Cancer Chemother Pharmacol (1996) 39: 113–121

ORIGINAL ARTICLE

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Effect of angiotensin II on immunotoxin uptake in tumor and normal tissue

Received: 31 October 1995 / Accepted: 25 March 1996

Abstract *Purpose*: To investigate the effect of the sarcosine analog of human angiotensin II ({sar}ATII) on the uptake and spatial distribution of immunotoxins (MW 210000 Da) in RD rhabdomyosarcoma xenografts in mice. This analog has a pressor activity similar to native angiotensin II (ATII) but a longer duration of action. *Method*: A period of elevated blood pressure of approximately 80 min, measured by noninvasive photoplethysmography, was achieved by a 40-min continuous i.p. infusion of {sar}ATII at 0.07 μg/min. Tumor-bearing animals were injected i.v. with ^{125}I-labeled specific and ^{131}I-labeled nonspecific immunotoxins and made hypertensive by i.p. infusion of {sar}ATII. Radioactivity was measured in plasma, tumor, liver, kidney and muscle at 2, 6 and 24 h. Plasma radioactivity was subtracted from tissue values to calculate tissue uptake. To assess the spatial distribution of immunotoxin in the solid tumor, ^{125}I-labeled specific immunotoxin was injected i.v. into tumor-bearing animals, and quantitative autoradiography was performed on tumor sections. *Results*: The uptake of specific or nonspecific immunotoxins in tumor and normal tissues was not significantly different in {sar}ATII-hypertensive animals compared with saline-treated controls. In control animals, the spatial distribution of ^{125}I-labeled specific immunotoxins was very heterogeneous and contained punctate accumulations throughout the tumor. Treatment with {sar}ATII did not affect this distribution qualitatively or quantitatively. To examine a possible reason for the lack of {sar}ATII effect, we measured the interstitial pressure of the RD tumor using a fluid-filled micropipette connected to a servo-null pressure transducer. The interstitial pressure in this solid tumor was unexpectedly low, only 0.6 ± 0.9 mm Hg. *Conclusions*: The sustained period of {sar}ATII-induced hypertension had no effect on RD tumor or normal tissue uptake or tumor spatial distribution of immunotoxin. In saline-treated controls, the heterogeneity of immunotoxin distribution does not arise from an elevated interstitial pressure. Further studies are needed to determine whether a correlation exists between responsiveness to ATII-induced hypertensive chemotherapy using macromolecular drugs and tumor type and/or physiological properties.

Key words Immunotoxin · Angiotensin II · Biodistribution

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Introduction

The vasoactive drug angiotensin II (ATII) stimulates arteriolar constriction. When given systemically, it produces a widespread increase in peripheral vascular resistance and alters kidney excretion of salt and water through an aldosterone feedback loop. These effects lead to an increase in blood pressure [9]. Suzuki et al. discovered that during ATII-induced hypertension, blood flow in a variety of hepatic tumors is increased while in normal tissues, it is decreased or unchanged [32, 34]. The mechanisms that exist for normal vessels to regulate blood flow appear to be absent or incompletely developed in the tumor vasculature [11]. Several different research groups have demonstrated that such a phenomenon could be exploited to increase tumor uptake of a number of chemotherapeutic drugs and model markers [1, 7, 33] or to reduce the toxicity in normal tissues [18]. Further, it has been shown that ATII can enhance the effectiveness of mitomycin C in an animal tumor model [32]. The concept has been tested clinically in Japan where ATII-induced hypertension has been shown to yield a significantly higher response rate in the treatment of gastric carcinoma with combination chemotherapy consisting of adriamycin, 5-fluorouracil and mitomycin C [28]. Jirtle, on the other hand, has found that ATII decreases blood flow in both a mammary carcinoma and the surrounding normal mammary tissue, but since the decrease is

greater in normal tissue, the tumor/normal tissue blood flow ratio is nonetheless increased [14]. Tozer and Shaffi have observed a similar result in a subcutaneous carcinosarcoma tumor [36].

Immunotoxins are macromolecular drugs comprised of an antibody (or binding ligand) linked to a protein toxin. Tumor cell-specific killing can be achieved by the use of an antibody that binds preferentially to tumor cells and allows internalization of the toxin [5]. A major obstacle to the successful application of antibodies and immunotoxins in cancer therapy is their low accumulation and poor penetration into solid tumors [15, 21, 22, 24, 31]. Use of certain microvascular-permeability-enhancing compounds has been found to increase tumor uptake of antibodies [16, 27]. Since tissue uptake of macromolecules such as immunotoxins is proportional to the product of capillary permeability and capillary surface area [25], another potential strategy to increase the delivery of these molecules is to increase capillary surface area. Visualization of the capillary bed of a hepatoma grown in a sandwich chamber has shown that ATII dramatically increases the number of perfused vessels [10]. Moreover, Trotter et al. have shown that ATII reduces the fraction of tumor vessels that experience periods of stasis [37]. We had developed an animal model to study the tumor uptake and spatial distribution of immunotoxins. Using this model, we have found that the tumor distribution of tumor-specific immunotoxins is spatially very heterogeneous [31]. The reported effects of ATII in increasing the number of perfused tumor capillaries motivated us to use our model to examine whether ATII-induced hypertension could increase the uptake of immunotoxins and/or distribute them more uniformly within a solid tumor.

