Cancer therapy by means of irreversible tumor blood flow stasis: Starvation tactics against solid tumors

Review Article

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Abbreviations: [18]Ffluorodeoxyglucose, (18)FDG; blood flow, (BF); combretastatin A-4 phosphate, (CA-4P); combretastatin A-4, (CA-4); interstitial fluid pressure, (IFP); tumor blood flow, (TBF)

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Summary

Despite extensive research efforts, effective therapies for advanced cancers have not yet been established, and development of successful treatment strategies remains the most important task in the field of oncology. Three significant problems in conventional chemotherapy using cytotoxic drugs require attention: (i) choosing the most effective drug for individual patients, (ii) delivering a sufficient dose of drug to tumor, and (iii) minimizing severe side effects of anticancer drugs. Because the cancer cells are themselves the direct target of the drugs, these three problems cannot be avoided. We recently showed that AC7700 (currently AVE8062) a derivative of combretastatin A-4, achieved irreversible stasis of tumor blood flow (TBF), thereby causing necrosis of tumor tissue by halting the supply of nutrients. Such effects were unrelated to cancer type, in that they were found for various solid tumors. In this review, we summarize our research on AC7700 and tumor vessels and discuss how AC7700 causes stasis of TBF and why the blood flow does not resume. This technique of attacking tumor by means of blocking TBF largely avoids the three problems typically encountered in conventional cancer chemotherapy that were mentioned above. We propose that such starvation tactics constitute a new therapeutic approach to solid tumors, including refractory cancers which are resistant to conventional cytotoxic drugs and recurrent cancers that have acquired drug resistance.

I. Introduction

Cancer is a leading cause of death in many industrialized nations. Despite development of many treatments, effective approaches for all but early-stage cancers have remained illusive. Chemotherapy is typically used for advanced cancers accompanied by metastasis. The ultimate goal in chemotherapy is elimination of all cancer cells from the body by means of anticancer agents; thus, the target of the anticancer drugs has been the cancer cells themselves. Unfortunately, substances with strong cytotoxic effects on cancer cells show similar effects on other, rapidly dividing normal cells, such as hematopoietic cells and mucous membrane cells of the alimentary canal, which results in severe side effects. Some 60 years have elapsed since the first use of an anticancer agent nitrogen mustard (Gilman, 1963), but a substance with selective toxicity for cancer cells has yet to be found.

In recent years, interest has grown in therapeutic methods that target tumor vessels rather than tumor cells themselves. These approaches can be divided into two groups: one that suppresses tumor angiogenesis and one that focuses on blocking the flow of blood in the vessel that provides nutrition to the tumor. The former approach is based on the hypothesis offered by Folkman, (1971) that the tumor can forced into a dormant state if construction of the new vascular lifeline needed for tumor cell proliferation can be prevented. Thus far, many angiogenesis-preventing substances have been screened, several of which are being used in clinical trials. Recently the anti-VEGF antibody bevacizumab (Avastin) has been approved (Ferrara, 2004). Because many reviews of
antiangiogenic therapy are available (Kerbel, 2000; Liekens et al, 2001; Eskens, 2004; Cao, 2004), these drugs are not discussed here.

Studies of the latter approach blocking the flow of blood began in the late 1940s, when podophyllotoxin (isolated from the rhizome of Podophyllum peltatum) was found to induce extensive necrosis within tumors (Kelly and Hartwell, 1954). In 1954, Algire et al demonstrated that this necrosis was caused by the blocking of tumor blood flow (TFB) by podophyllotoxin. Unfortunately, this toxin shows significant toxicity and has not been used clinically.

About 30 years later, in 1983, Denekamp et al, performed experiments in which the blood flow (BF) in vessels feeding the tumor was mechanically blocked; they found a linear relationship between the duration of blockage of tumor perfusion and the suppression of tumor proliferation. Thereafter, hyalurazine (Chaplin, 1989) and flavone acetic acid (Hill et al, 1989; Zwi et al, 1989) were found to greatly reduce TBF; many studies have examined the effects of these substances in combination with anticancer agents, radiotherapy, and hyperthermia (Chaplin et al, 1989; Horsemam et al, 1991; Sakaguchi et al, 1992; Kozin et al, 1994). However, in animal experiments, the decrease in TBF induced by these substances was accompanied by markedly reduced mean arterial blood pressure (Stone et al, 1992).

