Antitumor Effect of Interleukin 1α in Combination with Hyperthermia

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ABSTRACT

The combined effects of interleukin-1α (IL-1α) and hyperthermia on SCK tumors grown in the legs of A/J mice were investigated. When the host mice were given i.p. injections of 25 µg/kg IL-1α, the tumor blood perfusion, as measured with the 86Rb uptake method, significantly declined, reaching minimum blood perfusion in 3-5 h. Although the tumor blood perfusion started to rise thereafter, the recovery was still incomplete 1 day later. Hyperthermia at 42.5°C or 43.5°C for 1 h also caused a marked decline in tumor blood perfusion. When the tumors were heated 5 h after an i.p. injection of IL-1α at 25 µg/kg, at which time the tumor blood perfusion was low, the tumor blood perfusion decreased further. The heating of tumors at 42.5°C or 43.5°C for 1 h significantly reduced the clonogenic cell number in the tumors, delayed the tumor growth, and prolonged the survival time of host animals. The antitumor effect of 25 µg/kg IL-1α alone, as judged from the clonogenic cell number, tumor growth delay, and host survival, was smaller than that of hyperthermia. When the host mice were treated with IL-1α and the tumors were heated 5 h later, the decline in the clonogenic tumor cell number, tumor growth delay, and prolongation of host survival were significantly greater than those produced by either treatment alone. It was concluded that the prior reduction of blood perfusion by IL-1α potentiated the antitumor effect of subsequent heatings.

INTRODUCTION

Tumor cells cannot survive and proliferate without a properly functioning vascular network. Therefore, destruction of vascular networks in tumors may lead to cell death and necrosis in the tumors and, thus, tumor vasculature may serve as a target of tumor therapy (1). In fact, one of the mechanisms through which tumor cells in vivo are killed by hyperthermia is believed to involve injury to the tumor vasculature by heat accompanied by impairment of blood circulation (2-5). It has recently been reported that the cell death produced by certain cytokines in solid tumors is also mediated, at least in part, through vascular injury (6-14). IL-1α is a multifunctional cytokine which has been reported to cause numerous types of pathophysiological and metabolic damage in murine tumor systems, whereas its direct cytotoxic effect in vitro is rather limited (7, 9). Braunschweiger and colleagues (7, 13-15) reported that IL-1α caused marked vascular injury in murine tumor models, accompanied by reduction in blood perfusion, vasodilation, vascular congestion, extravascular hemorrhage, increase in extravascular water, and decline in bioenergetic status.

Hyperthermic damage to tissues is dependent on temperature, which in turn is dependent on heat dissipation from the tissues through blood circulation during heating (2-5). It follows that prior impairment of blood circulation in tissues would potentiate the hyperthermic damage in the tissues. Attempts have been made to enhance thermal damage in tumors by reducing the tumor blood circulation with vasoactive compounds, such as hydralazine (16, 17), or by inducing hyperglycemia (18-20). The possibility that vascular injury by IL-1α might enhance the hyperthermic damage in tumors has also been indicated (21). The purpose of the present study was to test the possibility that the vascular injuries produced in tumors by IL-1α and by hyperthermia might interact with each other and that reduction in blood perfusion by IL-1α might enhance the hyperthermic damage in tumors.

MATERIALS AND METHODS

Tumor and Drug Treatment. SCK tumors (adenocarcinomas) grown in the right hind legs of female A/J mice were used (22). This is a spontaneous tumor which developed in a female mice. The early generations of the cells are stored in liquid nitrogen. This tumor is nonimmunogenic to A/J mice and grows rapidly without metastasizing. The tumor cells also grow well in vitro, with a plating efficiency of about 70% for the subculture. The SCK cells in exponential growth phase in culture were dispersed to single cells by treatment with 0.25% trypsin solution (Difco Laboratories, Detroit, MI) at 37°C for 10 min. The resultant single cells were washed 3 times with RPMI 1640 culture medium. The IL-1α concentration was adjusted so 25 µg/kg IL-1α could be injected i.p. at a volume of 0.01 ml/g of body weight. Control tumor-bearing mice received saline alone at 0.01 ml/g of body weight.

