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ORIGINAL ARTICLE

Effect of TNP-470 (AGM-1470) on the Growth of Rat Rhabdomyosarcoma Tumors of Different Sizes

Willy Landuyt, Sr.Sc.,[1] Jan Theys, Ir.,[4] Sandra Nuyts, M.D.,[1] Maria Drijkoningen, M.D., Ph.D.,[3] Jack Fowler, Ph.D., D.Sc.,[1] Alex Reijnders, Ing.,[2] Sandra Liekens, B.Sc.,[5] Johan Neyts, Ph.D.,[5] Ernst de Bruijn, Ph.D.,[2] Jozef Anné, Ir., Ph.D.,[4] and Philippe Lambin, M.D., Ph.D.[1]

[1]*Experimental Radiobiology/*[2]*Oncology,* [3]*Histopathology, University Hospital, K.U. Leuven,* [4]*Experimental Bacteriology, and* [5]*Virology and Experimental Chemotherapy, Rega Institute, K.U. Leuven, Belgium*

ABSTRACT

Potential anticancer therapy with the fumagillin analog TNP-470 was investigated in the present project using subcutaneously growing rhabdomyosarcomas in rats. Specifically, influences of different tumor sizes at the start of treatment as well as dose/schedules were evaluated with this angiogenesis inhibitor. The results show a significant ($p = \leq 0.01$) reduction of the growth rate, even for relatively large-sized (>7 cm^3) tumors, when 50 mg/kg TNP-470 was used every other day for up to 3 or 5 injections. With 30 mg/kg TNP-470 injections, effects were seen only with tumors measuring <7 cm^3. The histologic examinations demonstrate an increase in necrosis, both in the center and in the peripheral part of TNP-470-treated tumors. Overall, both tumor volume and drug dose determine treatment outcome with the rat rhabdomyosarcoma. The results suggest that angiogenesis inhibitors could represent

Corresponding author: W. Landuyt, Experimental Radiobiology, Departments of Radiotherapy/Oncology, University Hospital Gasthuisberg, K.U. Leuven, Herestraat 49, B-3000 Leuven, Belgium. Fax: (32)16-34.69.01; E-mail: Willy.Landuyt@rega.kuleuven.ac.be

a valid component in the treatment of progressive tumor growth, also of large tumors as often encountered in clinics. The antivasculature therapy might also improve hypoxia/necrosis–related therapeutic approaches.

INTRODUCTION

To support cell survival and growth, a tumor relies on neovascularization and thus on endothelial cell proliferation (1–3). The consequent effect of angiogenesis on tumor growth and the establishment of metastases is well documented. During the last two decades much attention has focused on the development of antiangiogenic compounds. One such inhibitor of angiogenesis is the fumagillin analog TNP-470 (AGM-1470). The compound has shown in vivo antiangiogenic effects in various tumor models (4–8) and is currently under investigation in clinical trials for the treatment of a variety of solid tumors. Although the full mechanism of the antiangiogenic activity has not yet been clarified, important inhibition of proliferation and migration of endothelial cells and of capillary tube formation has been observed (9,10).

The present report describes the antitumor effect of TNP-470 on the syngeneic rhabdomyosarcoma in WAG/Rij rats. The subcutaneously transplanted tumor shows a regular growth pattern without the development of metastasis (11,12). Our study specifically focuses on the relation between dosing/scheduling of TNP-470 and the size of the tumor at the start of the treatments. Tumor growth delay and histological appearance as well as the profile of the major side effects were assessed.

MATERIAL AND METHODS

Male WAG/Rij rats, aged 12–14 weeks and weighing about 280 g were bred in our conventional animal housing facility. The rats had free access to water and food during the total experimental period. The syngeneic rat rhabdomyosarcoma was implanted subcutaneously (s.c.) in the lower flank of anaesthetized animals using tumor pieces of about 1 mm^3. This experimental tumor model has been firmly established in radiobiology research and described thoroughly in a vast number of publications (11–14). Briefly, the rhabdomyosarcoma originated in the jaw musculature of inbred WAG/Rij rats that received total body irradiations. From this irradiation-induced tumor, a cell line was established using the classical procedures, giving reproducibly growing tumors in WAG/Rij rats.

The TNP-470 was received from Takeda Chemical Industries (Japan). For each experiment, the TNP-470 solution was freshly prepared in 10% ethanol and 5% arabic gum in saline. Control rats were treated with the vehicle only. Volumes for injection varied between 1 and 1.5 ml (depending on the drug concentration and animal weight). Animals were injected s.c. in the neck region distant from the tumor. Several TNP-470 administration schedules and dosages were evaluated. Either 30 mg/kg or 50 mg/kg were given 3 or 5 times, with 2-day intervals between the successive injections. In the case of the 30 mg/kg dose, the effect of a single TNP-470 treatment was also evaluated.