In 1989, Pettit et al, discovered a new substance combrestatin A-4 (CA-4). CA-4, isolated from the bark of the South African bush willow Combretum caffrum, is a compound that inhibits tubulin polymerization and in vivo shows strong suppression of tumor perfusion (Dark et al, 1997). Many studies have since described the effects of CA-4 against various solid tumors (Horsman et al, 1998; Beaulardo et al, 1998; Tozer et al, 1999; Landuyt et al, 2000; Malcontenti-Wilson et al, 2001), and many CA-4 derivatives have been synthesized (Pettit et al, 1995; Hatanaka et al, 1998; Ohsumi et al, 1998; Nam, 2003). A water-soluble derivative of CA-4 with markedly enhanced antitumor effects known as AC7700 (currently AVE8062) was developed (Hatanaka et al, 1998; Ohsumi et al, 1998). We studied the effects of AC7700 on tumor microcirculation and showed that its antitumor effects are due to irreversible blockage of TBF (Hori et al, 1999) and that it is effective in various solid tumors (Hori et al, 1999, 2001, 2002; Nihei et al, 1999a, b). Moreover, in recent studies we clarified the microcirculatory mechanism of this irreversible TBF blockage (Hori and Saito, 2003; Hori, 2003; Hori and Saito, 2004).

In the present review, we summarize our studies on AC7700-induced irreversible TBF stasis and the consequences of this effect for treatment of solid tumors. For an proper understanding of the hemodynamics of TBF stasis, we first discuss the formation of the tumor vascular network and the unusual structural and functional characteristics of tumor vessels. We then focus on the effects of AC7700 on tumor microcirculation. Finally, we discuss how this drug disrupts function of the tumor microcirculation, which leads to necrosis of tumor tissue.

II. Formation of the tumor vascular network and blood supply

On the basis of intravital microscopic observations, we concluded that tumor angiogenesis is particularly active at the end of host terminal arterioles, which lead into true capillaries (Hori et al, 1990). Tumor blood vessels develop centrifugally from that region and form a larger vascular network (Figure 1) (Hori et al, 1992). The tumor and tumor vessels appear to develop at the trunk of terminal arterioles. In the microvascular system of normal tissue, it is possible to distinguish among arterioles, true capillaries, postcapillary venules, and venules (Wiedeman, 1984). The vascular system of tumor tissue, however, shows marked morphological changes, and it becomes impossible to identify anything but arterioles. In this arteriolar system, although the vessel diameter and framework become enlarged, few changes occur in the pattern of vessel distribution (Hori et al, 1991) and the constrictor function (Hori et al, 1993).

Figure 1 obtained by intravital microscopic observation illustrates that the blood supply to the tumor is clearly governed by a terminal arteriole from the start of formation of the tumor vascular network.

III. Structure and function of a tumor microcirculation unit

By randomly selecting a vessel within the tumor vascular network and following it upstream, one arrives at a single blood vessel (a modified terminal arteriole) that is the feeding vessel of the tumor. By following it downstream, one arrives at drainage vessels. TBF within a network perfused by a single tumor-feeding vessel typically converges into flow in a single or several drainage vessels (Figure 2). The microvascular network that includes all vessels from this feeding vessel to the drainage vessel is the smallest architectural unit of circulatory function and can be considered the microcirculation unit. Chambers and Zweifach, (1944) discovered that the normal vascular bed is composed of microcirculation units and that the size and structure of a microcirculation unit in normal tissue are extremely stable. In contrast, both size and structure of a microcirculation unit in tumor tissue change with alterations in tumor perfusion. A microvascular network within a tumor with a diameter of 500 μm in an early stage is made up of only one microcirculation unit. As the tumor and vascular network develop, the pressure within the feeding vessel increases (Hori et al, 1991), so the size of the microcirculation unit increases (Figure 1). As the tumor grows, its vascular network becomes composed of multiple microcirculation units (Horai et al, 1991).

On the basis of anatomical position and function, we classified vessels within a tumor microcirculation unit into three types (Hori, 2003): (i) tumor-feeding vessels that supply blood to the tumor vascular network, (ii) tumor capillaries that play a central role in exchange of substances within the vascular network, and (iii) vessels that allow removal of blood from the tumor vascular network, i.e., drainage vessels. In Section VII-B, we discuss how these different vessels respond to AC7700.
IV. Blockage of a tumor-feeding vessel by mechanical means

As previously mentioned, BF in the tumor vascular network is governed by feeding arterioles beginning early in the formation of the network. Thus, if the BF of the feeding vessels is blocked, BF in the microcirculation unit as a whole stops. Figure 3 shows the effect of mechanical blockage of BF in tumor-feeding vessels (Hori et al, 1991). Flow of blood to the whole region of perfusion stopped. As will be discussed in Section VII, BF of feeding vessels can also be selectively blocked by using AC7700.

