Halothane Potentiates the Antitumor Activity of Gamma-interferon and Mimics Calmodulin-blocking Agents

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This study reports effects of halothane on tumor cells in vitro. Cells from the human colon cancer cell line HT-29 were exposed to various concentrations of halothane for 8-72 h. The effect of this exposure on this colon cancer cell line, with and without coinubcation with the biologic response modifier gamma-interferon (IFN-γ), was studied. Using the tumor target cell survival (TTCS) assay, concentrations of halothane from 0.5 to 2% markedly augmented the antitumor activities of IFN-γ against HT-29. The tumor cell cytostatic effects of IFN-γ in the 0.75-6-unit/ml range were increased nearly 400% by concentrations of halothane as low as 1%. These results were confirmed in a separate cytolytic assay (Indium-111 release assay), which revealed that halothane concentrations in the 2-4% range markedly increased the cytolytic capacity of IFN-γ at doses of IFN-γ between 75 and 1,250 units/ml. The cytolytic activity of IFN-γ was increased nearly 300% by doses of halothane as low as 1%. A nearly identical pattern of augmentation of IFN-γ-induced antitumor activity was observed when the known calmodulin inhibitor trifluoperazine (TFP) was coinubcated with IFN-γ. At concentrations of 4-10 μM, the antitumor activity of IFN-γ was increased nearly 400%. These observations suggest that the pattern of halothane potentiation of the antitumor activity of IFN-γ is similar to that exhibited by known calmodulin inhibitors. (Key words: Anesthetics, volatile; halothane. Calmodulin inhibitors; trifluoperazine (TFP). Gamma-interferon. Tumor cell cytotoxicity.)

THE SHORT- AND LONG-TERM effects of anesthetic agents on patients with cancer are not presently known. Some studies using animal models suggest that anesthetic agents enhance tumor cell growth.1,2 Previous clinical studies have produced few useful conclusions, both because of a lack of understanding of the precise mechanisms of human cancer immunosurveillance and because of a failure to segregate anesthetic agent effects from many other perioperative factors involved in cancer surgery.3

The purpose of the experiments performed in this report was to assess the effect of halothane on human tumor cell growth directly by the use of two new in vitro assays—the tumor target cell survival (TTCS) assay and the Indium-111 release assay. The influence of halothane on the known tumor cytostatic and cytolytic effects of the biologic response modifier, gamma-interferon (IFN-γ),1-9 on the human colon cancer cell line HT-29 was investigated. The effects of halothane on this system also were compared to the effects of trifluoperazine (TFP), a known calmodulin inhibitor, on the same system.

Materials and Methods

CELL LINE

The human colon cancer cell line HT-29 was obtained from the American Type Tissue Culture Collection (Rockville, MD) and was passaged in cultures, as described previously.9

HALOTHANE ANESTHESIA DELIVERY SYSTEM

The in vitro anesthetic delivery system has been described previously in detail.10 Briefly, cultured HT-29 cells were exposed either to humidified 5% CO2 and room air or to halothane concentrations of 0.5, 1%, 1.5%, 2%, or 4% plus humidified 5% CO2 and room air for periods of time ranging up to 72 h. Exposure chambers were maintained inside a 37 °C incubator, with excess anesthetic gases scavenged from the experimental area through a conduit connected to a chemical exhaust fume hood. The halothane vapor was prepared by passing gas through newly calibrated Drager vaporizers, set to the above-cited concentrations, and then through humidifiers and precision flow meters (Mandset™, model 36-541-035). The control gas passed through the same apparatus but without the vaporizer. To ensure that each test chamber actually received the anesthetic concentration of gas indicated by the vaporizer setting, the halothane concentrations were monitored by a volatile anesthetic agent monitoring system (AAM222, Puritan Bennett, Wilmington, MA) at the point of gas entry and gas exit for each chamber; in ad-
dition, gas samples were monitored inside each Plexiglass chamber with the AAM222 apparatus. At no time during data collection did the actual halothane concentration inside the enclosure chamber vary more than 0.1% from the target halothane concentration.