Treatments started when tumors reached the predetermined volume, which were <3 cm^3, 3–7 cm^3, and >7 cm^3 and referred to as small, medium or large, respectively. Measurements of the tumor volumes were regularly performed with a calliper. The volume (V) was calculated according to the standard procedure for ellipsoid growth:

$$V = (a \times b \times c) \times \pi/6 \quad (a = \text{length};\ b = \text{width};\ c = \text{thickness});\ \text{expressed in } cm^3.$$

To enable an easy display of the antitumor effect, the curves representing the measurements of TNP-470 treated tumors were normalized with respect to the control (vehicle-only treatment) tumors. Starting volumes of the various TNP-470 groups thus superimpose the control growth curve at the equivalent volume. This is indicated in all graphs by an arrow. Student's t-test was used to estimate significance from the comparison of the tumor growth curves.

The histological characteristics of TNP-470 treated rhabdomyosarcomas were examined and compared with tumor sections of vehicle-only treated rats. The pieces were selected from both the center and the periphery of the tumor separately. They were fixed in Bouin followed by a 10% formalin solution and processed using the routine histological techniques. Hematoxylin-eosin (HE) and periodic acid (PAS) stained sections were evaluated by light microscopy.

The experimental procedures were approved by the University of Leuven Animal Care and Use Committee in compliance with national guidelines.

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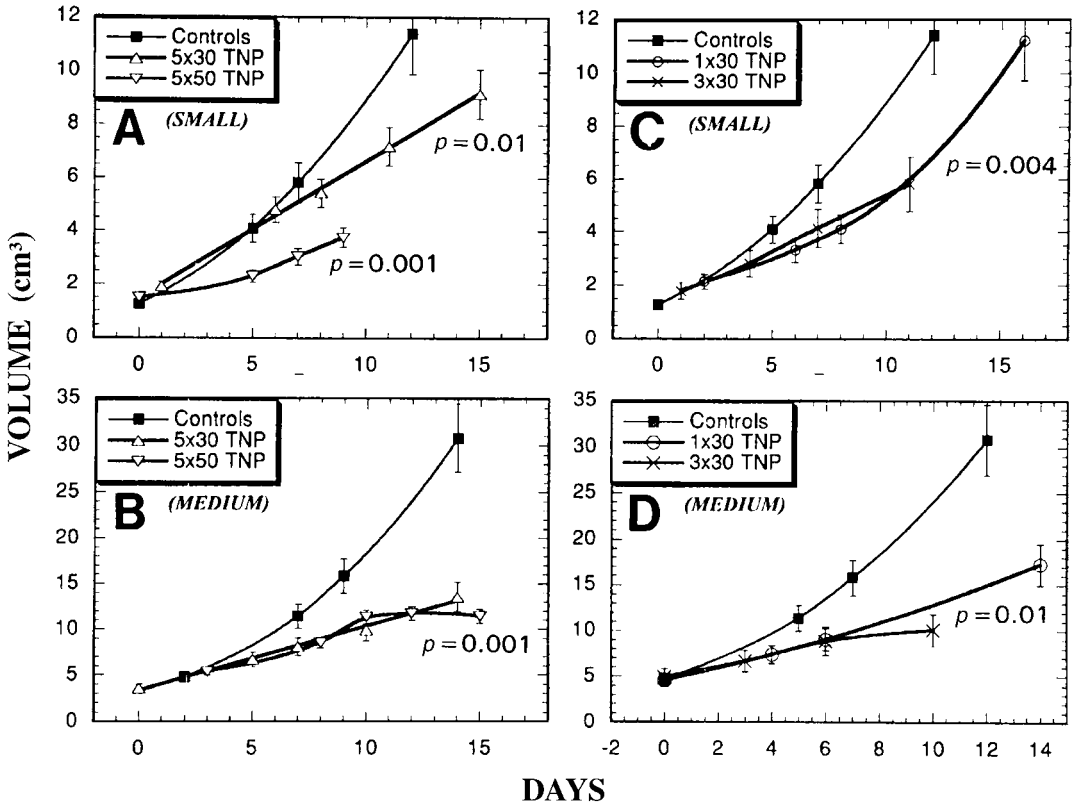

Figure 1. Growth of vehicle (controls) and of TNP-470 treated tumors. Panels A and C represent the results for small (<3 cm^3) tumors; panels B and D represent the results for medium (3–7 cm^3) tumors; vertical bars represent standard errors.