Figure 1. Enlargement of the tumor vascular network. A and B, photographs of the initial stage of tumor [Sato lung carcinoma (SLC)] vascularization (photographed at a 40x magnification). Arrows indicate the terminal arteriole, T, tumor. A, 0 h; B, 72 h. Bar scale, 500 µm. C and D, overlay tracing of each photograph: the network was photographed at a 400x magnification, individual photographs were assembled into a montage, a transparent sheet was placed over the photograph, and tumor vessels were traced onto the overlay. The black vessel is the terminal arteriole; shaded vessels are tumor capillaries; arrows show BF direction. Many tumor capillaries arose from the end portion of a terminal arteriole and developed centrifugally. The tumor microvascular network from one terminal arteriole makes up one microcirculation unit. Bar scale, 100 µm. (Reproduced from Hori et al, 1992 with kind permission from Tohoku Journal of Experimental Medicine (Sendai)).
V. CA-4 and related compounds

The chemical structure of CA-4, which has a trimethoxyphenyl group (the A ring), is shown in Figure 4A. Its structure is similar to that of colchicine (Figure 4B). In fact, CA-4 is known to bind to a colchicine-binding site (Li and Sham, 2002). Moreover, podophyllotoxin (Figure 4C), which was shown by Algire et al, (1954) to cause TBF stasis, also has a trimethoxyphenyl group and is of interest because it is a tubulin-polymerizing inhibitor (Kelleher, 1978). Because CA-4 has a simple structure, many derivatives have been synthesized; AC7700 (Figure 4D) is one of them.

VI. AC7700

Because the A ring of CA-4 (Figure 4A) is thought to play an essential role in the pharmacological effects of the inhibitor, the B ring is the primary target for changes as CA-4 derivatives are synthesized. The derivative
AC7700 is produced by exchanging the OH group with an NH$_2$ group on the B ring of CA-4. This structural change leads to markedly increased antitumor effects. By means of binding serinamide to the NH$_2$ group, water solubility of the compound is increased. In the following text, we describe the pharmacological effects of AC7700 on tumor microcirculation.

### A. Effects on TBF

We studied the effects of AC7700 in a variety of tumor models, as outlined below.

#### 1. Transplanted subcutaneous tumors

AC7700 blocked TBF in different transplanted subcutaneous tumors (Figure 5) (Hori et al., 1999; Hori, 2003). Figure 5A presents data for LY80 (a subline of Yoshida sarcoma)(magenta circle), AH109A (a Yoshida ascites hepatoma)(blue circle), SLC (Sato lung carcinoma)(green circle), respectively. Cells of these three tumor types had been transplanted subcutaneously into the back of Donryu rats. Figure 5B shows data for the human esophageal tumor line TE8, which was transplanted into the nude mouse. In all of these cases, TBF decreased markedly immediately after AC7700 administration, with almost complete stasis achieved after 30 minutes.

#### 2. Autochthonous primary tumors

The proliferation rate of the transplanted tumors whose TBF data appear in Figure 5A and 5B was rapid: the volume doubling time was 1.7-2.5 days. However, the proliferation rate of tumors in cancer patients is markedly slower than that of tumors transplanted into animals (Charbit et al., 1971). For this reason, before clinical trials of AC7700, it was also necessary to determine whether AC7700 blocks TBF in slowly proliferating tumors. We therefore administered the carcinogen methylcholanthrene subcutaneously to produce autochthonous primary tumors in rats. The volume doubling time of the tumors was 5.4-55.9 days (12.6 ± 9.0 days). AC7700 produced TBF stasis in all cases (Figure 5C) (Hori et al., 2001).

### 3. Tumors growing in internal organs and lymph node metastases

Most clinical cancers for which chemotherapy is used are cases in which the tumor develops within internal organs and is accompanied by metastases. Thus, we also investigated the TBF stasis effects of AC7700 in tumors within organs and lymph node metastases. Figure 6 illustrates that AC7700 produced TBF stasis in all tumors regardless of their location (Hori et al., 2002).

