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AT-125 in the modulation of EAC solid tumors

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Abstract

Solid tumors, namely carcinomas, are characterized by the formation of abnormal clumps of cells. These tumors depend highly on VEGF-mediated angiogenesis to grow beyond a certain size. The hostile microenvironment of solid tumors is characterized by lack of oxygen and nutrients. Glutamine, an important nutrient of cells, is avidly consumed by almost all tumor cells, including solid tumors. Malignant cells have a high oxidative glutamine metabolism and there is direct correlation between such oxidation and the degree of malignancy. AT-125 is an analogue of glutamine which is naturally found as a fermentation product of *Strepomyces svicei*. It is known to regulate tumor growth by hindering glutamine uptake by the rapidly multiplying cells. AT-125 or acivicin, is also known to block de novo purine and pyrimidine biosynthesis in growing cells by inhibiting a number of different glutmine amidotransferases including GMP synthetase and glutamyl transpeptidase. In this study, we report the effect of AT-125 on the growth of solid tumors by Ehrlich ascites carcinoma (EAC) cells in Swiss albino mice. The reduction in tumor growth suggests important role of AT-125 in regulation of solid tumors and warrants the need for further studies in this direction in the future.

Keywords:

Solid tumor,
At-125;
glutamine;
angiogenesis.
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1. Introduction

Solid tumors, characterized by rapidly growing cells, encounter the problem of lack of oxygen and nutrients in the surrounding microenvironment after reching a certain size. Glutamine serves as a major energy source for a variety of tumors.They are therefore avid glutamine consumersand highly depend on exogeneous supply of glutamine [1], [2]. AT-125 is a fermentation product of Streptomyces svicei [3] and a glutamine analogue. It is known to exert its antineoplastic effects by inhibiting glutamine amidotransferases including GMP sythetase [4], [5], CTP synthase [4], glutamyl transpeptidase [6], formylglycine amidine ribonucleotide synthetase [7] and carbomoyl phosphate synthase [8], in mammalian and protozoan systems. AT-125, also known as acivicin, is already uder PhaseII clinical trials [9] and has shown antineoplastic affects against some murine tumors and human tumor xenografts in nude mice [10]. Recent studies have shown that the cytotoxic effects of AT-125 might be mediated by downregulation of the aldehyde dehydrogenase ALDH4A1 by siRNA [11]

Previous reports indicate that low doses of chemotherapeutics can inhibit angiogenesis [12, 13]. The effects of AT-125 on tumoral angiogenesis have not been studied so far except in EAC ascites tumor model [14]. So, keeping the above points in mind, we hypothesized that low doses of the chemotherapeutic drug, AT-125, given in a frequent schedule (metronomic dosing) might have antiangiogenic effect. To test this hypothesis, in this study, we treated EAC tumor bearing mice with doses of AT-125 which are about 20 and 160 times lower than that used in earlier studies [15]. Thus, the present study is directed to find out the

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effects of AT-125 on modulation of EAC solid tumor growth and associated angiogenesis to add a better understanding to its mode of action as an anticancer agent.

2. Research Method

2.1 Drug

AT-125 or Acivicin [(alpha S, 5S)- alpha-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid] was purchased from SIGMA Chemical Co. (Cat No. A-2295). Two doses of acivicin (0.05µg/g and 0.4µg/g body weight per day) were used. The stock solution of 50µg/ml in physiological saline was appropriately diluted to prepare the doses.

2.2 Experimental design

Thirty male Swiss Albino mice of 6-7 weeks age were used. Each mouse was injected subcutaneously with 0.2 ml saline solution containing 1×10^6 EAC cells on the right flank region. This day was taken as day 0. These EAC bearing mice were randomly sorted into the following groups with five mice per group:

- Group I- Mice received i.p. saline injections for 15 consecutive days.
- Group II-Mice received i.p. injections with acivicin 0.05µg/g body weight for 15 days.
- Group III- Mice were injected with acivicin 0.4µg acivicin per gram body weight for 15 days.
- Group IV- Mice received saline for 20 consecutive days.
- Group V- Mice received i.p. injections with acivicin 0.05µg/g body weight for 20 consecutive days.
- Group VI- Mice received acivicin 0.4µg/g body weight for 20 days.
- Group VII- Mice received saline for 25 days.
- Group VIII- Mice received i.p. injections with acivicin 0.05µg/g body weight for 25 consecutive days.
- Group IX- Mice received acivicin 0.4µg/g body weight for 25 days.