RESULTS

The effect of TNP-470 on the growth of the s.c. transplanted rhabdomyosarcoma was evaluated at different tumor starting sizes and drug doses. The small (<3 cm^3), medium (3–7 cm^3), and large (>7 cm^3) sized tumors showed different responses, with TNP-470 either slowing or even inhibiting tumor progression.

With small tumors the effect of the 5 × 50 mg/kg TNP-470 treatments was significantly more prominent than for the 5 × 30 mg/kg dosing (Figure 1A). However, the growth delay was similar for both treatments with the medium-sized tumors (Figure 1B). The growth inhibitory effect of the 5 × 50 mg/kg treatment was also demonstrated with rats bearing tumors >7 cm^3 (Figure 2A).

The effect on tumor growth from 3 × 30 mg/kg was comparable to the delay measured with the larger TNP-470 dosage (see above). These results for both small- and medium-sized tumors are displayed in Figures 1C and 1D and are to be compared with the data in Figures 1A and 1B. The growth inhibitory effect of 3 × 30 mg/kg drug was not significant when tumors >7cm^3 were treated (Figure 2B).

In a final set of experiments we studied the use of a single TNP-470 treatment of 30 mg/kg. As can be deduced from Figure 1C and 1D, a clear-cut reduction of tumor growth was already obtained with this single TNP-470 treatment for rat rhabdomyosarcomas with volumes <7 cm^3.

On histologic examination, the nontreated rhabdomyosarcomas are very cellular. They consist of spindle cells, surrounding somewhat dilated vascular spaces. The cells show obvious atypia; mitoses are numerous. Necrosis is sparsely present in the periphery of the tumors, but is more pronounced in the center. With increase in tumor volume, necrotic areas become relatively larger, especially in the central part of very large tumors. After treatment with the repeated doses of TNP-470, necrosis is much more prominent in the medium and large tumors both in the central as well as in the peripheral areas. This is

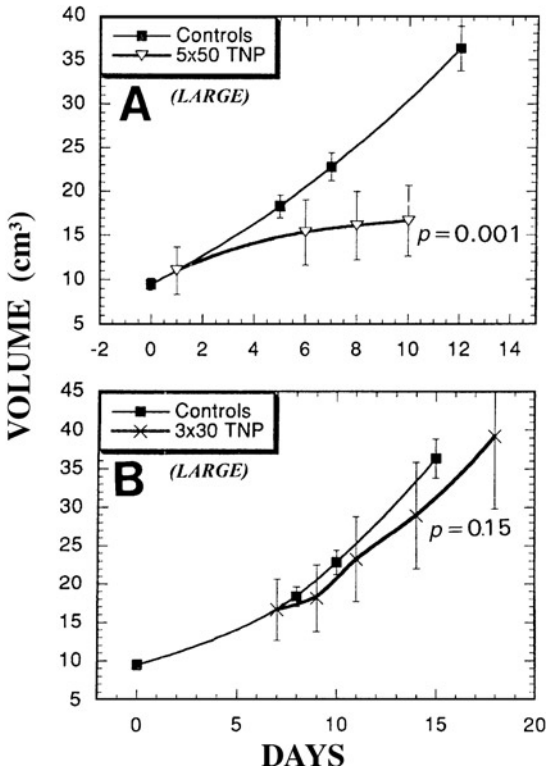

Figure 2. Growth of vehicle (controls) and of TNP-470 treated large (>7 cm^3) tumors. Vertical bars represent standard errors.

illustrated in Figure 3 for a TNP-470 treatment of 3 × 30 mg/kg only, and is representative for the other schedules. In the small tumors, this effect is also present, but less pronounced.

However, with most of these TNP-470 treatments toxicities were also observed. Overall, these were more severe with the 5 × 50 mg/kg than with the 5 × 30 mg/kg dosage and consisted of severe local skin damage at the site of the s.c. injection and of body weight loss. The loss in body weight occurred from 2 days after the first injection and was between 10% and 30% for both dosages, being progressive throughout the observation period. Lowering the total TNP-470 doses to 3 × 30 mg/kg did not fully resolve the problem of both skin and body weight effects. However, in these series, most of the animals started to recover their loss in body weight before the end of the follow-up period. The vehicle-treated tumor-bearing rats always showed an increase in body weight of 4–7% for the same time period as the TNP-470 series. The skin damage was a local ulceration present during the series of s.c. injections in all drug experiments, with healing thereafter.

DISCUSSION

Inhibition of tumor angiogenesis has been recognized as a potential adjuvant in the anticancer treatment arsenal. The fumagillin analog TNP-470 (AGM-1470) has retained special attention because of its strong inhibition of proliferation and migration of endothelial cells in tumor systems. This endotheliostatic effect was reflected in various laboratory in vivo biological models by a reduced growth rate of the primary tumor or a decreased formation of metastasis (4–6,8,9). So far, however, data on the effect of TNP-470 in rodent tumors are only available for very small primary tumors (<1 cm^3).