In this research, we administered human ATII (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) or {sar}ATII (sarcosine replaces aspartic acid) to mice by intraperitoneal (i.p.) infusion to achieve an elevation in blood pressure. Blood pressure was monitored noninvasively by photoplethysmography recordings from a tail cuff. The sarcosine analog gave us more consistent and prolonged elevation of blood pressure and was used in all the uptake studies. Tumor-bearing animals were injected intravenously (i.v.) with ^{125}I-labeled tumor-specific and ^{131}I-labeled nonspecific immunotoxins and then made hypertensive by i.p. infusion of {sar}ATII. At various times after injection, plasma and tissues were sampled for radioactivity. These animals were compared with saline-treated controls. In another set of experiments, quantitative autoradiography was performed on tumor sections from {sar}ATII- and saline-treated animals that had been injected with ^{125}I-labeled tumor-specific immunotoxin in order to assess the effect of {sar}ATII on the spatial distribution of the immunotoxin within the tumor.

Materials and methods

Materials

Human ATII was obtained from CalBiochem (La Jolla, Calif.), and {sar}ATII was obtained from Peninsula Laboratories (Belmont, Calif.). 454A12, an IgG_1 murine monoclonal antibody recognizing the human transferrin receptor, was generously provided by Lou Houston. MOPC21, an IgG_1 murine monoclonal antibody that has no known binding specificity, was obtained from Sigma Chemical Co. (St. Louis, Mo.). It was passed through a Sephadex-G25 M Column PD-10 (Pharmacia-LKB, Piscataway, N.J.) to remove sodium azide prior to use in any experiments. The immunotoxins 454A12-107 and MOPC21-107 are chemical conjugates of the monoclonal antibodies and a mutant form of diphtheria toxin linked via a thioether bond [29]. They were purified on a size-exclusion column, and the fractions corresponding to the one-to-one conjugate (MW 210 000 Da) were used in these studies. The antibody and the immunotoxins used in the biodistribution study were iodinated with ^{125}I or ^{131}I (ICN Radiochemicals, Irvine, Calif.) by means of immobilized lactoperoxidase (Bio-Rad, Richmond, Calif.). For the autoradiography study, 454A12-107 was radiolabeled with ^{125}I Bolton-Hunter reagent (NEN, Boston, Mass.). The radioiodinated proteins were purified on a Sephadex G-25 M column PD-10 and further dialyzed overnight prior to use. Radioactivity was $>90\%$ precipitable in TCA; the immunoreactive fraction [20] of the specific immunotoxin ranged from 0.4 to 0.5. The proteins were used within 3 days of radiolabeling.

Animal model

Athymic female mice (NIH Nu/Nu) 6 to 8 weeks old weighing 18 to 25 g were used in these experiments. They received a flank injection of 10^7 human rhabdomyosarcoma RD cells (ATCC, Rockville, Md.). The cells were obtained from confluent monolayers grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum. These cells express approximately 60 000 transferrin receptors/cell [29]. The animals were used in the experiments 2–3 weeks later. Median and mean tumor weights were 0.3 g and 0.5 g, respectively (range 0.04–1.6 g). The animals were divided so that test and control groups contained approximately the same distribution of tumor weights. Further details of this animal model are given in reference 29.

Blood pressure measurements

Because of the technical difficulty of placing an intraarterial pressure transducer in a mouse, we used a noninvasive photoplethysmography detection system to monitor blood pressure (Columbus Instruments, Columbus, Ohio). A mouse's tail is placed into an inflatable pressure cuff which contains a light emitting diode and a photodetector. Pulsations in blood flow through the tail artery bias the detector. The signals are sent to a signal filter, amplifier, and a chart recorder (Gould Instruments, Valley View, Ohio). The blood pressure is determined from simultaneous chart recordings of the pulse and cuff pressure. Because movement in an awake animal creates signal artifact which obscures the pulse signal, the animals were anesthetized with i.p. sodium pentobarbital (0.3 ml at 0.1 mg/ml). The animal's body temperature was supported with a 37 °C heating blanket (Harvard Instruments Homeothermic Blanket, South Natick, Mass.).

Blood pressure response to angiotensin II infusions

The blood pressure was monitored every 3 to 5 min in nontumor-bearing anesthetized animals as a function of time after initiating an i.p. infusion of ATII or {sar}ATII (0.044 mg/ml). Infusion rates ranged from 1.2 to 2.0 μl/min. Blood pressure measurements were also made after infusions were stopped. Control animals received infusions of saline over the same range of flow rates. Because sodium pentobarbital lowers vascular resistance and hence blood pressure, we sought to confirm that ATII or {sar}ATII could produce an elevation in blood pressure in pentobarbital-anesthetized animals. It is noteworthy that experiments done by Suzuki et al. [32–34] and Hori et al. [10–12], which showed that blood flow and capillary surface area in tumors increase following ATII infusion, used pentobarbital as the anesthetic agent. We therefore supposed that pentobarbital would not counteract the effects of ATII, but as an added precaution, used in our analyses

Table 1 Organ blood space in mice

Organ	Angiotensin IL-treated (%, mean ± SD, n = 8)	Saline-treated control (%, mean ± SD, n = 4)
Tumor	1.33 ± 0.15	1.65 ± 0.24
Muscle	1.12 ± 0.17	0.95 ± 0.58
Liver	27.7 ± 4.5	32.5 ± 7.1
Kidney	14.4 ± 4.1	14.3 ± 3.0

only animals that experienced an elevation in blood pressure following ATII or {sar}ATII infusion.

