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Differential relationship between changes in tumour size and microcirculatory functions induced by therapy with an antivascular drug and with cytotoxic drugs: implications for the evaluation of therapeutic efficacy of AC7700 (AVE8062)

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Abstract

A novel combretastatin A-4 derivative, AC7700, which is now in Phase I clinical trials under a new code, AVE8062, has shown strong antitumour effects against solid tumours in rodents because of its powerful and continued stanching of the tumour blood flow (TBF). Despite the strong tumour-suppressing qualities of AC7700, it does not produce an immediate reduction in tumour size. To elucidate the reason for this effect, we investigated the relationship between the change in tumour size in Sato lung carcinoma (SLC) and circulatory functions after therapy with AC7700, doxorubicin (Adriamycin [ADR]), or mitomycin C (MMC). To measure time-lapse changes in TBF with the hydrogen clearance method at the same site after drug administration, we developed a new apparatus for keeping electrodes within a tumour. AC7700 led to the destruction of both cancer cells and tumour vessels by interrupting the supply of nutrients. Intravenous (i.v.) administration of fluorescent dyes after AC7700 treatment revealed no fluorescence within the tumour vessels, which confirmed that the tumour microcirculation had been completely blocked. In contrast, ADR led to the destruction of SLC tumour cells, but did not have the same effect on tumour vessels. Intravenously administered fluorescent dyes immediately reached the tumour, which indicated that the tumour vasculature remained intact, and the TBF remained at the preadministration level, even 6 days after ADR treatment. In addition, although the size of the tumour increased slightly for 2 days with ADR treatment, possibly because of swelling of the cancer cells, thereafter it continued to decrease. MMC had virtually no effect on SLC tumour cells, tumour size or tumour vessels. We conclude that changes in tumour size brought about by cancer chemotherapy depend not only on the sensitivity of the cancer cells to the drug in question, but also on the nature of changes in the microcirculatory functions of the tumour brought about by the therapy. When both tumour cells and the tumour vasculature are destroyed, the effectiveness of therapy can not be determined from changes in tumour size alone.

Keywords: Combretastatin A-4; AVE8062; Tumour blood flow; Tumour vessel; Microcirculation; Antitumour

1. Introduction

We recently showed that a novel combretastatin A-4 (CS A-4) [1] derivative, AC7700, has remarkable antitumour effects against solid tumours in rodents [2–4]. We also demonstrated that its effectiveness is due to powerful and continued stanching of tumour blood flow (TBF) [3]. These strong effects on TBF are found not only in transplanted tumours, but also in primary tumours induced by chemical carcinogens [5]; in cancers of the liver, stomach and kidney; and in lymph node metastases and microfoci smaller than 2.5 mm [6]. These results suggest that AC7700 is effective in all types of solid tumours, including refractory cancers.

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AC7700 therapy leads to a significantly prolonged survival of tumour-bearing animals [3,4,7]. Solid tumours demonstrate coagulation necrosis, and, after scar formation, sloughing of scar tissue and cases of complete cure are observed [3]. Despite the strong tumour-suppressing qualities of AC7700, this drug does not usually produce a reduction in tumour size immediately after therapy. The fact that there is no change in tumour size for a lengthy period of time is markedly different from the effects of most other anticancer drugs. The target of most other anticancer drugs has been the tumour cells themselves, and drugs are selected as candidate anticancer agents when they show strong suppression of tumour-cell proliferation and a reduction in the size of a transplanted tumour.

Because AC7700 is now in Phase I clinical trials (under a new code, AVE8062), it is appropriate to ask why there is little reduction in tumour size, why the tumour continues to be maintained at its original size, and what conditions might lead to a reduction in tumour size. Elucidation of this problem is important not only for evaluation of the therapeutic effects of AC7700, but also for planning a reasonable therapeutic protocol. Despite the importance of these questions little research has been conducted in this area.

We believe that the main reason for the lack of reduction in tumour size after AC7700 therapy is that the tumour vessels are destroyed, which disrupts circulatory functions. As a consequence, there is no functional tumour vascular network, and this is the route used by cells that normally deal with the destroyed tumour cells (neutrophil leucocytes and macrophages) and carry cellular debris out of the tumour. To validate this hypothesis, we studied the relationship between the change in tumour size and circulatory functions after treatment of solid tumours (Sato lung carcinoma, SLC) with AC7700, doxorubicin (Adriamycin [ADR]) and mitomycin C (MMC). This tumour and these drugs were used as models of typical results obtained with cancer chemotherapy, because there are no changes in SLC tumour size after AC7700 therapy, the tumour size is reduced after ADR therapy, and the tumour size increases after MMC therapy.

The purpose of this study was therefore to determine the involvement of tumour vessels and TBF in changes in tumour size after drug administration.

2. Materials and methods

2.1. Rats and tumours

Male Donryu rats (Crj-Donryu; Nippon Charles-River, Yokohama, Japan) at 8–10 weeks of age and with an average weight of 250–300 g were used for all of the experiments. Rats were bred and maintained in a ventilated, temperature-controlled (24±1 °C), specific pathogen-free environment, on a bed of wood shavings, with food and water freely available and a 12-h light–dark cycle. They were usually housed two or three per cage. Rats that had been implanted with transparent chambers [8] for vital microscopic observations of their tumour microcirculation and rats implanted with an apparatus for time-lapse measurements of their blood flow were caged singly.