All the above-mentioned treatment injections were given intraperitoneally starting from day 1 after tumor inoculation. Mice were killed after respective time periods by anesthetic overdose. Tumor size was measured by slide calipers. Serum was also collected.

2.3 Measurement of tumor burden

Solid tumors such as EAC or B16F10 solid tumors or PA-1 tumor xenografts were measured with slide calipers and tumor volume was calculated according to the formula $V= \pi/6 \times D1 \times (D2)^2$ where V is the volume of the tumor, and D1 and D2 are the longer and shorter diameters of the tumor respectively.

2.4 Quantification of tumor-oriented blood vessels

Solid tumors were located and the number of capillaries oriented towards the tumor was counted under dissecting microscope [16].

2.5 VEGF ELISA

Serum VEGF level was measured by ELISA. Vascular endothelial growth factor was measured by using commercially available ELISA kit (Quantikine, Cat No MMV00).

2.6 Survivability

A parallel set of experiments was carried out with 5 mice per group receiving the same treatments. The numbers of surviving mice were recorded daily. Survivability was measured as: Percentage increase in life span= % ILS= [mean survival time (treated)/mean survival time (control)]-1 x 100.

Statistical analysis

The data between control and treated series were analyzed by Student's t-test. A p-value less than 0.05 was considered to be significant.

3. Results and Analysis

3.1. Tumor volume

Tumor size was found to be smaller in mice treated with the drug, moreover, the higher dose was more effective than lower dose. EAC solid tumor volume as measured by Vernier calipers was reduced by 27.65, 42.42 and 38.55% ($p<0.01$-0.001) respectively by 0.05 µg-acivicin/g body weight (TL) after 15, 20 and 25 days of treatment. As shown in Fig 1.2, the higher dose (0.4µg-acivicin/g body weight or TH) of acivicin led to 31.51, 63.63 and 76.71% reduction in tumor volume ($p<0.001$) when compared to control after treatment for 15, 20 and 25 days respectively (Figs 1& 2).

3.2. Quantification of tumor-oriented blood vessels

The number of blood vessels oriented towards EAC solid tumor was also counted to study the effect of acivicin on angiogenesis. Lesser numbers of tumor oriented blood vessels were obtained after treatment with TH than TL when compared to untreated control groups (Fig 2). Blood vessel count decreased by 13.33, 17.65 and 38.525 ($p<0.001$) with TL treatment compared to controls, while decrease was 60, 23.53 and 53.86% ($p<0.05$-0.001) with TH treatment after 15, 20 and 25 days respectively (Fig 3).

3.3. VEGF ELISA

Serum samples were assayed in triplicate and calibrated against VEGF standards. The level of VEGF in the serum of EAC tumor bearing mice was found to increase with tumor progression (Fig.4). It indicates a direct correlation between the increasing level of VEGF and the extent of induced angiogenic response. Acivicin was found to regulate VEGF secretion by tumor cells. The serum VEGF concentration was significantly reduced in high dose drug treated group compared to control after 25 days of consecutive treatment.

3.4. Survivability

A maximum ILS of about 40.5% was obtained after treating solid tumor bearing mice with 0.4µg acivicin/g body weight/day for 25 days (Table 1). Survivability of treated EAC tumor bearing mice was significantly higher ($p<0.0.01$) than control tumor bearing mice. However, the difference in %ILS was not significant between the two doses of acivicin in both the types of tumors.