### 4. Microtumors

In addition, we studied the effects of AC7700 on microtumors by means of tumors growing in a rat transparent chamber. With this intravitral microscopic observation system (Hori et al., 1990), we demonstrated that AC7700 also produced complete stasis of TBF in microtumors (Hori et al., 1999). This drug can target micrometastatic foci dispersed throughout the body.

### C. Influences of AC7700 on BF in normal tissues during TBF occlusion

To use AC7700 safely, it is important to know what BF changes occur in normal tissues during occlusion of BF to tumor tissue. Therefore, we measured changes in BF in normal tissues and organs after use of 10 mg/kg AC7700 (Hori et al., 1999).

BF in the brain cortex decreased by approximately 35%. However, the level returned to normal after 24 h. BF in the liver decreased about 50%, but it too returned to its pretreatment level, within 8 h. BF in the kidney cortex showed almost no changes. The decrease in BF in the bone marrow was about 80%. However, it returned to 80-90% of pretreatment level after 24 h. We therefore concluded that, except for kidney tissue, AC7700 caused a
reversible decrease in BF in normal tissues, which is thought to be a consequence of constriction of arterioles caused by AC7700 (see below).

**Figure 5.** TBF stasis effect of AC7700 on various subcutaneous tumors. A, magenta circle, LY80, a subline of Yoshida sarcoma (n = 13); blue circle, AH109A, a Yoshida ascites hepatoma (n = 12); green circle, SLC, a rat lung carcinoma (n = 18); B, TE8, a human esophageal cancer (n = 10). C, methylcholanthrene-induced fibrosarcoma [10 mg/kg AC7700 group (●), n = 24] vs 0.9% NaCl group (○, n = 10), P = 0.0082 (repeated measures ANOVA). AC7700 solution (10 mg/kg) was administered i.v. at 0 min. TBF in all tumors decreased markedly within 30 min. (Reproduced from Hori et al, 1999 with kind permission from Japanese Journal of Cancer Research; Reproduced from Hori et al, 2001 with kind permission from Medical Science Monitor).

**Figure 6.** TBF stasis effect of AC7700 on tumors growing in various tissues and organs. A, 0.9% NaCl group; B, 10 mg/kg AC7700 group. Blue circles, tumor growing in the kidney [AC7700 group (n = 10) vs 0.9% NaCl group (n = 8), P = 0.0004 (repeated measures ANOVA)]; brown circles, tumor growing in the liver [AC7700 group (n = 10) vs 0.9% NaCl group (n = 8), P = 0.0007]; black circles, tumor growing in the muscularis propria of the stomach [AC7700 group (n = 14) vs 0.9% NaCl group (n = 8), P = 0.0118]; green circles, metastatic foci in the cervical lymph node [AC7700 group (n = 10) vs 0.9% NaCl group (n = 8), P = 0.0065]; magenta circles, tumor growing in muscle [AC7700 group (n = 10) vs 0.9% NaCl group (n = 8), P = 0.0243]. AC7700 solution was administered i.v. at 0 min. TBF in all tumors decreased markedly within 30 min. (Reproduced from Hori et al, 2002 with kind permission from British Journal of Cancer).

**D. Changes in glucose metabolism in various tissues**

By using [18F]fluorodeoxyglucose ([18F]FDG), Kubota et al, (2002) investigated changes in metabolic functions of various tissues (tumor, brain, heart, kidney, intestine,
and bone marrow) after administration of 10 mg/kg AC7700. $^{18}$FDG uptake by tumor tissue 1 h after AC7700 administration fell to 1/10th the preadministration level, with virtually no recovery of uptake after 6 and 24 h. This finding indicates that metabolic functions of tumor tissue are lost at a relatively early time. In contrast, metabolic measures of the brain, kidney, intestine, and bone marrow at 1, 6, and 24 h after AC7700 administration did not differ significantly from control levels.

$^{18}$FDG uptake to the heart increased 2.8-fold 1 h after AC7700 administration. Uptake returned to normal and was not significantly different from the control level after 6 and 24 h, which indicates a transient change (Kubota et al, 2002). Nevertheless, this finding suggests that AC7700 induced a constriction of cardiac vessels and an increase in the load to cardiac muscle. Therefore, in clinical situations, caution should be paid to side effects on the heart and the dose of AC7700 should be determined with care.

E. Antitumor effects

We investigated antitumor effects of AC7700 resulting from TBF stasis as related to growth inhibition, hematogenous metastases, and survival rate.