SLC tumour cells were used for the experiments; the tumours had been induced in a male Donryu rat using 4-nitroquinoline 1-oxide and the method of Sato and Shimosato [9] and were maintained in our laboratory by successive subcutaneous (s.c.) transplantations. SLC tumours growing in the transparent chamber showed a clear demarcation between the edge of the growing tumour and the normal tissue [10]. All experimental protocols were reviewed by the Committee on the Ethics of Animal Experiments in our institute and were carried out in accordance with Guidelines for Animal Experiments issued by Tohoku University School of Medicine and the Law (No. 105) and Notification (No. 6) issued by the Japanese Government.

2.2. Drugs

AC7700 (AVE8062) ((*Z*)-*N*-[2-methoxy-5-[2-(3,4,5-trimethoxyphenyl) vinyl] phenyl]-L-serinamide hydrochloride), a combretastatin derivative, was synthesised [11,12] and provided by Ajinomoto Pharmaceutical Research Laboratories, Kawasaki, Japan. ADR (Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan), to which SLC is sensitive, and MMC (Kyowa Hakko), to which SLC is insensitive, were used as comparative cytotoxic drugs. AC7700, ADR and MMC were dissolved in 0.9% NaCl solution immediately before use to give final concentrations of 10.0 mg/ml, 8.0 mg/ml and 2.0 mg/ml, respectively. These drugs were injected via an infusion pump (Compact syringe pump; Harvard Apparatus, South Natick, MA, USA) into the tail vein at a rate of 0.15 ml/min to give single doses of 10.0 mg/kg, 8.0 mg/kg (maximum tolerated dose, 13 mg/kg), and 2.0 mg/kg (MTD, 3 mg/kg), respectively.

Fluorescein sodium solution (Fluorestin; Kobayashi Seiyaku Co., Tokyo, Japan) was used for the visualisation of functioning vessels. Pentobarbital sodium (pentobarbital sodium salt; Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) and enflurane (Ethrane; Abbott Laboratories, North Chicago, IL, USA) were used for anaesthesia. Pentobarbital sodium was administered intramuscularly (i.m.) at a dose of 30 mg/kg 10 min before the experiment. The concentration of enflurane was maintained at 1% (v/v) in the inhaled carrier gas at 1 litre/min by means of an anaesthetic apparatus for small laboratory animals [13].

2.3. Apparatus for maintaining the electrode position

We developed a new apparatus for keeping electrodes within the tumour so that we could measure, via the hydrogen clearance method [6,13], daily changes in TBF at the same site during tumour growth or after drug administration. Fig. 1 shows the design of the apparatus, which is composed of a pair of aluminum shields to support the double layer of skin. One shield has a centre hole 20 mm in diameter, and the other shield with two holes, each 2 mm in diameter, has a hangar in which two electrodes can be placed. For measurements of TBF, SLC cells (2×10^6 cells) in 0.2 ml were first prepared from the s.c. tumour of a donor rat by a routine method and were injected s.c. into the 20-mm centre hole region of the aluminum shield after implantation of the apparatus into the rat. Tumour spread in two dimensions is relatively restricted by the shield, but growth is allowed in the third dimension. The double value of tumour thickness (T) shown in Fig. 1 is the tumour volume, because the volume (cm^3) is calculated by the following formula:

$$\text{Tumour volume } (cm^3) = (\pi/6) \times a \times b \times c \div 2$$
$$= (1/2) \times 2 \times 2 \times 2T \div 2 = 2T$$

where a, b and c are the three dimensions of the tumour nodule. When tumour volume reached 1.2–1.5 cm^3, 8 days after tumour cell implantation, the sensitive part of the wire electrode, which can detect hydrogen gas, was placed in the area at a depth of 5 mm below the tumour surface, and the remaining part of the wire was placed in the hangar by passing it through a 2-mm hole of the shield.

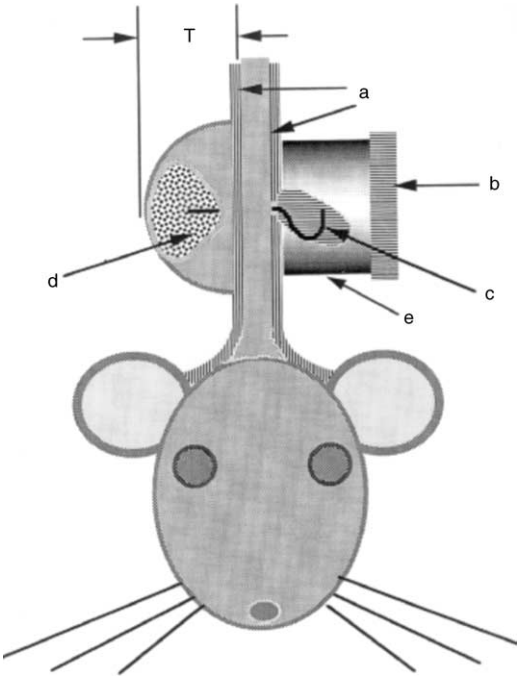

Fig. 1. A front sectional view of the structure of the apparatus for keeping electrodes at the same site within a tissue. (a) Aluminum shield, (b) screw cap, (c) electrode, (d) tumour, (e) hangar. The double value of the tumour thickness (T) equals the tumour volume (see text).