Organ blood space measurements

Organ blood space was determined using ^{125}I-labeled MOPC21 immunoglobulin as a plasma volume marker. Measurements were made in animals receiving an infusion of ATII or saline. The tumor-bearing animals were anesthetized as described above, and several baseline blood pressure measurements were recorded. An i.p. infusion of ATII at 0.044 mg/ml was administered at a rate of 1.6 µl/min with a Harvard Infusion Pump (Harvard Instruments), and blood pressure measurements were taken every few minutes. By 10 min, if the blood pressure had not increased by 35 mm Hg, the infusion rate was increased, but never exceeded 2.0 µl/min. When the blood pressure reached 35 to 45 mm Hg above the baseline level, the animal was injected with 0.1–0.6 MBq of ^{125}I-labeled MOPC21 (0.5 Mbq/µg, 2.2 MBq/ml). Two samples of blood were obtained 1 min later from the tail vein, one for hematocrit determination, the other for counting radioactivity (LKB Clinigamma Counter 1272, Pharmacia-LKB Nuclear, Gaithersburg, Md.). The animal was sacrificed and tumor, muscle, liver and kidney samples were obtained. All samples were weighed and counted. Blood samples were also counted after precipitation in trichloroacetic acid. The radioactivity of the injectate and all blood samples was >95% precipitable. The blood space was calculated by dividing the radioactivity per gram of tissue by the radioactivity per gram of blood. Control animals received infusions of saline.

Biodistribution experiments

Tumor-bearing animals were anesthetized as described above and several blood pressure measurements were obtained. From the above experiments, we found that after starting infusion of {sar}ATII, injecting substances into the tail vein became more difficult. Because {sar}ATII elevates blood pressure within 5 min of injection, whereas the plasma clearance of the immunotoxin is slow ($t_{1/2\alpha}$=45 min, $t_{1/2\beta}$=13 h), if the immunotoxin is injected before the start of {sar}ATII infusion, little immunotoxin will be cleared before the blood pressure becomes elevated. Thus, in the following procedure, the animals were first injected in the tail vein with 0.2 ml of a solution containing 0.125 pmol ^{125}I-454A12-107 and 0.125 pmol ^{131}I-MOPC21-107 immunotoxins. Immediately after injection, an i.p. infusion of {sar}ATII (0.044 mg/ml) or saline at 1.6 µl/min was started. Blood samples were obtained 2 min after injection for hematocrit and radioactivity measurements (before and after precipitation in 25% trichloroacetic acid). The blood pressure was measured again 30 min later and its change from the initial blood pressure was recorded. The infusion was continued for a total of 40 min. Blood samples were obtained from the tail vein 2, 6, and 24 h after injection of the immunotoxins and the animal was sacrificed. Tumor, muscle, liver and kidney samples were obtained. All tissues were weighed and measured for radioactivity content. The counts were corrected for background and crossover, and the ^{131}I counts were also adjusted for decay. The average interstitial concentration of the immunotoxins was calculated by subtracting the contribution of the radioactivity in the blood (Table 1) and dividing by the average interstitial volume fraction of the organ [13, 29]. Results are expressed as a percentage of the initial (2 min) plasma concentration.

Quantitative autoradiography experiments

The method used was similar to the biodistribution experiments except that only one immunotoxin, ^{125}I-454A12-107 (0.125 pmol) was injected. A blood sample was obtained 2, 6, and 24 h after injection of the immunotoxin, and the animal was sacrificed. The animals were perfused with 10 ml heparin solution (100 U/ml phosphate-buffered saline, PBS) followed by 5–10 ml 10% formalin at 3 ml/min from a 25 gauge butterfly needle placed in the heart. This procedure was done in order to clear the radioactivity from the vasculature. The tumor was then removed and cut approximately in half. Both pieces were weighed. One piece was placed in a test-tube containing 10% formalin, and the radioactivity was counted. The other piece was coated with Tissue Tek embedding medium (Miles Laboratories, Elkhart, Ind.) and frozen by submersion in isopentane cooled in dry ice. The tumors were stored at –20 °C. They were then sectioned into 20-µm thick sections on a cryotome (Zeiss, Thornwood, N.Y.) at –20 °C and transferred to gelatin-coated slides. The slides were placed in light-tight cassettes with ^{125}I standards (Amersham, Arlington Heights, Ill.) and covered with Hyperfilm ^{3}H Film (Amersham). The cassettes were stored in a desiccator at room temperature. The film was developed in GBX developer (Kodak, Rochester, NY) 2–3 months later. The sections were then stained with Gill's hematoxylin (Polysciences, Warrington, Pa.). Film images were digitized, quantitated and analyzed as described previously [31].

Interstitial tumor pressure measurements

Interstitial tumor pressure measurements were made by a servo-null method similar to one described by Boucher et al. [3]. The servo-null instrumentation was the 900A Micropressure System (World Precision Instruments, Sarasota, Fl.). The system uses an electrolyte-filled micropipette with a tip diameter of 2–5 µm. The micropipettes were made with a vertical pipette puller (Model 720, Kopf Instruments, Tujunga, Calif.) and were bevelled on an air-controlled, rotating grinding stone. Tumor-bearing mice were anesthetized with i.p. sodium pentobarbital as described above and placed on a heating blanket. Tumor diameters ranged from 1 to 2 cm. The animal and heating blanket were then placed on an adjustable stage to permit positioning of the animal near the micropipette tip which was mounted in a micromanipulator. With the aid of an operating microscope (Zeiss), a small nick in the skin surface over the tumor was made with a 30-gauge hypodermic needle at the position where the micropipette was to be inserted. This reduced the likelihood of micropipette breakage upon insertion into the tumor. A small drop of PBS was placed on the nick, and the micropipette was advanced into the drop approximately perpendicular to the face of the tumor to a point just above the surface of the skin. The pressure measured at this position was used as the reference pressure. The micropipette then was advanced slowly approximately 4 mm into the tumor and then gently moved back and forth 0.1 mm to dislodge any tissue that may have adhered to the tip during entry. The micropipette was also flushed using positive pressure to unclog the tip of any tissue. Pressure measurements were then taken at this position and at various positions as the micropipette was withdrawn from the tumor.