1. Growth inhibition

Effects of AC7700 on growth of tumors (LY80 and SLC) and on the body weight of rats are shown in Figure 7 (Hori et al, 1999). LY80 is resistant to various anticancer drugs in clinical use. We found, however, that AC7700 markedly suppressed proliferation of these LY80 tumors (Figure 7A) without body weight loss (Figure 7C). Moreover, symptoms commonly associated with anticancer drugs, such as anemia, and diarrhea were not

![Figure 7](image-url)  
**Figure 7.** Effects of AC7700 on tumor growth and body weight. ●, 10 mg/kg AC7700; Y, 0.9% NaCl. A, growth of LY80 tumor; B, growth of SLC tumor; C, body weight of LY80 tumor-bearing rats; D, body weight of SLC tumor-bearing rats. In LY80 tumor-bearing rats, AC7700 or NaCl was given i.v. at 8, 11, 14, 17, 20, 23, and 26 days after tumor implantation. In SLC tumor-bearing rats, AC7700 or NaCl was administered i.v. at 10, 13, 16, 19, 22, 25, and 28 days after tumor implantation. In LY80 tumor-bearing rats, differences in tumor size between the AC7700 group (n = 6) and the NaCl group (n = 6) on days 13-33 after tumor implantation were highly significant ($P < 0.0001$). In SLC tumor-bearing rats, the differences in tumor size between the AC7700 group (n = 8) and the NaCl group (n = 8) on days 15-33 after tumor implantation were highly significant ($P < 0.0001$). No obvious side effect such as anemia or diarrhea was observed at the dose used in this experiment. (Reproduced from Hori et al, 1999 with kind permission from Japanese Journal of Cancer Research).
seen after administration of AC7700. This finding is thought to reflect the fact that the target of AC7700 is not the actively proliferating cells, so that the side effects of bone marrow suppression and intestinal malfunction do not occur.

SLC tumor growth inhibition was more marked (Figure 7B). Although cachexia was induced in SLC tumor-bearing rats at an early stage of tumor growth, the overall condition of the rats undergoing AC7700 therapy was favorable, with the animals showing an increase in body weight (Figure 7D). In addition, in mice into which colon 26 adenocarcinoma was transplanted to the cecum (orthotopically transplanted tumor), tumor growth was markedly suppressed by AC7700 (Nihei et al, 1999a).

In rats with methylcholanthrene-induced autochthonous primary tumors, two of five animals showed a complete cure, with no subsequent tumor proliferation after a single dose of 10 mg/kg AC7700 (Hori et al, 2001). The effects of anticancer drugs on slow-growing tumors are usually weak, but the effectiveness of treatment by means of TBF blockage was unrelated to the rate of tumor proliferation.

2. Effects on hematogenous metastases

Injection of LY80 cells into the tail vein results in formation of foci of cancer cells in nearly all internal organs except kidneys. This model of hematogenous metastases was used to evaluate AC7700 therapy. Five treatments were given at intervals of 2 days, starting on day 7 after tumor cell injection. Measurement of the number and size of tumors in the internal organs after therapy showed that tumor cell proliferation was significantly suppressed in all sites examined, including lung, liver, cardiac muscle, skin, and internal lymph nodes (mediastinale, celiacum, mesentericum, lumbare)(Hori et al, 2002). These results support our conclusion that AC7700 blocks BF to tumors growing in internal organs.

3. Effects on survival

The most important criterion for evaluating drug treatments is survival rate. In general, even when drugs suppress tumor proliferation, they often have little effect on survival rate, because both the growth rate of transplanted tumors and the reproliferation of tumor remaining after therapy are rapid.

We previously reported that AC7700 significantly prolonged survival of various tumor-bearing animals (Hori et al, 1999; Nihei et al, 1999a). In the case of LY80, survival was prolonged by 7 days, a significant increase. As mentioned earlier, LY80 is resistant to nearly all anticancer drugs in clinical use. For this reason, we believe that the significant increase in survival obtained with AC7700 is particularly important and again indicates the effectiveness of the technique of blocking BF to tumor tissue.

In the case of SLC, the tumors showed strong coagulation necrosis, and two of eight tumor-bearing rats were cured completely after scab formation and desiduation (Figure 8) (Hori et al, 1999).