2.4. Measurement of tissue blood flow

Blood flow in the tumour and s.c. tissue was measured by use of the hydrogen clearance method [6,13]. The blood flow by this method is not the total blood flow [14]; rather, it is the local blood flow [15]. According to our experience, the local blood flow responses after the drug administrations are similar, irrespective of the measuring sites within a tumour, because the tumour vascular network consists of many microvascular units [16], which respond to drugs in the same manner. The hydrogen clearance method also allows the measurement of the absolute value of blood flow. Thus, our methodology was adequate for the purposes of our study.

The hydrogen clearance method was originally developed by Aukland and colleagues [17]. In brief, after saturation of the tissue with hydrogen following the inhalation of 9% (v/v) hydrogen gas in air (at 1 litre/min), the blood flow value (in ml/min/100 g tissue) is calculated from the half-life of the clearance curve obtained. In the present experiment, we used a tissue blood flow meter with two separate amplifiers (PHG-201; Unique Medical Co., Tokyo, Japan). Two hydrogen electrodes with a diameter of 80-μm and a length of 3 cm (TT200-010; Unique Medical) were placed within the tumour. For the measurements, two small incisions were made in the dorsal skin for insertion of two rod-type Ag/AgCl reference electrodes, each 2 mm in diameter (TT-98012; Unique Medical). Throughout the blood flow measurement procedure, rats were prone on a heated stage at 34 °C. Rectal temperature monitored with a thermistor for small animals (PTC-201; Unique Medical) was 33.5–35.5 °C and the condition was maintained. All experiments were performed with animals that were anaesthetised in a controlled-temperature box (25 °C) fitted with a suction duct.

2.5. TBF during tumour growth and after drug administration

To examine changes in TBF during tumour growth, measurements were started 8 days after implantation of tumour cells, when the tumour size was 1.2–1.5 cm^3, and were continued every day or every other day until the blood flow became approximately zero. As a control, tissue blood flow at the same site within normal s.c. tissue was measured at 3-day intervals for 24 days, starting on day 3 after implantation of the apparatus.

Measurements of changes in TBF that were induced by drug administration were started 10 days after tumour implantation. TBF was measured before and after the drug administration, and the measurement continued for 6 days.

2.6. Histological examination

Other tumour-bearing rats were treated with AC7700, ADR or MMC, and tumours were excised 48 h later. Control tumour-bearing rats received 0.9% NaCl solution. After fixation of tissues in 15% formalin and standard processing with paraffin wax blocks, sections (4 μm) were prepared and stained with haematoxylin and eosin.

2.7. Intravital fluorescence microscopy for detecting functioning tumour vessels

For vital microscopic observation, transparent chambers were implanted under aseptic conditions into the dorsal skin flaps of rats [8]. Each chamber consisted of two identical titanium frames containing a circular quartz glass window that was 300 μm thick. After administration of anaesthesia to a rat with a transparent chamber, the animal was placed in the right lateral position on a heated (34.5 °C) stage (MATS-SFA, Tokai HIT Co., Ltd., Tokyo, Japan), which was attached to the mechanical stage of the microscope. Tumour microcirculation was observed via a light microscope (Eclipse E800; Nikon Corporation, Tokyo, Japan) with a 10× ocular (CFI UW; Nikon) and 2–20× objectives (CFI Plan Flour; Nikon). The tumour microvascular network within the chamber was transilluminated by a 12-V 100-W halogen lamp. For intravital fluorescence microscopic observation, the light source was changed to a 100-W mercury lamp.

Fluorescein sodium solution (10 mg/ml) was injected intravenously (i.v.) as a single bolus (1 ml/kg). Tumour vessels within the chamber were epi-illuminated through a primary filter (420–490 nm), a 505-nm dichroic interference mirror, and a 520-nm barrier filter. The fluorescein microscopic images were photographed by use of a silicone-intensified video camera (C2400-08, Hamamatsu Photonics, Hamamatsu, Japan), were displayed on a TV monitor (PVM-14M4J; Sony Corporation, Tokyo, Japan), and were recorded using a S-VHS video recorder (SVO-2100, Sony Corporation). A CCD video camera (CS-900; Olympus Kogaku K.K., Tokyo, Japan) was used for routine colour photography. A video timer was superimposed on images for record keeping. Segments of the videotape containing desired images were transferred to the hard disk of a computer (Power Macintosh 8600/200, Apple Japan, Inc., Tokyo, Japan) through a video frame grabber board (IQ-V50PCI, Hamamatsu Photonics). Final images were outputted by a digital printer (Pictrography 4000, Fuji Photo Film, Tokyo, Japan).

2.8. Statistics

Results are expressed as means ± standard deviation (S.D.) The statistical significance of changes in TBF and tumour size at each time point after drug administration was evaluated with repeated measures ANOVA. P values of 0.05 or below were considered significant.

3. Results

3.1. Daily blood flow changes at the same site within the tumour and in the normal subcutis

Fig. 2 shows the daily blood flow changes at the same site within the tumour and in the normal subcutis. The TBF initially increased as the tumour grew larger; the mean peak TBF was 36.3 ± 12.7 ml/min/100 g ($n = 10$). However, with the continued growth of the tumour, the TBF began to fall and finally became almost zero. By contrast, the mean blood flow in the normal subcutis was relatively constant (14.0 ± 1.8 ml/min/100 g) ($n = 6$) throughout the experimental period. Although the s.c. tissue around an electrode showed a slight demarcation at 24 days after insertion of the electrode, we did not observe any foreign body reactions involving macrophages (data not shown).