**IFN-γ**

Recombinant IFN-γ was supplied by the Genentech Corporation (South San Francisco, CA); as a confirmatory supply, IFN-γ was also obtained from the Biogen Research Corporation (Cambridge, MA). In the TTCS assay (see below), IFN-γ was used in a range of concentrations between 0 and 25 units/ml for varying culture time periods. For the Indium-111 cell lysis assay (see below), IFN-γ was used in a range of 0–5,000 units/ml for varying culture time periods.

**CALMODULIN INHIBITOR**

TFP obtained from Sigma Chemical (St. Louis, MO) was employed at ranges of 0–9 μM for various culture time periods.

**TUMOR TARGET CELL SURVIVAL ASSAY**

The TTCS was performed as described by Carmichael et al. The assay depends on the cellular reduction of dimethylthiazol diphenyltetrazolium bromide (MTT) by the mitochondrial dehydrogenase of viable cells to a blue formazan product that can be measured spectrophotometrically. After 72 h of incubation of the tumor cells with halothane or TFP (± IFN-γ), the MTT test solution was added to the plate wells. Two hours later, the corresponding MTT-solution-exposed wells were read. Cell survival values presented in this report were calculated by the following formula:

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\text{Tumor target cell survival (\%)} = 100 \times \frac{\text{MTT reading of control cells} - \text{MTT reading of exposed cells}}{\text{MTT reading of control cells}}
\]

**TUMOR CELL LYSIS ASSAY**

The Indium-111 tumor lysis assay was performed in the following manner. Single cell suspensions were obtained from the disaggregation of the adherent cell line, HT-29, by EDTA/trypsin treatment of monolayer cultures. These nonadherent cells were then checked for viability by the trypan blue exclusion method and manual counting. The HT-29 cells were washed twice and labeled with Indium-111 (Amersham, Arlington Heights, IL) and then washed again and subsequently plated in polystyrene plates in 10% fetal calf serum. At this time, the IFN-γ and the calmodulin blocking agents were added to the individual wells. The cells were allowed to incubate for 72 h and then were harvested in a Scatron harvester. Percent cytolyis was calculated as the percent of Indium-111 released into the supernatant of each well, as previously described.

**STATISTICAL ANALYSIS**

Statistics were performed with the Scheffe multiple-comparison test after a one-way analysis of variance (ANOVA) test was performed on the pooled data. A P value ≤0.05 was considered significant.

**Results**

**AUGMENTATION OF THE ANTITUMOR ACTIVITIES OF IFN-γ BY HALOTHANE**

As shown in figure 1, the addition of IFN-γ to the human colon cancer cell line HT-29 produces a decrease in tumor cell survival, as has been described previously. A 72-h exposure to halothane alone exerts an approximately 20% decrease in the survival of HT-29 in the 0.5–1.5% concentrations; 2% halothane alone has a 50% inhibitory effect on tumor cell survival. Within the range of 0.75–6 units/ml, IFN-γ inhibits the survival of HT-29 cells from 2 to 20%. The addition of 0.5% halothane produced a 20% increase in the inhibitory effect of IFN-γ over the 0.75–6.0-units/ml concentrations of the 72-h assay. Higher halothane concentrations (between 1 and 2%) produced even more marked IFN-γ-mediated inhibition of cell survival (generally in excess of 400% augmentation). This augmentation of IFN-γ activity was noted at all concentrations of halothane tested and at all IFN-γ concentrations between 0.75 and 6.0 units/ml (P ≤ 0.01). The shortest time period of exposure of the cultured cells to halothane that produced this IFN-γ-potentiating effect was 8 h (data not shown).