Figure 8. Effect of AC7700 on survival rate of tumor-bearing rats. A. Rats received either 0.9% NaCl solution (group I, control) or 10 mg/kg AC7700 (group II) i.v. at 10, 13, 16, 19, 22, 25, and 28 days after SLC tumor implantation. AC7700 significantly prolonged the survival of tumor-bearing rats [0.9% NaCl group (n = 8) vs 10 mg/kg AC7700 group (n = 8), P = 0.0022 (log rank test)]. B. Two of the eight rats were cured completely after scab formation (arrow). (Reproduced from Hori et al, 1999 with kind permission from Japanese Journal of Cancer Research).
Ohno et al, (2002) also reported that AC7700 significantly prolonged survival of rats bearing Yoshida ascites hepatoma AH130 transplanted to the liver (orthotopically transplanted tumors). Moreover, a markedly prolonged survival rate was found in colon 38 tumor-bearing mice (Nihei et al, 1999a), which suggests that no species differences exist with regard to the effectiveness of AC7700.

VII. Microvascular mechanism of TBF stasis and induction of necrosis

In Section VI, we showed that AC7700 blocks TBF and induces necrosis of solid tumors. In this section, we address questions about the microcirculatory effects of AC7700.

A. Does AC7700 act directly on tumor vessels?

To clarify the mechanism by which AC7700 induces irreversible TBF stasis, it is essential to know the effects of this drug on microcirculation. The first question is therefore whether AC7700 acts directly on tumor vessels to bring about stasis or whether it acts on the host vascular response and thus has indirect effects on tumor vessels.

In these investigations, we dropped a small quantity of AC7700 directly on the region at which BF could be measured and followed BF changes in these regions after topical application of the drug. We found markedly greater sensitivity of normal vessels to AC7700 compared with the sensitivity of tumor vessels (Hori and Saito, 2003). Even with application of AC7700 to tumor vessels at a concentration greater than that thought likely to reach the tumor after intravenous administration, TBF did not change to a great extent. However, when AC7700 was given intravenously to the same animals, complete stasis of TBF occurred within 30 min at sites that showed no changes after topical application (Hori, 2003).

This finding strongly suggests that the main site of AC7700 action may not be the tumor vessels themselves. If AC7700 does not act directly on tumor vessels, how does it induce blockage of TBF, and why do tumor vessels occluded by AC7700 show little tendency to resume normal BF?

B. Vessel reaction to AC7700

To address these questions, we used a transparent chamber for direct observation of responses of host arterioles and tumor vessels to AC7700. The results reported below are based on experiments with 36 rats.

1. Host arterioles

The arteriolar system in subcutaneous tissue, as in other organs, shows a hierarchical structure, with bifurcation of arterioles and eventually, after several branchings, arrival at terminal arterioles (Hori et al, 1990). AC7700 produces constriction but not blockage of all arterioles of this hierarchy, which leads to an increase in vascular resistance (Hori and Saito, 2003) and thus an increase in systemic blood pressure (Hori et al, 1999).

2. Tumor-feeding vessels

As a result of the increased vascular resistance in host arterioles caused by AC7700, vessels feeding the tumor downstream show BF stasis (Hori and Saito, 2003). As discussed in Section II, one feeding vessel provides all blood to the tumor microcirculation unit and tumor vascular network usually consists of many microcirculation units supplied blood by each feeding vessel. BF stasis in feeding vessels thus leads to stasis of BF in the entire tumor vascular network.

3. Tumor capillaries

Tumor capillaries are usually composed of one layer of endothelial cells (Papadimitriou and Woods, 1975; Kornerding et al, 1989). Even when many pericytes and smooth muscle cells are present around the tumor vessels, the relation between the tumor endothelial cells and pericytes or smooth muscle cells is weak (Morikawa et al, 2000; Baluk et al, 2003; Inai et al, 2004). Therefore, tumor vessels cannot maintain a constant morphology; rather, lumens show passive enlargement (mechanical distension) when TBF increases (Suzuki et al, 1984) and passive reduction when TBF decreases (Hori et al, 1993). Even in vessels that had initially been maintained in an enlarged state because of abundant BF, AC7700 produces occlusion of BF or stricture or complete closure of the vascular lumen, such that the vessel often becomes thread-like (Figure 9A) (Hori and Saito, 2003). Stricture or complete closure of the lumen makes the tumor vessel highly resistant to subsequent reflow.