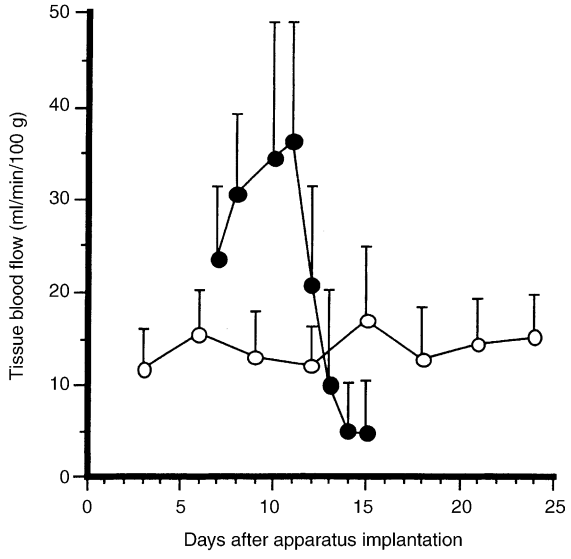

Fig. 2. Daily blood flow changes at the same site within the SLC tumour transplanted subcutaneously (s.c.) (●) and in the normal subcutis (○). Implantation of the apparatus for maintaining the position of the electrodes and tumour implantation were performed on day 0.

3.2. Growth curves for SLC tumours and daily TBF changes at the same site within the tumour after administration of AC7700, ADR, MMC and 0.9% NaCl

Growth curves for tumours are shown in Fig. 3a, and daily TBF changes at the same site after a single administration of AC7700, ADR, MMC or 0.9% NaCl are shown in Fig. 3b. Although the mean values of TBF and tumour size before drug administration were not significantly different among these four groups, both tumour size and TBF after administration of each drug showed significantly different changes (both the tumour size and TBF: ADR vs AC7700, $P<0.0001$; ADR vs MMC, $P<0.0001$; MMC vs AC7700, $P<0.0001$). However, there were no significant differences in both the tumour size and TBF between the MMC group and the 0.9% NaCl group (tumour size, $P=0.2860$; TBF, $P=0.5635$).

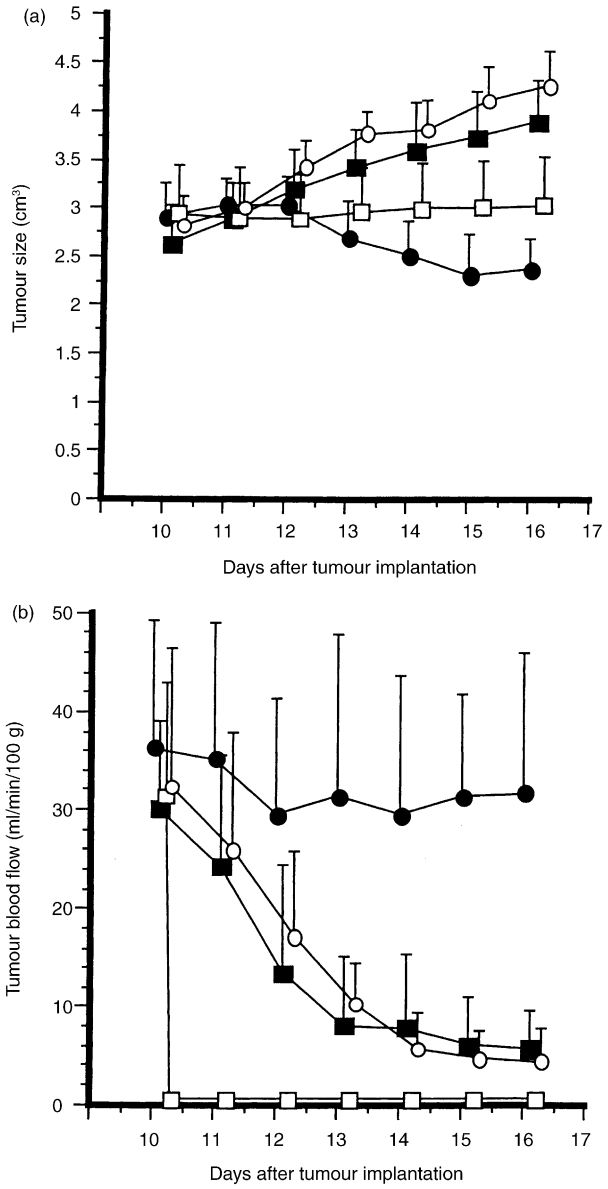

Fig. 3. Growth curves of SLC tumours and daily TBF changes at the same site within the tumours after a single administration of AC7700 (□, $n=12$), ADR (●, $n=12$), MMC (■, $n=12$), or 0.9% NaCl (○, $n=10$). (a) Growth curves of tumours. (b) Daily TBF changes at the same site. Drugs were administered on day 10 after tumour implantation.

For those treated with AC7700 ($n=12$), the TBF decreased to zero 30–60 min after the i.v. administration of 10 mg/kg AC7700 and never recovered. Tumour size did not change at all during the experimental period of 6 days.

