours to an angiogenic phenotype and keeping micrometastatic tumours dormant, so as to prevent clinically relevant tumour development or recurrence (Scott and Harris, 1994). Moreover, combination with conventional anticancer treatment is also a promising therapeutic strategy for antiangiogenesis.

Currently, a novel angiogenesis inhibitor O-(chloroacetyl-carbamoyl)fumagillol (AGM-1470, TNP-470), which is a synthetic analogue of the fungal antibiotic fumagillin (Ingber *et al.*, 1990), is reported by many investigators to be a very promising antiangiogenic compound without obvious toxicity and gender dependence (Brem and Folkman, 1993; Yamaoka *et al.*, 1993; Yanase *et al.*, 1993; Tanaka *et al.*, 1995; Yazaki *et al.*, 1995; Fujioka *et al.*, 1996). In many *in vitro* and *in vivo* models, AGM-1470 is very effective in inhibiting endothelial cell proliferation (Yamaoka *et al.*, 1993; Antoine *et al.*, 1994; Kusaka *et al.*, 1994; Yamamoto *et al.*, 1994), endothelial cell migration (Brem *et al.*, 1991), and capillary tube formation (Kusaka *et al.*, 1991) without a substantial effect on tumour cells (Brem and Folkman, 1993; Yamaoka *et al.*, 1993; Yanase *et al.*, 1993) and then prevents tumour growth and metastasis *in vivo*.

In this study, our goal was to evaluate the effectiveness of AGM-1470 on local tumour growth of an NPC cell line transplanted into nude mice. The cell line applied in this study was CNE-2, which is a cell line of undifferentiated nasopharyngeal carcinoma (Zhang *et al.*, 1983).

Materials and methods

Cell line

CNE-2 was provided by the pathology department, Institute of Oncology, SUMS. It was maintained in RPMI-1640 culture medium supplemented with 10 per cent calf serum at 37°C. A single cell suspension of approximately 2×10^6 cells in 0.5 ml culture medium was inoculated subcutaneously in two male BABL/c nude mice to make source tumours. These source tumours were excised when they grew to 1 cm^3, and then 2–3 mm^3 of morcelled tumour tissue was implanted subcutaneously into the left axillary region of the flank of each BALB/c nude mouse.

Animals

Four-week-old BALB/c nude mice (nu/nu) four were purchased by Animal Laboratory of Cancer Center, SUMS, and reared under specific pathogen-free conditions. Twenty-one of 22 nude mice (eight males and 13 females) were available for the experiment when the tumours were 54–180 mm^3 on day 5 after implantation. The mice were then randomized into a treatment groups (four males and seven females) and a control group (four males and six females). Treatment began on day 6 after implantation. The average tumour volume and weight in the treatment group and control group were approximately equal at the beginning of treatment.

Angiogenesis inhibitor

AGM-1470 was donated by Takeda Chemical Industries, Ltd. (Osaka, Japan). It was stored dry at −20°C. A stock solution of 10 per cent (w/v) AGM-1470 in 100 per cent ethanol could be stored in 4°C for about three months. Immediately prior to the injections, a treatment solution was made by diluting the stock solution of AGM-1470 in 0.9 per cent normal saline (30 μl stock solution/1 ml saline). Treatment was 30 mg/kg of AGM-1470 given by subcutaneous injection every other day in each treatment mouse at a site remote from the tumour. Control mice were given the injections of a comparable volume of vehicle (three per cent ethanol solution in 0.9 per cent saline) alone every other day.

Tumour volume

Tumour dimensions were measured every third day with callipers. Tumour volumes were calculated by width^2 × length × 0.52. Tumour volume is also expressed by the ratio of mean tumour volume in treated animals to mean tumour volume in the control animals (T:C ratio). The T:C ratio before treatment was 1.04.

Animal weights

The mice were weighed every third day after treatment began.

Autopsy

The experiment was terminated on day 18 after treatment. Animals were killed by continuous inhalation of ether. All animals were weighed prior to autopsy, at which time tumour weights were also obtained. The net animal weights at the time of autopsy were calculated by subtracting the tumour weight from the animal weight prior to autopsy.