Our results demonstrate that TNP-470 is also an active modulator of rhabdomyosarcoma growth, even for relatively large-sized tumors. Indeed, a substantial reduction of growth was measured with tumor volumes between 1 and 7 cm^3 at the start of the TNP-470 treatments. These changes in growth rate were seen with all the drug-fractionation schedules tested. With the large tumors (>7 cm^3), however, no significant effect was observed with the reduced TNP-470 dosage. This could indicate a threshold volume-dose-response relation with this tumor type. The overall examination of the stained tumor slices revealed the enlargement of necrotic areas in TNP-470 as compared with vehicle-treated tumors. There is ample literature information on the effect of TNP-470 on the endothelium and the inhibition of angiogenesis (9,10,15). These published observations grossly explain the presence of a decreased growth rate and enlarged necrosis seen in the present rhabdomyosarcomas treated with TNP-470. Interestingly, even a single TNP-470 dose of 30 mg/kg slowed the tumor growth. The latter effect is important to quote, as it is measured in rats with a firmly developed tumor (up to 7 cm^3). Using this single treatment, no growth delay was obtained with larger tumors (data not shown).

The reductions in growth capacity observed in the rat rhabdomyosarcoma following TNP-470 treatments seem consistent with the hypothesis that rapidly proliferating tumors are more angiogenesis dependent (16). Cell kinetic measurements with the rhabdomyosarcoma indeed showed a short cell cycle time of about 20 hours (13). Also, from our tumor growth curve for rats injected with the vehicle only, a volume doubling time of 4 to 6 days can be deduced. These rapid tumor growth characteristics could partly explain the TNP-470 effect on the evolution of s.c. implanted tumors whatever the size at the start of treatment.

A recently published clinical phase I study cited effectiveness of TNP-470 in large tumors as also clearly demonstrated in our study with rat rhabdomyosarcomas.

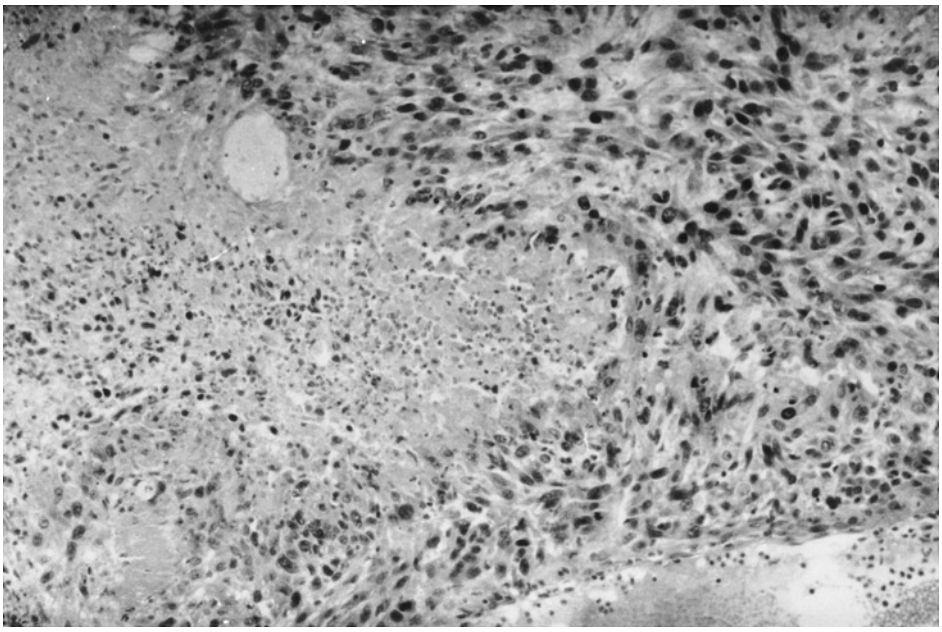

Figure 3. Representative light microscopy image of rhabdomyosarcoma treated with TNP-470 (3 × 30mg/kg i.p. injections). Large necrotic areas are intermingled with remaining areas of viable tumor cells. Magnification ×360.

Notwithstanding the small number of patients, it was shown that repeated TNP-470 administration affected pulmonary cervix carcinoma metastases with a volume of about 14 cm^3 (17). This observation coincides with the results obtained from our investigations. Such data further invite the use of compounds that specifically attack the neovasculature, not only in small but certainly also in large-sized tumors using appropriate dose schedules. Moreover, the presence of necrosis and vascular damage inherently parallels increased hypoxic tumor cell fractions. This could provide a basis for combination therapy with hypoxia targeting drugs to improve tumor control.

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