Heating. The tumors grown in the mouse legs were heated without anesthesia, as previously reported by us (22, 23). Mice were placed on specially designed Plexiglas jigs and the tumor-bearing legs were extended and anchored to a supporter on the jig. The jigs were then placed on a Plexiglas shelf, immersing the anchored legs into a preheated water bath (Thermonix 1400; B. Braun Co., Melsungen, Germany). When the water was heated to 42.5°C or 43.5°C, the intratumor temperature was 0.1-0.3°C lower than the water temperature, as measured with copper-constantan thermocouples. The animal rectal temperature rose to 38-39°C after heating of tumors with 42.5°C or 43.5°C water for 1 h.

Measurement of Blood Perfusion. The blood perfusion in tumors and normal tissues was measured with the use of the 86RbCl uptake method, as described elsewhere (22, 23). Note that the 86RbCl uptake method does not provide an absolute value (in terms of ml/min/g) for blood perfusion in tissues; it merely indicates the fraction of cardiac output into each tissue. About 5 µCi of 86RbCl in 0.1 ml of 0.9% NaCl, pH 7.4, were injected into the tail veins of mice, which had been anesthetized with an i.p. injection of sodium pentobarbital (60 mg/kg) about 10 min earlier. The tumor-bearing legs were amputated with a pair of sharp scissors 90 s later, and the animals were immediately killed by cervical dislocation. When the 86RbCl uptake during heating was to be determined, the injection of 86RbCl was done while the tumor-bearing legs were still in the heating water, and then the animals were removed from the water bath 60 s later. The removal of the animals from the water bath and freeing of the animals from the heating jig took about 20 s, so that the leg amputation could be done 90 s after the isotope injection. The tumors and other tissues or organs were then dissected and weighed and their radioactivities were counted with a well-type gamma counter (1282 Compugamma Cs; Pharmacia LKB Nuclear, Inc., Gaithersburg, MD). The tail of each animal was also removed and the radioactivity was counted. Whenever the 86Rb radioactivity in the tail was >5% of the injected 86Rb, the tumor and tissues from that mouse were excluded from further analysis. Since heating...
causes varying degrees of edema, the dry weight of tissues was obtained by
drying them overnight in a 100°C oven and the percentage of 86Rb accumu-
lated per gram of dried tissue was calculated.

Clonogenicity of Tumor Cells. The changes in clonogenic cell numbers in
tumors produced by hyperthermia and IL-1α alone or in combination were
determined with an in vivo-in vitro excision assay method. The clonogenic
assay was done 5 h after IL-1α injection or immediately after heating, which
was applied 5 h after IL-1α injection. The tumors were excised under sterile
conditions, weighed, minced, suspended in trypsin solution containing a small
amount of DNase (30 ml of 0.25% trypsin plus 1 ml of 0.05% DNase), and
continuously agitated for 30 min at room temperature. The cell suspension was
filtered through 8-ply sterile gauze and washed with RPMI 1640 medium
containing 10% calf serum, and the number of viable cells able to exclude
trypan blue per gram of tumor was determined. The number of clonogenic cells per gram of tumor was
calculated from the number of recovered cells per gram of tumor and the
plating efficiency of tumor cells. Eight to 10 tumors were used for each
treatment.

Tumor Growth Study. The host mice bearing 7-8-mm tumors were given
i.p. injections of 25 μg/kg IL-1α (in 0.01 ml/g of body weight) or of an
equivalent volume of 0.9% NaCl. As shown in “Results,” the tumor blood
perfusion declined to its smallest value 3-5 h after an i.p. injection of IL-1α.
In order to heat the tumors when the blood perfusion is lowest, the tumors were
heated, as described above, at 42.5°C or 43.5°C for 1 h starting 5 h after the
IL-1α injection. A group of mice received IL-1α treatment alone. The tumor
size was measured with a caliper and the tumor volume (V) was calculated
with the following formula: 

\[ V = \frac{1}{2} ab \]

where a and b are the shortest and longest diameters of the tumor, respectively.