Histological evaluation

During autopsy, the tumour tissue, the heart, part of the liver and a kidney were removed from each mouse and fixed in 10 per cent formalin solution for at least 72 hours. The liver tissue and transverse slices (2–3 mm) of the heart and kidney of each mouse were then dehydrated through graded ethanol, embedded in paraffin, and cut into 4 μm sections for routine haematoxylin-eosin staining. The sections were evaluated by one of us (Lin HL) without any knowledge of the randomized treatment.

Statistics

Students *t* test for independent samples was used for comparing the means of tumour volume, T:C ratio, tumour weight, and body weight between both groups. The correlation between tumour weight and tumour volume was analyzed by the Pearson correlation test.

Results

Tumour growth

One day before treatment, the tumour volume in the control group was 106 ± 33 mm^3, versus 110 ± 40 mm^3 in the treatment group ($p = 0.824$). Significant differences in tumour volume could be calculated from day 9 of treatment. On day 15 of treatment, the tumour volume was 4251 ± 559 mm^3 ($n = 10$) in the vehicle-treated group and 3122 ± 967 mm^3 ($n = 11$) in the AGM-1470 treated group ($p = 0.004$) (Figure 1). The T:C ratio decreased gradually during the experiment, and was 0.73 on day 15 treatment (Figure 2).

Identifiable central necroses of tumours occurred on day 15 of treatment in five mice in the control group and six mice in the treatment group. Consequential shrinkage of tumour volume occurred at the terminal day (18 days after treatment) in four mice in both groups respectively, obvious central necroses occurred in most tumours as well (nine out of 10 in the control group and eight out of 11 in the treatment group). After histological analysis, central necroses were found in all tumour tissues. The shrinkage of tumour in both groups increased standard deviations which resulted in no significant difference in tumour volume at terminal day (4458 ± 1834 mm^3 in control group versus 3184 ± 1251 mm^3 in treated group, $p = 0.076$), although the T:C ratio could be calculated as 0.71 at that time.

Although shrinkage of tumours occurred at terminal day, the mean tumour weights in both groups were still significantly different: 2.8 ± 1.4 grams in the control group versus 1.9 ± 0.9 grams in AGM-1470 treated group ($p = 0.49$). There was significant correlation between the tumour weight and tumour volume at terminal day (Correlation coefficient was 0.877, $p<0.01$).

Toxicity

All animals gained body weight throughout the experiment. The net body weights of nude mice at terminal day were 20.8 ± 1.5 grams in the control group versus 20.4 ± 2.0 grams in AGM-1470 treated group ($p = 0.605$). There was no physical drug-related toxicity (i.e. diminished activity, diarrhoea, anorexia or seizure activity).

After histological analysis, no pathological change was found in the murine tissues of heart, liver, and kidney from the mice treated with AGM-1470.

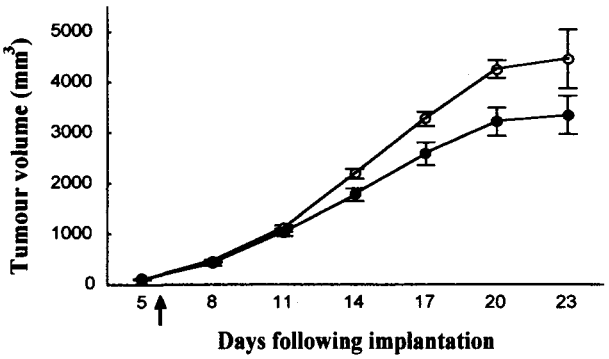

FIG. 1

Inhibition of growth of CNE-2 after administration of AGM-1470. Treatment started from Day 6 (arrow). Significant differences were seen from Day 14 to Day 20. After Day 20, necroses occurred in both groups. Points mean±SE. ○, control; ●, AGM-1470 treatment.

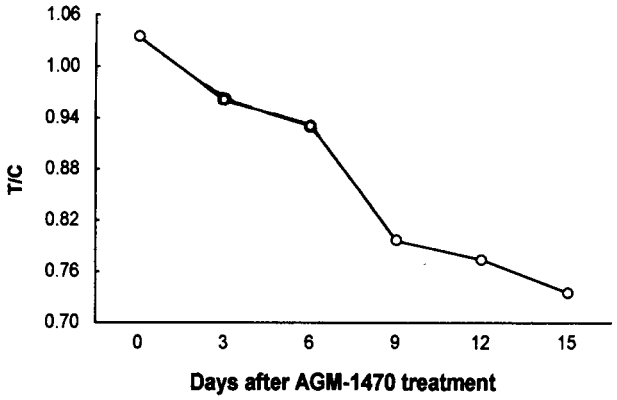

FIG. 2

Inhibition of growth of the human NPC cell line CNE-2 with administration of AGM-1470 before necroses, which was indicated by the T:C ratio. T:C was 0.73 after 15 days of treatment ($p = 0.004$).