In contrast, the morphology of the vast majority of normal blood vessels was largely unaffected by changes in BF. The lumens of true capillaries 10-15 μm in diameter, unlike the lumens of tumor vessels, remained unchanged after AC7700 administration (Figure 9B). The stability of the morphology of normal capillaries is due to the support of the continuous basement membrane and pericytes (Simionescu and Simionescu, 1984; Baluk et al, 2003; Inai et al, 2004). The normal vascular lumen was maintained even with complete BF stasis after the death of the animal. Because of this structural stability, BF in normal vessels recovers within a short time, even with some decrease in BF as a result of AC7700.

4. Drainage

The most dramatic change seen after AC7700 administration occurs in the drainage vessels of the tumor vascular network. Such vessels have a sinusoidal structure, and most are dilated. BF in the drainage vessels is normally slow, and AC7700 causes further slowing. Thirty minutes after AC7700 administration, many erythrocytes stagnated within the drainage vessels, and complete stasis of BF ensued. Erythrocytes trapped within the lumen lysed after 2-3 h of BF stasis, and complete embolization of the lumens of the tumor vessels occurred (Hori and Saito, 2003). The relationship between lysis and embolization remains unclear at present.
C. Changes in tumor interstitial fluid pressure (IFP)

Tumor IFP is an important index for understanding the movement of water within the tumor interstitium. We measured AC7700-induced changes in tumor IFP by using a diffusion chamber method (Hori et al., 1986). IFP within the tumor fell immediately after AC7700 administration. When TBF was about zero, the IFP was 40-50% of the initial value. During the subsequent 6 h, neither TBF nor IFP returned to normal levels (Hori and Saito, 2003). This finding leads us to exclude the possibility that the cause of TBF stasis induced by AC7700 is compression of tumor vessels brought about by increased tumor IFP.

D. The process leading from TBF stasis to tumor necrosis

From our observations and measurements described above, we summarized the process by which AC7700 leads to blockage of TBF and necrosis of tumor tissue, as shown in Figure 10.

Initially, AC7700 produces sustained constriction of host arterioles, which leads to an increase in vascular resistance. The continuing high arteriolar vascular resistance causes a fall in perfusion pressure in the vessels feeding the tumor downstream and brings the inflow of blood to the tumor vascular network to a halt. This change reduces water volume within the tumor interstitium and is the cause of the fall in tumor IFP.

Because of the complete blockage of TBF, the lumens of tumor vessels, with their inherently weak morphology, become extremely narrow or entirely closed. Even if BF to the tumor subsequently increases, the closed tumor vessels are highly resistant to reflow.

The decrease in TBF and ultimate stoppage produce a marked reduction in the water volume within the tumor. Drainage vessels then become filled with erythrocytes, followed by hemolysis within 2-3 h. The hemolysis leads to local fibrin embolism within the tumor (data not shown) and irreversible blockage of TBF.

The complete stoppage of TBF also results in dysfunction of convection in the tumor interstitium, and a decrease in the diffusion efficiency within the tumor. These decreases in turn prevent the supply of nutrients to the solid tumor and the removal of waste and thereby lead to the death of tumor cells (Hori and Saito, 2003).

CA-4 phosphate (CA-4P) has been reported to enhance tumor vascular permeability (Tozer et al., 2001), and it has been argued that the increase in tumor IFP brought about by increased vascular permeability is an important mechanism in CA-4P-induced blockage of TBF (Tozer et al., 2002). However, blockage of TBF produced by AC7700, as just described, is unrelated to either vascular permeability or IFP. The mechanism of action of these two drugs might be different.
AC7700 administration → Continuing constriction of arterioles

Increased vascular resistance → Increase in systemic blood pressure

Continuing closure in the tumor-feeding vessels → Fall in tumor interstitial fluid pressure

Passive constriction of tumor capillaries → Stagnation of erythrocytes in the drainage vessels

Constriction or disappearance of the tumor vessel lumen → Necrosis of solid tumor

Irreversible tumor blood flow stasis

Hemolysis

Irreversible tumor blood flow stasis

Cessation of interstitial convection

Decrease in the efficiency of diffusion

Starvation

Prevention of nutrient supply to the tumor

Figure 10. Process of AC7700-induced irreversible TBF stasis and tumor necrosis. AC7700 prevents nutrient supply to the tumor, which is ultimately the cause of necrosis of the solid tumor tissue. See text. (Reproduced from Hori and Saito, 2003 with kind permission from British Journal of Cancer).