Statistical Analysis of Data. The Student’s t test was used to determine the
statistical significance of any changes observed. Differences at the P < 0.05
level were judged to be statistically significant.

RESULTS
The effect of IL-1α on the blood perfusion in SCK tumors, as
measured with 86Rb uptake, is illustrated in Fig. 1. The 86Rb uptake
in control tumors was 17.6%/g (dry). The 86Rb uptake in tumors markedly decreased upon treatment of the host with IL-1α at 25
μg/kg, reaching less than one third of control (P < 0.05) in 3-5 h and
then slowly recovering. At 10 h after IL-1α treatment, the 86Rb uptake
in tumors was about 50% of control and it recovered further to about
75% of control at 24 h. The 86Rb uptake in the muscle and skin was
not affected by IL-1α. The 86Rb uptake in the control kidney and
spleen was 152.2 ± 6.4%/g (dry) and 47.2 ± 3.3%/g (dry), respec-
tively, and it slightly declined to 129.4 ± 6.9%/g (dry) and 42.8 ±
3.7%/g (dry), respectively, 5 h after an i.p. injection of 25 μg/kg
IL-1α. (The dry weights of kidney and spleen are about 0.037 g and
0.031 g, respectively.) When the mice were given injections of IL-1α at 25
μg/kg and the tumors were heated 5 h later, the 86Rb uptake reached 23.0%/g (dry) from the control value of
17.6%/g (dry). The 86Rb uptake in the tumors then declined to
10.8%/g (dry) 1 h after heating and slowly recovered to control levels
1 day later. The aforementioned increase in 86Rb uptake at the end of
1 h of heating and the decrease in 86Rb uptake 1 h after heating were
statistically significant (P < 0.05). When the host mice were given
injections of IL-1α at 25 μg/kg and the tumors were heated 5 h later
at 42.5°C for 1 h, the 86Rb uptake declined to as little as 3.5%/g (dry).
One day after a combined treatment with heating at 42.5°C for 1 h and
IL-1α injection, the 86Rb uptake was 13.4%/g (dry), which was
slightly but not statistically smaller than that in the tumors which
received heating alone. When heated at 43.5°C for 1 h, the 86Rb uptake
further decreased to about 4.6%/g (dry) at 5 h. At 24 h after heating
at 43.5°C, the 86Rb uptake in the tumors was still significantly smaller
than the 86Rb uptake in the control tumors (P < 0.05). When the host
mice were treated with IL-1α and the tumors were heated 5 h later at
43°C for 1 h, the 86Rb uptake declined to 3.1%/g and remained at a
similar level for up to 1 day after treatment.

The changes in the number of clonogenic cells per gram of tumor,
\textit{i.e.}, product of recoverable cell number per gram and plating efficiency
of the cells, following various treatments are shown in Fig. 3. The plating efficiency of control tumor cells was 61.0 ± 3.7%. The number of clonogenic cells/g was 8.29 ± 0.68 × 10^7 in control tumors and it decreased to 2.87 ± 0.35 × 10^7 and 9.03 ± 1.14 × 10^7 following heating at 42.5°C and 43.5°C, respectively, for 1 h. These decreases were due both to the decrease in the number of cells recovered and the decrease in plating efficiency. The clonogenic cell number in the
tumors 5 h after an i.p. injection of 25 μg/kg IL-1α was 8.25 ± 2.11
× 10^6. When the mice were given i.p. injections of 25 μg/kg IL-1α and
the tumors were heated 5 h later at 42.5°C or 43.5°C for 1 h, the
clonogenic cells/g declined to 7.64 ± 1.23 × 10^6 and 1.12 ± 0.35 × 10^6,
respectively.
The control tumor volume increased 4-fold in 4 days (Fig. 4). When heated at 42.5°C for 1 h, the tumor volume increased 4-fold in 5.5 days, a growth delay of 1.5 days. Treatment of host mice with 25 μg/kg IL-1α alone delayed the tumor growth by 1 day. When 25 μg/kg IL-1α was injected and the tumors were heated 5 h later at 42.5°C for 1 h, the tumor volume increased 4-fold in 8 days, a growth delay of 4 days. Heating at 43.5°C for 1 h alone resulted in a tumor growth delay of 3 days. When the host mice were given i.p. injections of 25 μg/kg IL-1α and heated 5 h later at 43.5°C for 1 h, the tumors grew to 4 times the size of the initial tumor in 11 days, a growth delay of 7 days.