For those treated with ADR ($n=12$), although the tumour size increased slightly for 2 days after the i.v. administration of 8.0 mg/kg ADR, it continued to decrease thereafter. However, the TBF was constant despite the shrinkage of the tumour and was maintained, even 6 days after the ADR administration. In one case in the ADR group, TBF was high before treatment, but decreased to zero 72 h after the ADR administration and never recovered (Fig. 4b). In this case, the tumour size did not increase or decrease (Fig. 4a), as was the case for those receiving AC7700. Dissection of the tumour confirmed that there was extensive coagulation necrosis within it (data not shown).

For those treated with MMC ($n=12$), although body weight loss was observed after treatment with 2.0 mg/kg MMC (data not shown), tumour growth was not suppressed and the tumours continued to grow. The pattern of TBF changes after MMC administration was similar to that for the 0.9% NaCl group ($n=10$). That is, TBF decreased markedly with the enlargement of the tumours and finally approached zero.

3.3. Histology of the tumour tissue after the administration of AC7700, ADR, MMC or 0.9% NaCl

Fig. 5 shows the changes in tumour tissues 48 h after the i.v. administration of 10 mg/kg AC7700 (a), 8.0 mg/kg ADR (b), 2.0 mg/kg MMC (c) or 0.9% NaCl solution (d). Histological examination revealed that both the SLC cells and the tumour vessels were severely damaged by AC7700 (Fig. 5a): pyknosis of the tumour cells and degradation of most of the cells and tumour vessels was noted.

In the case of ADR administration, the tumour cells, but not the tumour vessels, were severely damaged (Fig. 5b) compared with the control cells (Fig. 5d). Most ADR-treated cells showed an increased cell size, with enlargement of the nuclei and numerous abnormal mitotic features. However, many intact vessels remained within the tumours.

In contrast, administration of MMC did not damage either the tumour cells or the tumour vessels (Fig. 5c).

3.4. Changes in tumour microcirculatory function after the administration of AC7700, ADR or MMC

Fig. 6a and b show a SLC tumour growing in the transparent chamber and its vascular network before

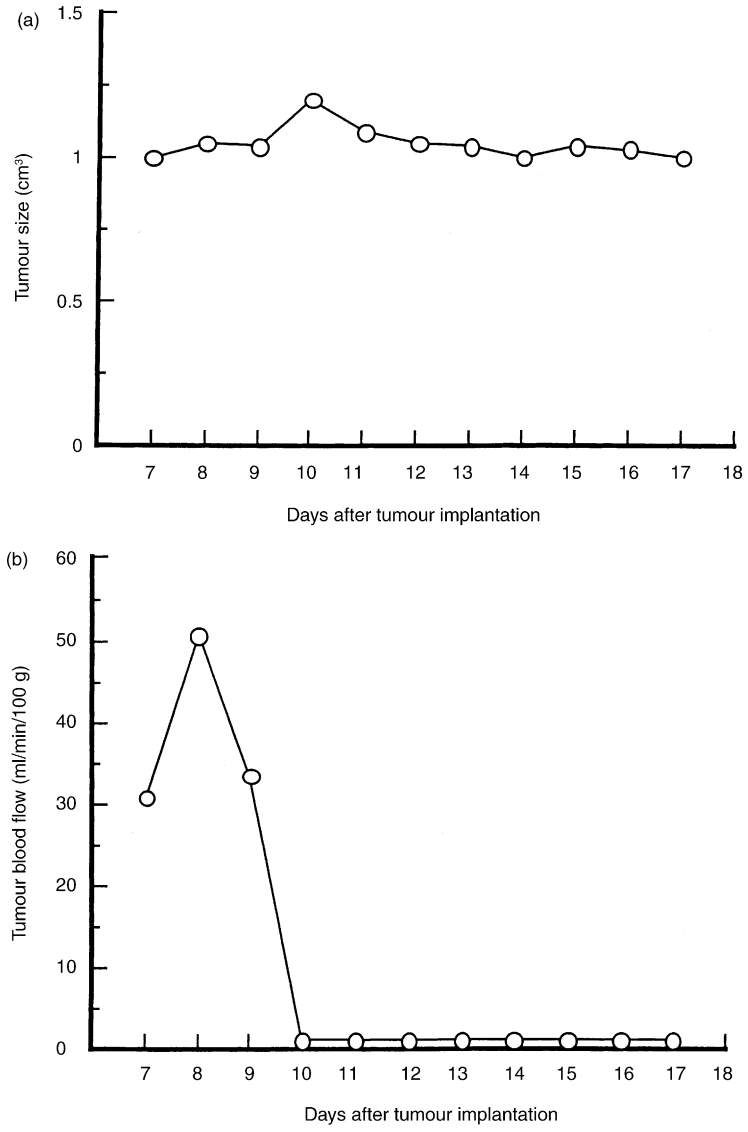

Fig. 4. Constancy in the tumour size in one case in which TBF stanched completely after ADR administration. ADR was administered on day 7 after implantation of the tumour. (a) Growth curve of SLC tumour transplanted subcutaneously. (b) Daily blood flow changes at the same site within the tumour.