Results

Blood pressure response to angiotensin II infusions

An example of the signal output from the photoplethysmograph is shown in Fig. 1A, B. The upper strip is a record of pulsations through the tail artery, the lower strip is a record of the cuff pressure. When the cuff pressure exceeded the blood pressure, pulsations were not detectable. As the cuff pressure was released and dropped below the blood pressure, pulsations were recorded. The point at which pulsa-

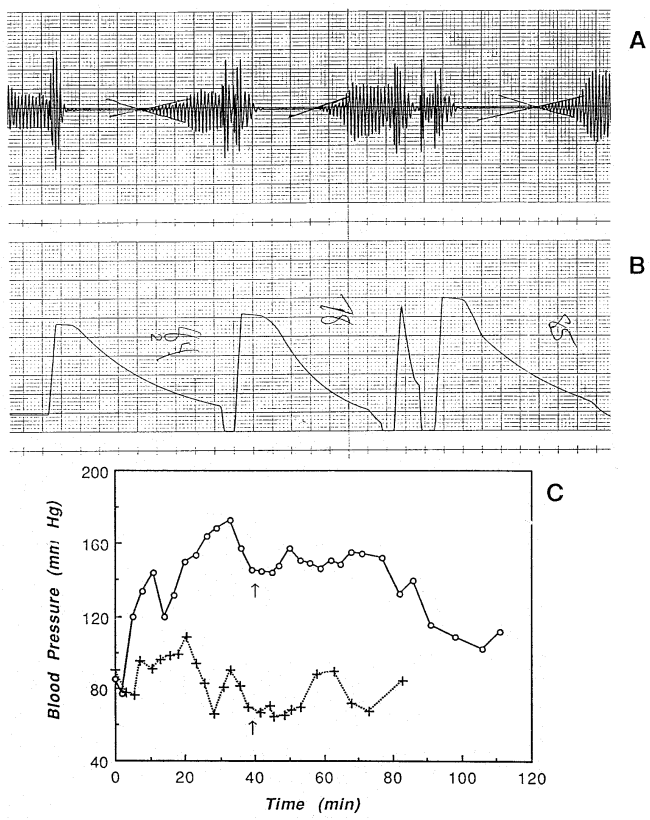

Fig. 1A–C A, B Illustrative blood pressure by photoplethysmography. **A** Pulse recording; **B** simultaneously recorded cuff pressure (0–300 mm Hg full scale). From left to right, cuff pressure was held at 30 mm Hg and a steady pulse was detected. Cuff pressure then was rapidly increased to approximately 170 mm Hg and slowly released. At a cuff pressure greater than the animal's blood pressure, pulse waves are not detected. As the cuff pressure drops below the animal's blood pressure, pulse waves appear. Extrapolation of the pulse wave "envelope" to the point where pulsations are first detectable yields the animal's blood pressure. Three successive measurements are taken, and the pressures averaged. **C** *Open circles* effect of i.p. infusion of {sar}ATII on blood pressure in a mouse, crosses blood pressure in a control animal receiving i.p. infusion of saline. *Arrows* represent the times that infusions were stopped. Following the end of {sar}ATII infusion, blood pressure is elevated for 40–50 min. Measurements were discontinued when animals recovered from the anesthetic

tions were first detectable was the blood pressure, found by the intersection of the two lines drawn along the pulse envelope. Following i.p. infusion of ATII, we observed a rise in blood pressure within 5 min. The blood pressure returned to baseline values within 5 min after the infusion was stopped. In later studies, we used {sar}ATII in which the non-naturally occurring amino acid sarcosine replaces the terminal aspartic acid of ATII. This analog produces a longer duration of action presumably owing to its higher affinity for the ATII receptor and slower degradative metabolism [17]. The upper curve in Fig. 1C shows an example of the blood pressure response to an infusion of {sar}ATII at 1.6 μl/min (open circles). As with native ATII, within 5 min of the start of infusion, an elevation in blood pressure was detectable. The blood pressure was elevated for the 40-min infusion period, and it remained elevated for approximately another 40 min following the end of infusion. The average pressure during the period from 5 to 80 min was 148 ± 13 mm Hg. The sustained increase in blood pressure was advantageous for our purposes because it allowed us, in subsequent experiments, to extend the period of elevated pressure beyond the time of infusion. The blood pressure response in a control animal receiving an infusion of saline is shown by the lower curve in Fig. 1C. The blood pressure measurements fluctuated somewhat, averaging 81 ± 13 mm Hg during the period from 5 to 80 min following infusion of saline.

Organ blood space measurements

The blood spaces of RD tumor, muscle, liver and kidney are listed in Table 1 for animals whose blood pressure was increased 35 mm Hg or greater by infusion of ATII. The blood space of the tumor in the ATII group was slightly lower than in the control group ($P < 0.05$, two-tailed Student's *t*-test). For all the normal tissues, the blood spaces did not differ significantly between the ATII and the control groups.

Biodistribution studies

In the biodistribution and autoradiography studies, the blood pressure was monitored periodically during the infusion period. In 2 of 20 animals, we found that the pressure was less than 15 mm Hg above the baseline at 30 min following the start of infusion, and these animals were excluded from the analysis. Figure 2 illustrates the biodistribution of tumor-specific and nonspecific immunotoxins in {sar}ATII-treated animals and saline controls 2, 6 and 24 h after injection. The plasma kinetics of the two immunotoxins were unaffected by {sar}ATII treatment. The tumor concentration of both specific and nonspecific immunotoxins appeared to be slightly, though not significantly, higher in {sar}ATII-treated animals than in control animals at 6 h, but not at 2 or 24 h. Results from the autoradiography study (Fig. 3) indicated that the uptake of specific immunotoxin in {sar}ATII-treated animals was the same as in control animals at all time-points.