The percentage of survival of tumor-bearing mice after various treatments is shown in Fig. 5. The control mice which received no treatment started to die from day 4, and the animals all died by day 10, with 50% death occurring by day 7. The major cause of death in host mice appeared to be the growth of primary tumors, since SCK tumors rarely metastasize. An i.p. injection of IL-1α at 25 μg/kg or heating of the tumors at 42.5°C for 1 h slightly prolonged the survival of the host animals. Heating the tumors at 43.5°C for 1 h significantly increased the survival of host animals, with 50% death occurring by day 13. The survival time of mice was markedly increased when IL-1α treatment and hyperthermia were combined. When the host mice were given i.p. injections of 25 μg/kg IL-1α and the tumors were heated 5 h later at 42.5°C or 43.5°C for 1 h, the mice started to die on day 11. One half of the mice treated with IL-1α in combination with heating at 42.5°C and 43.5°C died by days 16 and 17, respectively, as compared with day 7 for the untreated control mice. Whereas all control mice died within 10 days, some animals could live as long as 30 days after receiving the combined treatment of IL-1α and tumor heating, although all of these treated mice eventually died from regrowth of the primary tumors.

DISCUSSION

The purpose of the present study was to reveal the combined effects of IL-1α and hyperthermia on tumors. It was postulated that a reduction of tumor blood perfusion produced by IL-1α might augment the tumor damage produced by subsequent heating. Therefore, first we treated the host mice with IL-1α and then the tumors were heated 5 h later, at which time the tumor blood perfusion was significantly reduced. The magnitude and kinetics of the decline in 86Rb uptake or blood perfusion in SCK tumors caused by an injection of 25 μg/kg IL-1α (Fig. 1) are similar to those observed in the RIF-1 fibrosarcoma of C3H/HeJ mice (7, 13, 14). In both SCK tumors (Fig. 1) and RIF-1 tumors (7, 14), the 86Rb uptake declined to about one third of control by 3–5 h after an i.p. injection of 25 μg/kg IL-1α and gradually recovered thereafter, although the 86Rb uptake at 24 h was still significantly smaller than that in the control (P < 0.05). The decline in 86Rb uptake in Panc02 pancreatic adenocarcinoma of C57BL/6J mice produced by 25 μg/kg IL-1α was faster and more extensive than that in RIF-1 tumors (7).

The decline in blood perfusion in murine tumors produced by IL-1α injection has been reported to accompany an increase in vascular permeability, intravascular congestion, and hemorrhagic necrosis (6–15). Such pathophysiological changes in tumors produced by an i.p. injection of IL-1α are apparently related to the acute inflammatory reactions in normal tissues following intradermal or s.c. injection of IL-1α or TNF-α (24, 25). It has been demonstrated that inflammation induced by either IL-1α or TNF-α is accompanied by stimulation of endothelium. IL-1α and TNF-α render endothelial cells superadhesive to circulating polymorphonuclear leukocytes by inducing endothelial cell surface expression of leukocyte adhesion molecules (26–31).

Massive adhesion and accumulation of leukocytes on the surface of capillary endothelium would impede blood perfusion through the capillaries. IL-1α and TNF-α have also been reported to stimulate endothelial cells to produce procoagulants (30–32), plasminogen activator inhibitor (33–35), and prostaglandins (36). In addition, IL-1α and TNF-α directly cause damage and induce morphological changes in endothelial cells in vitro (37, 38).