AC7700 administration, respectively. Note that the vascular density is quite high. Administration of AC7700 caused the TBF to stop completely, and the tumour ceased growing. Fig. 6c shows degradation of the tumour caused by AC7700, and Fig. 6d shows a typical result of fluorescent angiography performed 48 h after AC7700 administration. Although fluorescent dyes were administered by a single, bolus, i.v. injection, the dyes did not reach the tumour tissue at all because of the complete blockage of the tumour microcirculation. These findings occurred in all five rats, without exception, that were studied by means of intravital fluorescence microscopy.

SLC cells within the chamber were destroyed by ADR, and tumour growth stopped completely 48 h after drug administration. However, at that time, there were many vessels within the tumour tissue (Fig. 6e). A typical fluorescence microscopic finding 48 h after ADR administration is shown in Fig. 6f. TBF was well preserved, and fluorescent dyes could be delivered to tumour tissue, indicating that those tumour vessels were functioning. Tumour vascular permeability was enhanced after ADR administration (Fig. 6g and h). These findings were noted in all five rats.

In contrast, administration of MMC hardly damaged either the SLC cells or tumour vessels, and the tumours continued to grow. MMC did not enhance or suppress tumour vascular permeability (data not shown).

These data obtained by microscopy after the administration of each drug supported the results for TBF changes measured by the hydrogen clearance method.

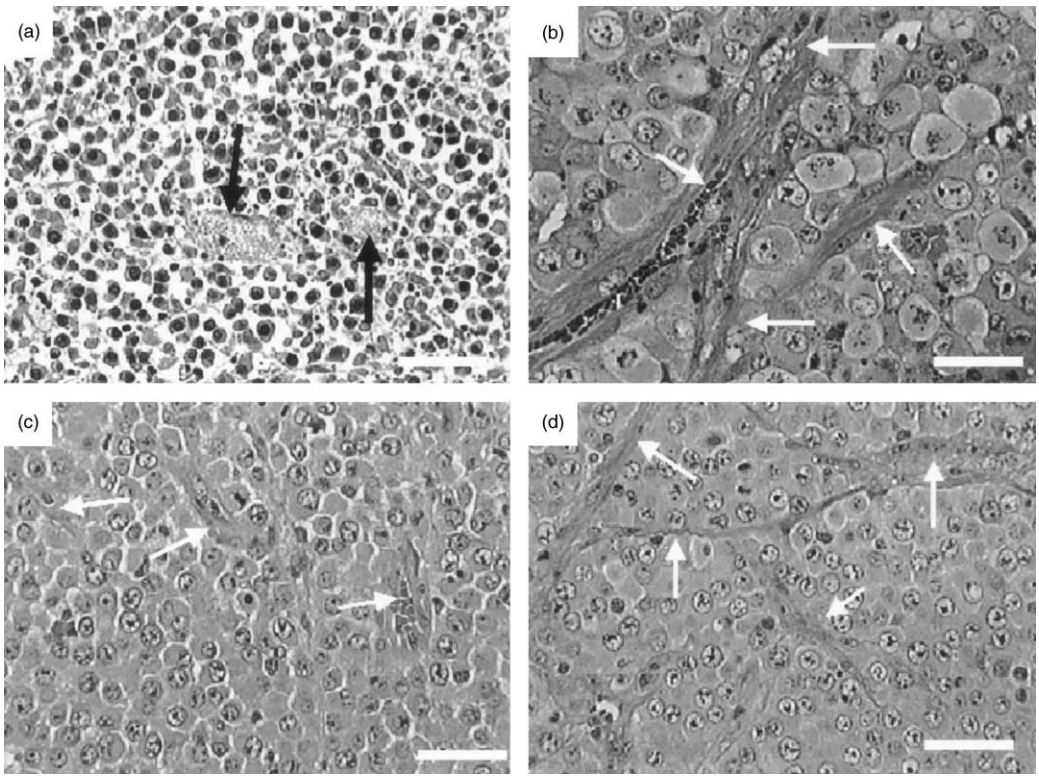

Fig. 5. Histology of SLC tumour tissue 48 h after the i.v. administration of the drugs: (a) 10.0 mg/kg AC7700; (b) 8.0 mg/kg ADR; (c) 2.0 mg/kg MMC; (d) 0.9% NaCl; original magnification ×200. Haematoxylin and eosin staining. Arrows, tumour vessels; bars, 50 μm.

4. Discussion

The results of the present study strongly suggest that changes in tumour size (a decrease or increase, or no change at all) brought about by cancer chemotherapy depend not only on the sensitivity of the cancer cells to the drug, but also on the nature of the drug-induced changes in the tumour microcirculation. We were able to reach this conclusion by developing a new method for continual monitoring of blood flow at the same site within the tumour over several days. As no tissue reaction related to the platinum hydrogen electrode occurred, even when the electrode had been in place subdermally for approximately 3 weeks, the blood flow values measured in this study are thought not to be affected by inflammation. This technique has made it possible to measure changes in TBF brought about by drug therapy, as well as changes in TBF caused by tumour proliferation.