Figure 2 also reveals that there was no difference in tumor concentration between the tumor-specific and nonspecific immunotoxins. Results with tumor-specific immunotoxin were in accordance with previously published experiments, but the nonspecific immunotoxin MOPC21-107 did not clear from the RD tumor as rapidly as previously observed [29]. The sources of MOPC21 antibody were different between these studies (Cetus and Sigma) because the Cetus product was no longer available. It appears that the presently used MOPC21 has a higher degree of nonspecific binding to the RD tumor. Changes in production conditions may alter the glycosylation pattern of a monoclonal antibody [8] which in turn may affect its nonspecific binding characteristics.

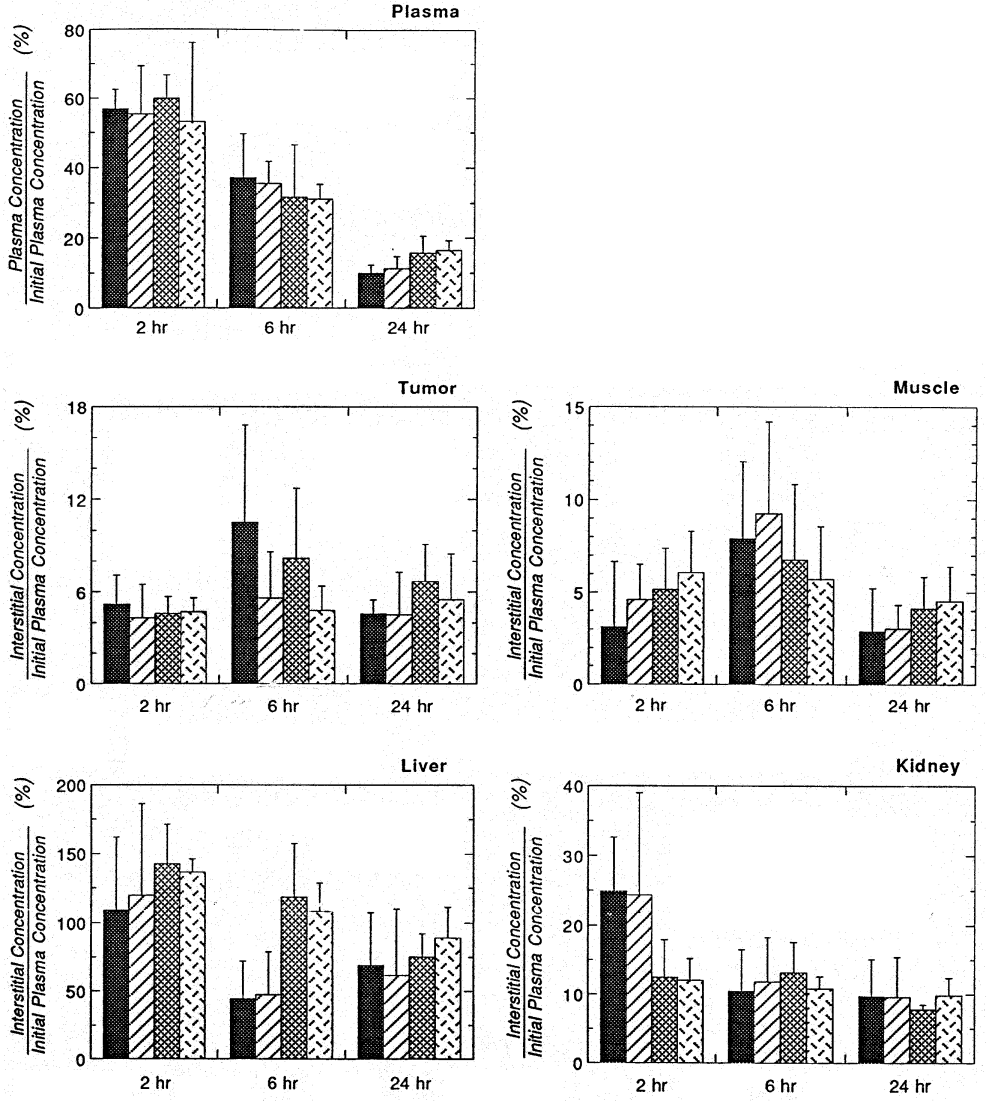

Fig. 2 Biodistribution (mean normalized concentration ±s.d.) of ^{125}I-labeled immunotoxin or ^{131}I-lableled nonspecific immunotoxin in {sar}ATII-treated animals and saline-treated controls 2, 6 and 24 h after injection of the radiolabeled compounds. Animals receiving: ■ tumor-specific immunotoxin and {sar}ATII, ▨ tumor-specific immunotoxin and saline, ▩ nonspecific immunotoxin and {sar}ATII, and ▽ nonspecific immunotoxin and saline