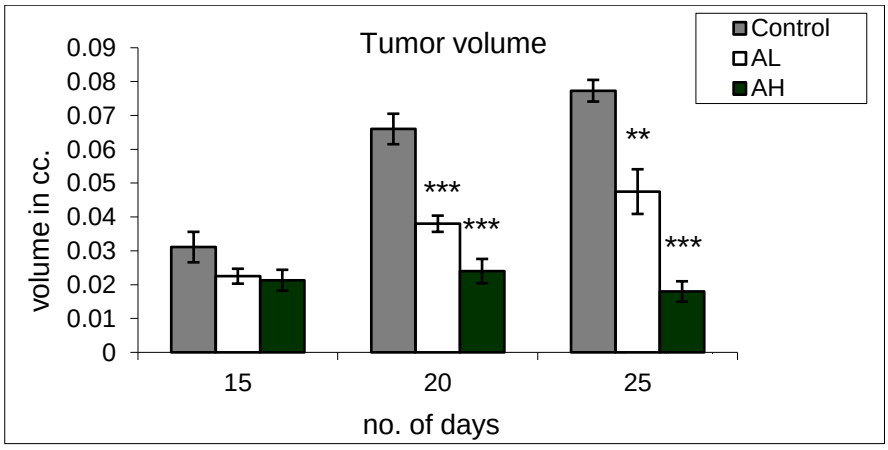

Fig 1: Effect of acivicin treatment on EAC solid tumor growth. The volume of solid tumor in EAC tumor bearing mice in control and acivicin treated groups after 15, 20 and 25 days (mean ± SE, n=5); Here, TL = 0.05 µg-acivicin/g body weight; TH = 0.4µg-acivicin/g body weight per day. **$p<0.01$ and ***$p<0.001$, when treated groups were compared with controls.

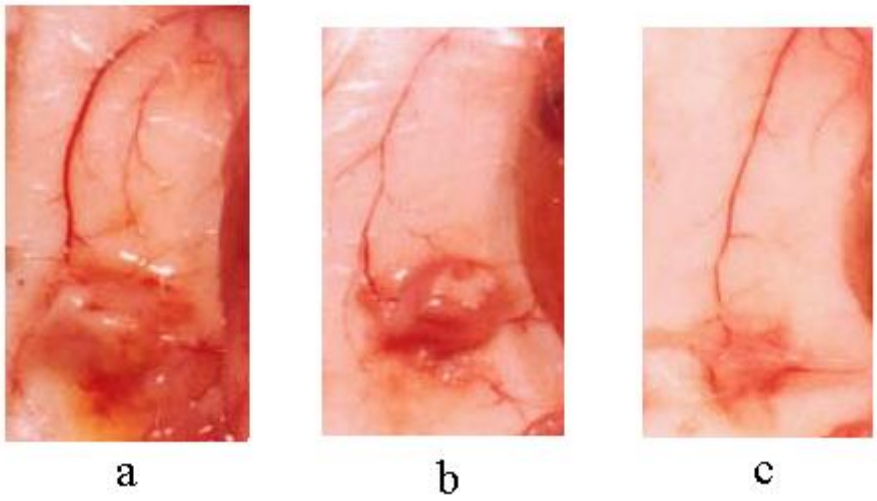

Fig 2: Effect of acivicin on tumor size and tumor induced angiogenesis in EAC solid tumor after 25 days of treatment. a–tumor bearing control; b– injected with acivicin 0.05µg/g body weight/day; c– injected with acivicin 0.4µg/g body weight per day.

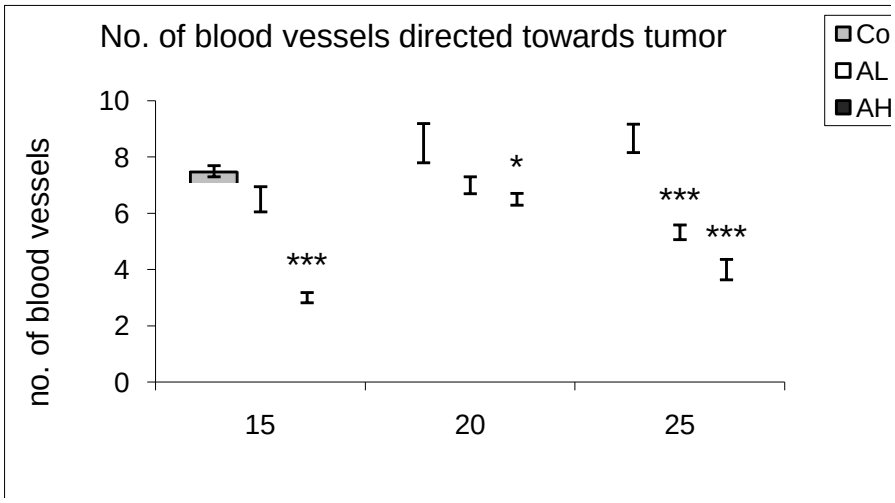

Fig 3: Effect of acivicin on blood vasculature count in EAC tumor. In solid tumor, the number of vessels oriented towards the tumor are shown (mean ± SE, n=5); TL: injected with acivicin 0.05µg/g body weight/day; TH: injected with acivicin 0.4µg/g body weight per day. *p<0.05, **p<0.01 & ***p<0.001, when treated groups were compared with their respective control groups.