The natural course of tumour growth includes, an initial marked increase in TBF, together with tumour growth, with a peak TBF value being achieved quickly. When the rate of tumour growth decreases, the TBF begins to fall [18] and eventually reaches a value of zero. This series of changes was previously reported by Paskins-Hurlburt and colleagues in Ref. [19]. Using a rat transparent chamber, Yamaura and Sato [20] showed the morphological process by which tumours initially undergo neovascularisation and exponential growth, followed by necrosis. Our measurements of TBF provide functional support for their findings: the increased TBF at the outset is thought to reflect an increase in the number of tumour vessels. The subsequent decrease in TBF is thought to reflect a decrease in the number of tumour vessels caused by larger necrotic areas. The reason for the decrease in TBF with the continued growth of the tumour may be due to the increase in tumour interstitial fluid pressure together with tumour proliferation and subsequent compression of the tumour vessels by the increased pressure [19,21–23].

Characteristic changes in TBF occurred for several days after the administration of AC7700, ADR or MMC. Both AC7700 and ADR have been reported to have strong antitumour effects against SLC, resulting in some cases in a complete cure [3,24], but the present study showed large differences in the response to therapy using these agents with regard to changes in both the tumour size and TBF. Use of AC7700 led to the destruction of both the cancer cells and tumour vessels. Complete stanching of the TBF of the SLC tumour occurred within 30 min of AC7700 administration, with no subsequent recovery of the blood flow. However, there was no notable change in the tumour size during 6 days of observation. Histological specimens obtained after 48 h revealed pyknosis of tumour cells and necrosis

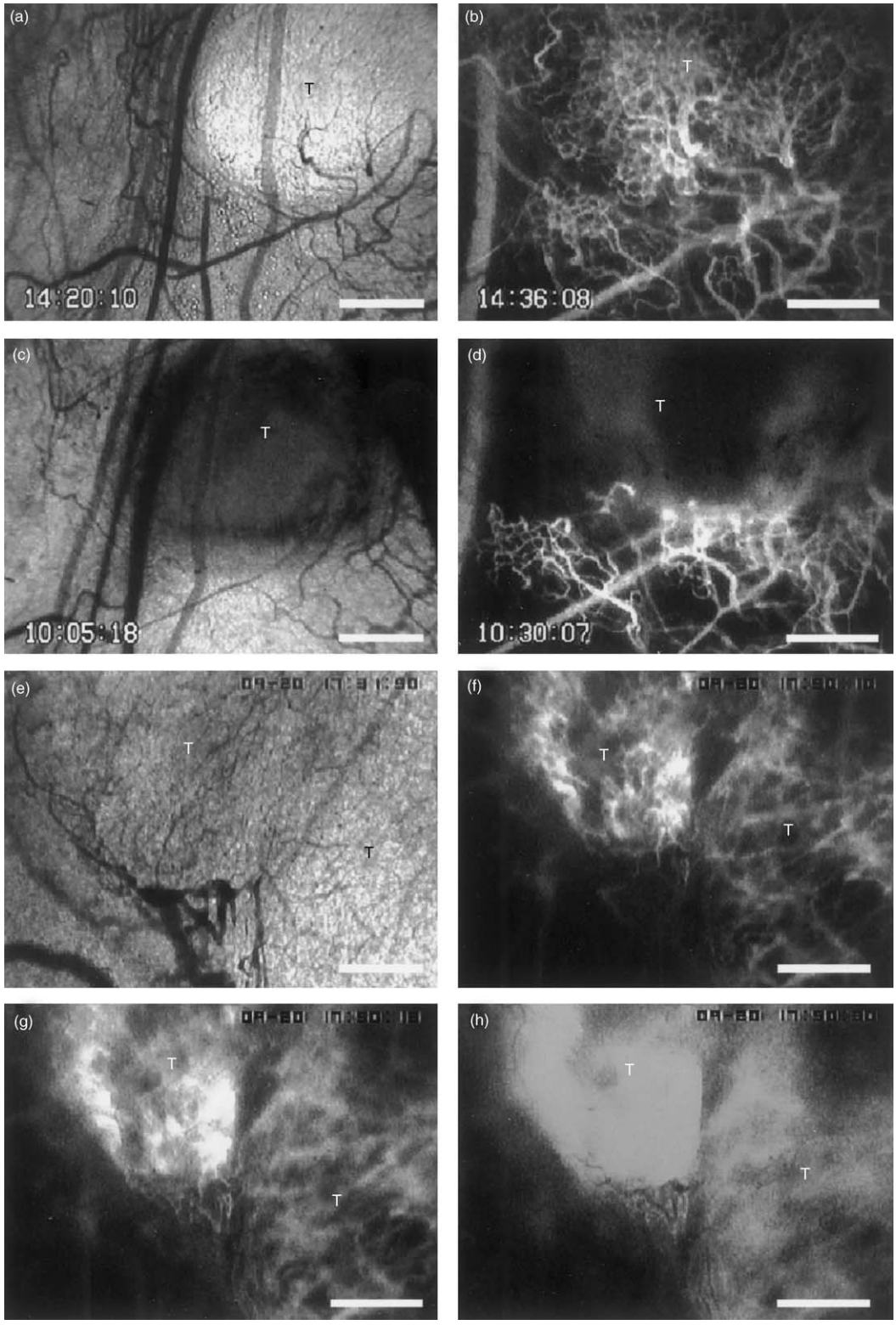

Fig. 6. Intravital fluorescence microscopic observation of the tumour vascular network after the administration of AC7700 or ADR. Vital microscopic observation was performed with the use of a transparent chamber. Original magnification ×40. Fluorescein sodium solution (10 mg/kg) was injected i.v. as a single bolus (1 ml/kg) 48 h after drug administration. (a, c, e) photographed by transmitted light. (a) SLC tumour before drug administration; (b) fluorescent angiography of the tumour shown in a; (c) the identical tumour 48 h after administration of 10.0 mg/kg AC7700; (d) fluorescent angiography of the tumour shown in c; (e) another SLC tumour 48 h after the administration of 8.0 mg/kg ADR; (f–h) fluorescent angiography of the tumour shown in e. f, 10 s later; g, 13 s later; h, 30 s later. T, tumour; bars, 500 μm.

of most cells and vessels. Intravenous administration of fluorescent dyes produced no fluorescence within the tumour vessels, which also suggested that the tumour circulation had been completely blocked.