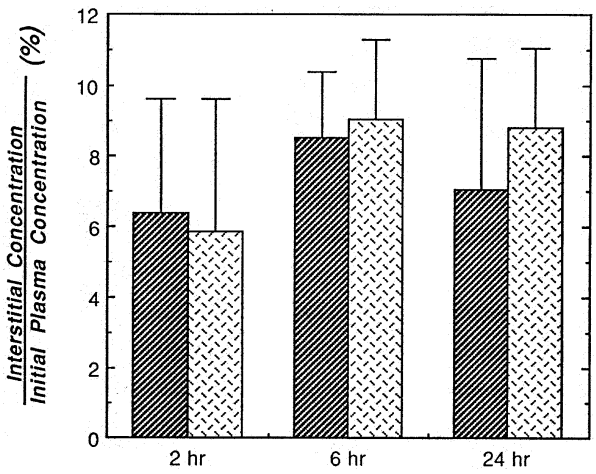

Fig. 3 Tumor uptake of ^{125}I-labeled immunotoxin in animals used in the autoradiography study. Animals received a 40-min infusion of ▨ {sar}ATII or ▽ saline following injection of the immunotoxin. Tumors were removed 2, 6 and 24 h after immunotoxin injection. Tumor uptake was not significantly different between animals receiving {sar}ATII and those receiving saline

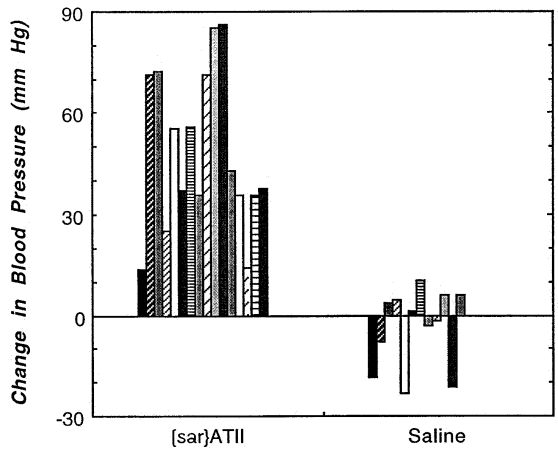

Fig. 4 Change in blood pressure 30 min after the beginning of ■ {sar}ATII or saline infusion among animals used in the autoradiography study

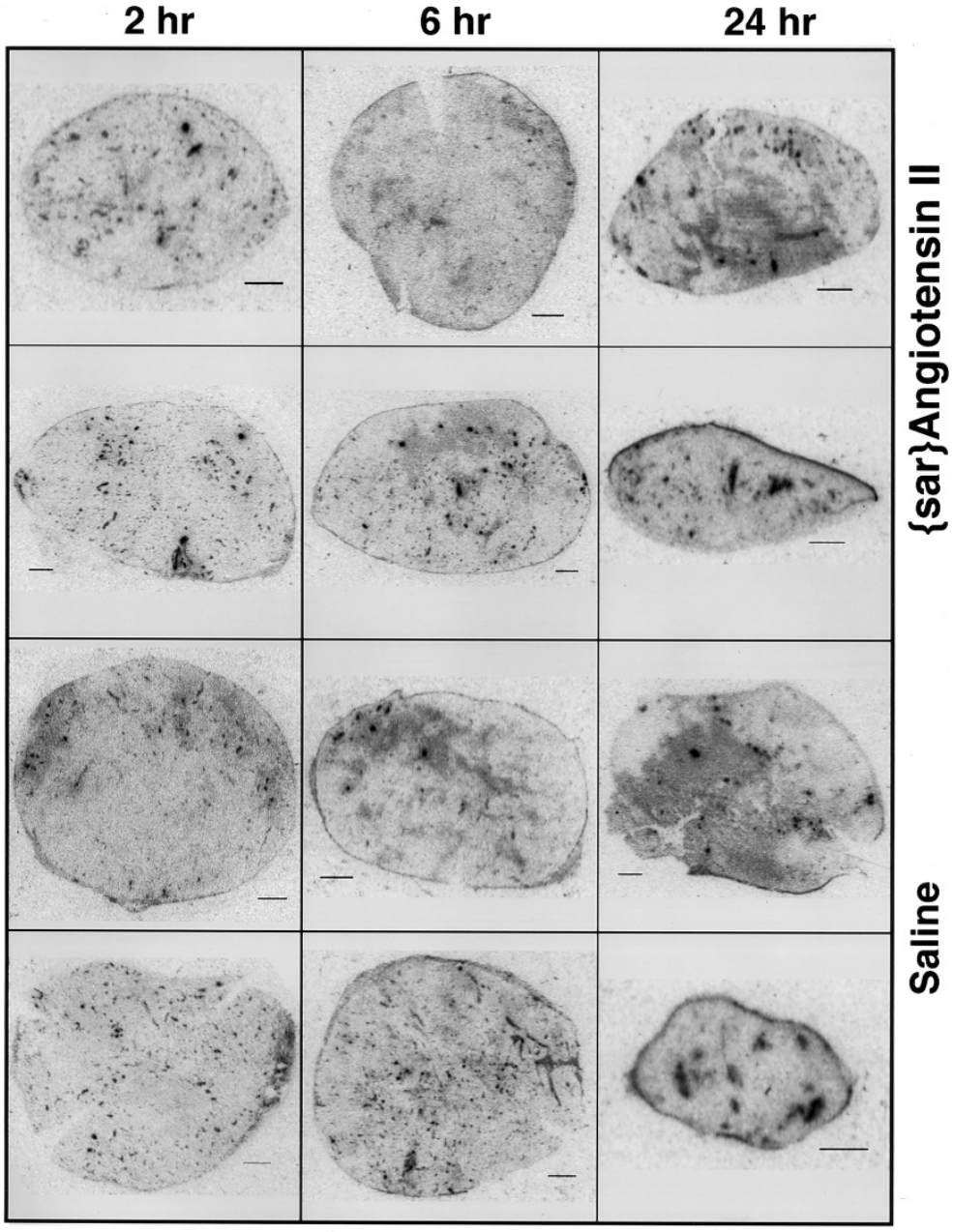

Fig. 5 Autogradiograms of tumors from animals receiving ^{125}I-labeled specific immunotoxin. Top six panels represent tumors after {sar}ATII treatment, and lower six panels represent tumors from saline-treated controls (bars=1 mm)

The biodistribution data show that the liver received the highest concentrations of immunotoxins. Liver uptake of the nonspecific immunotoxin at 6 h was somewhat higher than that of the tumor-specific immunotoxin, while kidney uptake of the tumor-specific immunotoxin was somewhat higher at 2 h than that of the nonspecific immunotoxin. However, in all normal tissues (plasma, muscle, liver and kidney) at all time-points, no differences in uptake for a particular immunotoxin were observed when comparing {sar}ATII-treated and saline-treated control animals.