E. Verification of the hemodynamic mechanism by means of epinephrine

If the hemodynamic mechanism for irreversible TBF stasis induced by AC7700, as discussed above, is a general one, drugs other than AC7700 that produce persistent blockage of BF in tumor-feeding vessels should cause irreversible TBF stasis, similar to that caused by AC7700. We tested this hypothesis by using epinephrine (Hori and Saito, 2004). We used epinephrine because its site of action for increasing arteriolar vascular resistance is extremely similar to that of AC7700 (Hori et al., 1993b; Hori and Saito, 2003), although the duration of the effect is notably different. The effects of AC7700 persist for 2-3 h after administration, whereas those of epinephrine disappear when the drug is no longer given. We therefore hypothesized that prolonging the administration of epinephrine for 2-3 h could induce tumor necrotic effects similar to those of AC7700.

We found that TBF returned to normal immediately after 0.3 mg/ml epinephrine administration when the drug was applied for only 30 min (at a rate of 0.015 ml/min). After a 60-min administration, TBF recovered, but not to the original level. After a 120-min administration, however, TBF did not recover the BF stoppage was irreversible like the case of AC7700. Moreover, extensive necrosis within the tumor was found, as predicted (Hori and Saito, 2004).

We conclude that the hemodynamic mechanisms by which AC7700 and epinephrine stop TBF are extremely similar. Although it is still uncertain why AC7700 causes stricture of host arterioles, it is likely that the site of action of AC7700 is similar to that of epinephrine, i.e., vascular smooth muscle. Further study is required to determine whether specific receptors for AC7700 exist on smooth muscle and whether the epinephrine α receptor is involved in the increased vascular resistance induced by AC7700.

VIII. Evaluation of the therapeutic effect

The degree of reduction in tumor size is generally thought to be important for evaluation of cancer treatment. Our own research has shown that tumor size is markedly reduced when tumor cells are destroyed but that tumor vessels still function. In contrast, however, the degree of tumor size reduction is relatively small when both tumor cells and tumor vessels are destroyed, as is the case with AC7700 (Hori et al., 2003). To obtain prominent reductions in tumor size, the destroyed tumor cells must be moved to outside of the tumor region. For that purpose, scavenger cells (neutrophilic leukocytes and macrophages) must enter the tumor mass and process the tumor cells destroyed by the treatment. Because blockage of BF to the tumor prevents the arrival of scavenger cells, tumor debris that follows necrosis remains at the site of the once-active
tumor. For this reason, therapy with AC7700 does not reduce tumor size to a great degree, even though the tumor itself has been destroyed (Hori et al, 2003).

In light of these findings, we conclude one cannot use tumor size reduction as the basis of the evaluation of the effectiveness of drugs such as AC7700 against solid tumors. For a more precise evaluation, greater attention should be paid to intratumor hemodynamics (Gee et al, 2001; Anderson et al, 2003; Stevenson et al, 2003; Thoeny et al, 2005) and changes in tumor markers (Bocci et al, 2004).

IX. Conclusion

Three significant problems in conventional cancer chemotherapy must be addressed. The first concerns drug sensitivity. Because cancer cells in each patient have diverse properties, it is essential to select drugs that have appropriate therapeutic effects. Recent research has focused on drug sensitivity at the genetic level (Zembutsu et al, 2002), but clinical application of the research will take time. The second problem concerns drug delivery. Delivery of anticancer drugs to tumor tissue depends greatly on TBF (Suzuki et al, 1981, 1984; Jain and Ward-Hartley, 1984). However, TBF decreases with increasing tumor volume (Gullino and Grantham, 1961; Vaupel et al, 1987; Hori et al, 1993a). To enhance delivery of anticancer drugs to tumor tissue, we must increase TBF when the drugs are administered (Suzuki et al, 1981, 1984; Sato et al, 1995). The third problem pertains to side effects. Substances thus far screened as candidate anticancer drugs have been those with cytotoxic effects on rapidly dividing cells; therefore, side effects on rapidly proliferating normal tissues are, in principle, inevitable.

As discussed above, treatments that focus on blockage of TBF contrast sharply with anticancer drug therapies. By causing selective dysfunction of tumor vessels, we can attack solid tumors through starvation tactics rather than a direct assault on the cancer cells themselves. Therefore, the three problems in cancer chemotherapy that were just mentioned can be largely avoided. Starvation may become an effective strategy for all solid tumors, including refractory cancers, because the therapeutic effect is independent of tumor location and type.

References


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