In contrast, ADR led to the destruction of SLC tumour cells, but did not have the same effect on tumour vessels. Although the size of the tumour continued to decrease after ADR administration during the 6 days of observation, the TBF remained at its pre-administration level. A histological study after 48 h revealed considerable damage to the cancer cells, including swelling and widespread abnormal mitosis, but many blood vessels were found within the tumour tissue. Fluorescent dyes administered i.v. immediately reached the tumour vessels, which confirmed that the tumour vasculature remained intact. Moreover, tumour vascular permeability was markedly enhanced and led to a leakage of the fluorescent dye. Enhancement of vascular permeability was observed, not only with low molecular weight molecules, but also with fluorescein isothiocyanate-dextran (which has a molecular weight of 70 000) (data not shown). The fact that TBF was maintained in the ADR group demonstrates the continued presence of functional blood vessels within the tumour.

MMC had virtually no effect on either the SLC cells or tumour vessels, as evidenced by the findings that the TBF remained unchanged, there were no changes in tumour size, and no histological changes that could distinguish the MMC group from the control group treated with 0.9% NaCl.

We believe that, to reduce the size of a tumour, not only must the tumour cells be destroyed, but also the continued presence of functional blood vessels within the tumour is necessary. Rubin and Casarett [25] found that tumours showing regression after irradiation had a marked abundance of tumour vessels in comparison with a control group. We also found that when there was regression immediately after irradiation, an increase in tumour circulation occurred (data not shown). Moreover, one animal in the ADR-treated group showed complete stanching of TBF after administration of this agent, and that animal showed no changes in tumour size, similar to the effects observed in the AC7700 group. Furthermore, Sato [26] reported a clinical case showing no long-term changes in tumour size after angiotensin II-induced hypertension chemotherapy [27], but a biopsy revealed complete coagulation necrosis of that tumour. All these findings indicate a strong relationship between TBF and a reduction in tumour size.

For scavenger cells (neutrophil leucocytes and macrophages) to enter the tumour mass and process the destroyed tumour cells, tumour vessels and some degree of TBF are essential. Moreover, TBF is needed to carry macromolecules released from the destroyed tumour cells and cells that have phagocytosed tumour debris out of the tumour. In tumours that have been treated with ADR, tumour vascular permeability increases markedly, thus macromolecules can easily pass through the vessel walls, and these effects accelerate the reduction in tumour size. However, when the tumour tissue has been completely affected by coagulation necrosis, TBF is brought to a halt and the scavenger process stops. As a result, the removal of destroyed tumour cells must be performed by the blood and lymph vessels of the normal tissue that surrounds the tumour. Small tumours within a transparent chamber responded to AC7700 in the same manner as large s.c. tumours [6]. When coagulation necrosis had occurred in the small tumours, the tumours sometimes regressed. However, considerable time was necessary for the complete absorption of the necrotic tissue (data not shown).

In our experience, the overall therapeutic effectiveness of AC7700 is clearly superior to that of ADR for SLC tumours, but, as discussed above, AC7700 does not produce a rapid reduction in tumour size. For this reason, there is a strong possibility of an incorrect evaluation of the therapeutic effectiveness of drugs such as AC7700 that destroy tumour vessel functions in solid tumours. Therapy with AC7700 should be seen as effective when there are no long-term changes in tumour size. In such cases, we should keep in mind that extensive coagulation necrosis may have occurred within the tumour. Further increases in drug dosage when there is no decrease in tumour size will only lead to unfavourable side-effects.

In recent years, facilities for monitoring aspects of tumour haemodynamics in a non-invasive manner have been used to evaluate the effectiveness of vascular targeting therapies [28–30]. Clinical trials of AC7700 have begun in both the United States and Europe under a new code, AVE8062, and it is clear that the accurate evaluation of this therapy will require the introduction of such monitoring techniques. In addition, the change in tumour markers after therapy should be followed more carefully.

In conclusion, the effectiveness of therapy can be determined from changes in tumour size in cases in which tumour cells are destroyed, but the tumour vasculature remains intact. However, when both tumour cells and the tumour vasculature are destroyed, no changes in tumour size will occur for an extended period of time, and the effectiveness of therapy cannot be determined from changes in tumour size alone. Thus, current methods for evaluating the effectiveness of chemotherapy in solid tumours, on the basis of changes in size, are inappropriate for drugs that have mechanisms of action that rely on stanching of the TBF. When such drugs are used, it is essential to monitor the condition within the tumour to understand the tumour haemodynamics and therefore evaluate the therapy accurately.

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