Quantitative autoradiography experiments

Although the average uptake of immunotoxin was the same in {sar}ATII-treated and saline-treated control animals, we thought it possible that the spatial distribution of the immunotoxin in the tumor could differ in the two cases. Evidence that ATII treatment opens previously unperfused capillary beds in tumors [10] raised the possibility that such treatment would increase the homogeneity of immunotoxin distribution. Figure 4 shows the change in blood pressure in animals included in the analysis of the autoradiography studies. The {sar}ATII-treated animals had a 49 ± 23 mm Hg (mean $\pm$ s.d.) elevation in blood pressure compared with a -4 ± 11 mm Hg (mean $\pm$ s.d.) change in the control group. Examples of autoradiograms of immunotoxin distribution in tumors in {sar}ATII-treated and saline-treated control animals are shown in Fig. 5. In both groups, the immunotoxin distribution was very heterogeneous, characterized by punctate accumulations of high concentration and diffuse areas of relatively low concentration. Previously, we have

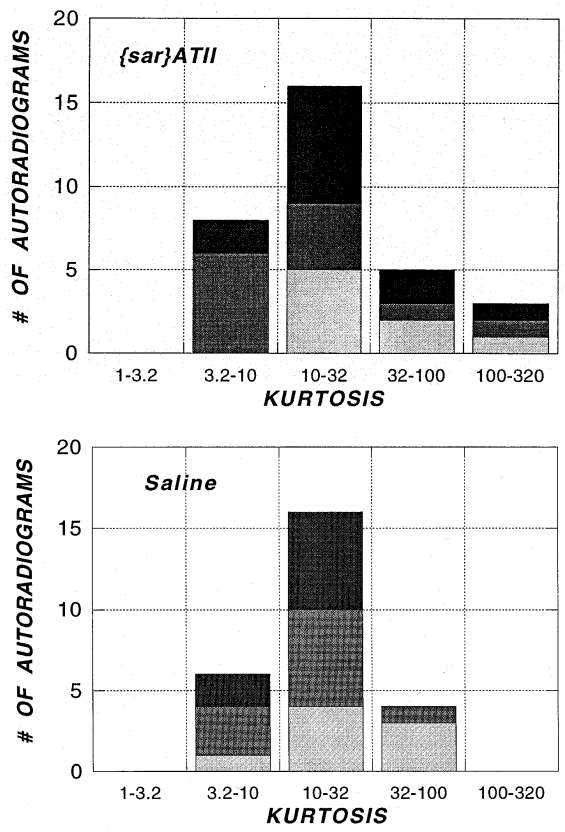

Fig. 6 Kurtosis of quantitative autoradiography images of tumors from animals receiving {sar}ATII or saline infusions after injection of ^{125}I-labeled tumor-specific immunotoxin at ⊡ 2 h, ▒ 6 h and ■ 24 h. Treatment with {sar}ATII does not increase the homogeneity of immunotoxin distribution in the tumor

shown that the degree of heterogeneity can be quantitatively characterized by the kurtosis of the distribution of concentrations [31]. The kurtosis is the dimensionless fourth moment about the mean. A normal distribution has a kurtosis of 3 while a distribution with a long tail will have a large kurtosis because points that are far from the mean are weighted strongly in the calculation. Figure 6 shows the kurtosis values of tumor images from {sar}ATII-treated animals and saline-treated control animals, 2, 6 and 24 h after injection of immunotoxin. The distribution of kurtosis values did not differ significantly between the two groups (Fisher's exact test for $r \times c$ contingency tables).

Interstitial tumor pressure measurements

The lack of effect of {sar}ATII infusion on immunotoxin uptake and distribution led us to consider possible mechanisms for the result. Elevation of interstitial pressure in solid tumors has been stressed as an impediment to delivery of macromolecules [3]. We therefore sought to characterize the interstitial pressure of this tumor. The mean interstitial pressure $\pm$S.D. in the tumors at a position 3 mm deep, measured by the servo-null micropipette method, was 0.6 ± 0.9 mm Hg (16 measurements in 7 animals). Experiments done independently with the use of the wick-in-needle methodology [4] confirmed that the interstitial pressure of this tumor was low: 0.9 ± 1.1 mm Hg (10 measurements in 9 animals; Y. Boucher, personal communication). The pressure in this tumor was unusally low in comparison with values reported for a wide variety of other tumors [3, 4]. Thus, the RD tumor may be a useful control in studies on tumor interstitial pressure. A theoretical model of fluid dynamics in a tumor predicts that interstitial pressure will be elevated under conditions of high capillary permeability and capillary surface area/volume of tumor [2]. A possible mechanistic explanation for the low interstitial pressure in the RD tumor is that such conditions do not exist in this tumor.