In contrast to the marked reduction in blood perfusion in mouse tumors produced by IL-1α, as measured with the 86Rb uptake method, the blood perfusion in the normal muscle and skin of C3H mice was affected little by IL-1α (9). In mice bearing Meth A tumors, TNF-α
injection induced massive thrombus formation in the tumor vasculature but not in the vessels of normal tissues (8). We also noticed that reduction in 82Rb uptake produced by IL-1α in the normal tissue of A/J mice was rather small (see “Results”). The cause of such preferential perturbation of blood perfusion or damage to vessels in tumors, relative to those in normal tissues, produced by IL-1α or TNF-α is unknown. The differences in the structure of vessels and/or differences in the density of receptors for effectors or inhibitors of cytokines may account for the differential response to the cytokines by vessels in tumors and normal tissues (7). It was reported that tumor-derived mediators may augment the procoagulant response of tumor endothelial cells to TNF-α (39). In this connection, hypoxic conditions have been reported to suppress the procoagulant cofactor thrombomodulin and to induce an activator of factor X in endothelial cell cultures (40, 41). It is conceivable that such a decline in procoagulant factor in tumors due to hypoxia enhances the intravascular coagulation produced by IL-1α or TNF-α. The bioenergetic status in murine tumors, as measured with 31P-nuclear magnetic resonance spectroscopy, has been shown to be decreased by IL-1α and similar cytokines (12, 13). Such a deterioration of bioenergetic status in the tumors with the cytokines could be attributed to the deterioration of the microcirculation. Note that, although IL-1α and TNF-α cause similar pathophysiological changes in tumors, there has been a report that IL-1α-induced microvascular injury in vivo is not mediated through TNF-α (42).

The effects of hyperthermia on the blood flow in SCK tumors have been extensively investigated by us (2, 22, 43). One of the hypotheses for the cause of the heat-induced decrease in tumor blood flow (Fig. 2) is adhesion of leukocytes to the tumor endothelial cells (5), as is the case for the reduction of tumor blood flow by IL-1α or TNF-α. It is not surprising that heating the tumors 5 h after IL-1α injection further reduced the 82Rb uptake, which was already low due to the IL-1α injection, and that the cell death (Fig. 3) and tumor growth delay (Fig. 4) produced by IL-1α injection followed by heating 5 h later were significantly greater than those produced by IL-1α injection or tumor heating alone. Note that, whereas the differences in the clonogenic cell numbers in the tumors treated with IL-1α or heating at 42.5°C for 1 h alone or in combination were insignificant (Fig. 3), the tumor growth delay produced by IL-1α injection in combination with heating at 42.5°C for 1 h was greater than that produced by IL-1α or heating alone (Fig. 4). It appears that the clonogenic cell assay in the present study underestimated the actual cell death produced by heating alone or by the combined treatment of IL-1α and heating; the clonogenic cell assay was done immediately after heating, whereas it has been known that additional cell death occurs over several hours after heating due to vascular damage and ischemia (2, 22, 44). In light of the fact that SCK cells are nonimmunogenic to A/J mice, it is highly unlikely that an immune reaction is involved in the delayed cell death in the treated SCK tumors. The antitumor effect of IL-1α in combination with hyperthermia is dramatically demonstrated in the survival of host mice, as shown in Fig. 5. The mice treated with IL-1α and hyperthermia started to die as late as 10 days after treatment, at which time all the control mice as well as the mice which received IL-1α treatment alone were already dead.

In conclusion, our results strongly suggest that the greater tumor cell killing and tumor growth delay produced by the combination of IL-1α and hyperthermia, compared with those produced by IL-1α or hyperthermia alone, resulted from an increase in vascular damage. However, the possibility of an increase in direct killing of tumor cells by the combination of IL-1α and hyperthermia, relative to cell killing by either treatment alone, may not be completely eliminated. Regardless of the mechanisms, the results obtained in the present study strongly indicate that the combination of vascoactive cytokines, such as IL-1α, with hyperthermia may be potentially useful for the control of human tumors.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Peter T. Lomedico, Hoffman-LaRoche, for the generous gift of IL-1α and Peggy Evans for her assistance in the preparation of the manuscript.

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