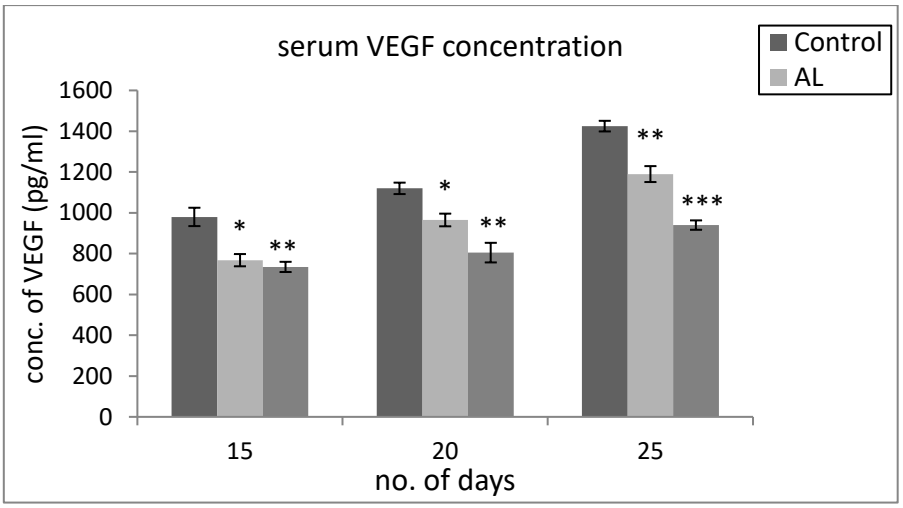

Fig 4: Effect of VEGF concentration in serum of EAC solid tumor bearing mice (mean ± SE, n=5); TL: injected with acivicin 0.05µg/g body weight/day; TH: injected with acivicin 0.4µg/g body weight per day. *p<0.05, **p<0.01 & ***p<0.001, when treated groups were compared with their respective control groups.

Table 1: Survivability of EAC tumor bearing mice with and without AT-125 treatment

	Group	Mean survival time in days ±SE	% ILS
After 25 days of treatment	Control (tumor bearing)	37 ± 0.05	-
	TL	52 ± 0.43	37.84
	TH	51 ± 0.27	40.54

4. Conclusion

With the earlier information on the antineoplastic properties of AT-125 or acivicin, the present chapter was focused to find out whether antiangiogenic effect can be obtained by metronomic scheduling using much lower doses of the drug. At the same time it was equally necessary to observe the effects on tumor volume and life span of the host.

The results obtained in the present study for the first time showed that low doses of AT-125 significantly reduced tumor volume when injected daily into EAC tumor bearing mice. Solid tumor growth depends on the ready availability of glutamine and acivicin works by exploiting this event. This analogue of glutamine hinders the formation of nucleotides in the rapidly growing tumor cells by preventing the utilization of glutamine by the amidotransferases and implicates an enzyme involved in pyrimidine biosynthesis, CTP synthase as a possible crucial target of acivicin-mediated inhibition [17]. Moreover, the survivability of acivicin treated mice was found to be higher than untreated tumor bearing hosts.

Induction and maintenance of angiogenesis requires interaction of many growth factors with their respective receptors, which then activate endothelial cells. Vascular endothelial growth factor (VEGF) is one such factor and is abundantly expressed and secreted by most human and animal tumors examined [18], [19]. This particular cytokine is very important for cancer progression because it plays crucial role in angiogenesis besides helping in tumor growth VEGF level is reported to increase in serum of EAC solid tumor bearing mice with days [20]. In the present study, AT-125 reduced number of blood vessels feeding the tumors.

The results obtained in the present study indicate that tumor induced angiogenesis is inhibited by acivicin. The degree of tumoral angiogenesis was assessed by counting the peritoneal blood vessels and measuring the VEGF level. Moreover, the rate of tumor angiogenesis was correlated with tumor volumetric growth rate. Acivicin decreased serum VEGF levels in treated groups of mice and this correlated with the reduced angiogenesis. However, the indications of the antiangiogenic effects of acivicin in tumors as evident in the studies of this chapter need to be confirmed through further experimentations.

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