Discussion

The reported effects of ATII motivated us to explore the possibility of using ATII-induced hypertension to increase the delivery of macromolecular drugs to a solid tumor. We reasoned that the low transcapillary transport rates of immunotoxins would require a relatively extended period of ATII hypertension in order to obtain measurable differences from untreated controls. By means of i.p. infusion of {sar}ATII for 40 min, we were able to elevate blood pressure for approximately 80 min. If the transcapillary transport rate were doubled for the 80-min period, we estimated that uptake of the tumor-specific immunotoxin would be higher by approximately 70% at 2 h (the first sampling time) on the basis of a compartmental model of the transport of immunotoxins [29]. In this model, we assume that the dominant mechanism for solute transport is filtration [26]. Therefore, an increase in transcapillary fluid influx would be accompanied by an equivalent increase in the interstitial loss rate (by fluid efflux). If the transcapillary transport and interstitial loss rates returned to baseline levels at 80 min, we estimate that tumor uptake would be approximately 30% higher at 6 h compared with controls. By the same reasoning, a tripling of the transcapillary transport rate would be expected to produce an increase in immunotoxin uptake of 140% and 60% at 2 and 6 h, respectively. In cell culture, the number of cells killed rises more than proportionately with immunotoxin concentration [30]. This observation suggests that even modest increases in tissue concentrations of an immunotoxin can have a large impact on cytotoxicity. Thus, it was disappointing to find that in the RD rhabdomyosarcoma xenografts, average uptake of tumor-specific and nonspecific immunotoxins was not increased significantly by a sustained period of hypertension with {sar}ATII at any of the time-points compared with saline-treated controls. The uptake in normal tissues (muscle, liver, kidney and lung) was also unchanged.

Interstitial fluid pressure is increased in many types of solid tumors [3]. Furthermore, it increases after adminis-

tration of ATII [38]. When interstitial pressure exceeds microvascular pressure, vessels collapse and perfusion ceases. Hori et al., however, found that ATII raises microvascular pressure above the interstitial pressure [11]. Such a phenomenon would allow perfusion of previously collapsed tumor capillaries. Unfortunately, the duration of such an effect upon prolonged infusion was not reported. The increase in hydrostatic pressure difference between the tumor vasculature and interstitium will also increase fluid filtration, and if the additional fluid is not removed at a higher rate, interstitial pressure will rise. Thus, it is unclear whether the effect of ATII on increasing the capillary surface area of perfused vessels can be sustained. The dynamics of ATII action on capillaries needs to be documented with a tumor preparation that permits direct visualization of the microvasculature. A sustained opening of solute exchange vessels would be expected to produce a more uniform interstitial distribution of a macromolecular drug like an immunotoxin. In this case, however, {sar}ATII-induced hypertension was not associated with any significant change in the heterogeneity of immunotoxin distribution.

Another possible explanation for the lack of measurable effect of {sar}ATII on tumor uptake of the macromolecular immunotoxin may be the low interstitial pressure of the RD rhabdomyosarcoma. If these tumors already have a uniform distribution of perfused vessels, it may be difficult to obtain a significant change in the status of the tumor microvasculature that could significantly alter immunotoxin uptake and distribution after {sar}ATII treatment. The heterogeneous distribution of immunotoxin in the RD tumors evidently was not a consequence of elevated interstitial pressure. Given the low dose of immunotoxin and high affinity of the immunotoxin for the human transferrin receptor on the tumor cells, the heterogeneous distribution may simply reflect a high degree of binding to perivascular cells (binding-site barrier effect [6]).

In contrast to our results, an elevation in macromolecular drug uptake with ATII-induced hypertension has been obtained in other animal models. The intratumoral concentration of fluorescein-isothiocyanate-labeled neocarzinostatin (MW 10700 Da) in a rat hepatoma at 10 min has been shown to be twice control values following a 10-min period of ATII-induced hypertension [1]. Uptake of tumor-specific monoclonal antibodies (MW 150000 Da) in human colon carcinoma xenografts has been shown to be increased by 35% to 45% at 48 to 72 h after injection when mice are coinjected with ATII [23, 35]. More recently, in a study using rats bearing the Walker 256 carcinoma, Li et al. have found that injection of macromolecular SMANCS (MW 16 000 Da) and BSA (MW 68 000 Da) during ATII-induced hypertension (15 min) leads to a 20% to 80% increase in tumor uptake 1 or 6 h later [19]. None of these reports, however, describe the effect of ATII on the spatial distribution of the drugs within the tumors. It would be interesting to learn if ATII produces a redistribution of drug in those tumor models.

In summary, we found no effect of an extended period of {sar}ATII-induced hypertension upon uptake of either specific or nonspecific immunotoxins in solid RD human rhabdomyosarcoma xenografts over a 24-h period. The spatial distribution of the tumor-specific immunotoxin was also unaltered. The absence of effect may be related to the unexpectedly low interstitial pressure of these tumors. Another possibility is that the microvasculature of the RD tumor does not increase in surface area or increases only transiently during the period of hypertension. Further studies are needed to determine the types of tumors and/or their physiological properties that correlate with increased uptake of macromolecular drugs under ATII-induced hypertension. Such studies will enable better discrimination of the clinical circumstances that are and are not likely to benefit from chemotherapy conducted under ATII-induced hypertension.

Acknowledgements We wish to express our appreciation to Patricia Johnson for technical support with the cell cultures. Eli Walker and Seth Goldstein provided valuable assistance on the photoplethysmography and servo-null instrumentation. We also would like to thank Shigeru Tanda for stimulating discussions as we planned the experiments and Yves Boucher for graciously contributing his resources to measuring interstitial pressures in the RD tumors by the wick-in-needle methodology. Finally, we gratefully acknowlege the advice and helpful suggestions given by Robert Dedrick and Richard Youle.

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