Discussion

NPC is a common malignancy in south China. Because the nasopharynx is in a deep anatomical region, NPC is often discovered when locally advanced or already spread to lymph nodes (Fandi *et al.*, 1994). In spite of being sensitive to radiotherapy, local control failure is common in cases of advanced stage of primary tumour. Cytotoxic chemotherapy has been recommended in metastatic and/or recurrent NPC for many decades. In recent years, the study of induction chemotherapy showed a statistically significant improvement in local control and disease-free survival (Cvitkovic *et al.*, 1994). The best cure rate for metastatic NPC by intensive chemotherapy is estimated as 10 per cent (Fandi *et al.*, 1994).

In this study, we evaluated a new approach to the treatment of NPC by preventing the outgrowth of new blood vessels towards a tumour. We found that the angiogenesis inhibitor AGM-1470 had local effectiveness in the treatment of the NPC cell line CNE-2 *in vivo*, although it did not prevent tumour development. The mice treated with AGM-1470 had a 27 per cent reduction in tumour size (T:C ratio = 0.73) without significant toxicity. In this rapidly growing cell line, we also found that necroses of tumour and shrinkage of tumour volume occurred quickly in both control and treatment groups. Because of the cytostatic rather than cytotoxic character of AGM-1470, mature tumour vessels are not affected (Voest, 1996). Therefore, in theory distinct regression of tumour is unlikely following treatment by AGM-1470, which agreed with our results.

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However, there is differential sensitivity to the treatment of AGM-1470 between the different types of cancer cells (Yanase *et al.*, 1993). It has been reported that AGM-1470 was especially promising in the treatment of murine haemangioendothelioma (O'Reilly *et al.*, 1995), in which the T:C ratio 0.1. Compared with this angiomatous tumour, human oesophageal and gastric cancers did not show very high sensitivity to the treatment of AGM-1470 with 27 per cent of tumour growth inhibition (Yano *et al.*, 1995). Moreover, the efficacy of AGM-1470 is not only dose-dependent (Yamaoka *et al.*, 1993; Yanase *et al.*, 1993), but also timing-dependent. In an experiment on BALB/c mice inoculated with Renca murine tumour (Fujioka *et al.*, 1996), delayed administration of AGM-1470 on day 6 after inoculation did not result in inhibition of tumour growth, while it inhibited the tumour growth significantly when the treatment began on day 1. The timing-dependent characteristic indicated that AGM-1470 is more effective against small tumours than large tumours. To our knowledge, this is the first time AGM-1470 has been shown to be effective in the inhibition of growth of the human NPC cell line. Compared with other experiments, the relatively delayed administration of AGM-1470 in the present study may negatively influence the efficacy of the agent. More effective antitumour activity is possible by earlier administration.

The attractiveness of antiangiogenic therapy with AGM-1470 is not only because it has few side-effects (Ingber *et al.*, 1990; Brem and Folkman, 1993; Yamaoka *et al.*, 1993; Scott and Harris, 1994), that was consistent with our results in the present study, but also because some other therapeutic benefits had been reported. When combined with cytotoxic agents, it enhances the suppression of tumour growth by increasing tissue levels of certain anticancer drugs (Yamaoka *et al.*, 1993; Kato *et al.*, 1994; Teicher *et al.*, 1995). These features are promising in development of a multidisciplinary therapeutic modality including antiangiogenic therapy for treatment of local advanced, recurrent, and/or metastatic NPC. Although the exact mode of action of AGM-1470 remains to be elucidated, it widely inhibits the responses of endothelial cells to angiogenic factors (Scott and Harris, 1994). Recent studies indicate that the action of AGM-1470 on the cell cycle of endothelial cells is complex and could affect several key points (Castronovo and Belotti, 1996). Further research should be focused on the evaluation of the efficacy of the angiogenesis inhibitor on other NPC cell lines, and the efficacy of combined therapeutic modalities in NPC.

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