**AUGMENTATION OF THE ANTITUMOR ACTIVITIES OF IFN-γ BY TFP**

As shown in figure 2, the calmodulin-inhibiting agent TFP also inhibits the baseline tumor cell survival after exposure to IFN-γ.

In the 4 μM dose range, TFP did not alter IFN-γ activity in the 0.75–3-units/ml range. However, higher concentrations of TFP increased the antitumor effect of 0.75–3.0 units/ml of IFN-γ by over 400% (P ≤ 0.01).

**AUGMENTATION OF THE TUMORICIDAL ACTIVITIES OF IFN-γ BY HALOTHANE**

As figure 3 illustrates, IFN-γ displayed a baseline tumoricidal activity against HT-29 in the concentration ranges between 75 and 5,000 units/ml. Exposure of the cells to 0.5% halothane for up to 72 h did not significantly alter this baseline tumoricidal activity. However, halo-
HALOTHANE POTENTIATES THE ANTITUMOR ACTIVITY OF IFN-γ

Discussion

Anesthesiologists and surgeons have long been concerned that anesthetic agents used during operative procedures might influence the course of unresectable cancer or might contribute to metastatic disease development after total macroscopic resection of tumor. The effects of clinically used anesthetic agents on tumor cells and cancer immunosurveillance are not yet well established.

The effects of anesthetic agents on overall human immune system function have been studied extensively for many decades; several reviews have been published.2,12–16 Despite extensive data, it has been difficult

Fig. 1. Augmentation of gamma interferon–mediated tumor cell inhibition by halothane. The MTT tumor target survival assay was performed for 72 h. All cultures received gamma interferon plus the concentrations of halothane shown. Results displayed are means and standard error bars from triplicate samples. The halothane-potentiated percent survival was significantly different (P > 0.01) than nonexposed cells at gamma interferon concentrations greater than 0.

THANE CONCENTRATIONS BETWEEN 1 AND 4% MARKEDLY POTENTIATED THE TUMORICIDAL ACTIVITY OF IFN-γ IN THE IODINDUM-111 RELEASE ASSAY. ALL HALOTHANE CONCENTRATIONS TESTED ABOVE 1% PRODUCED CYTOLYTIC AUGMENTATION OF 300–400% (P ≤ 0.01).

AUGMENTATION OF THE TUMORICIDAL ACTIVITIES OF IFN-γ BY TFP

As shown in figure 4, IFN-γ-mediated HT-29 tumor cell cytolysis was augmented by the calmodulin inhibitor TFP. TFP concentrations above the 4 μM range markedly potentiated the tumoricidal activity of IFN-γ in the Indium-111 release assay (P ≤ 0.01).

Fig. 2. Augmentation of gamma interferon–mediated tumor cell inhibition by TFP. The TTCG was performed for 72 h. All cultures received gamma interferon plus the concentrations of TFP shown. Results displayed are means and standard error bars from triplicate samples. The 6 and 8 μM TFP potentiated percent survival was significantly different (P > 0.01) than non-TFP-exposed cells at gamma interferon concentrations greater than 0.
Although some specific elements of immunity are depressed postoperatively, it has not yet been established how this relates to the patient presenting for cancer surgery, since the precise immune mechanisms of destruction of cancer cells are not yet fully known. Only a small fraction of the currently known elements of immunity related to cancer immunosurveillance have been studied after anesthetic exposure. In addition, very little is known about the direct impact of halogenated anesthetic agents on tumor cell metabolism and viability.

Our current laboratory study investigated the effect of halothane and IFN-γ on human tumor cell viability. Interferons can modulate cellular immunity by decreasing delayed hypersensitivity and bone marrow cell growth. Interferons can also decrease macrophage, NK cell, and T-cell cytotoxicity. Interferons can modulate cell differentiation and proliferation, depending on the dose and timing of interferon administration. The cellular response to interferons includes the induction of at least 24 proteins, including enzymes that affect nucleic acid synthesis and second-messenger protein kinases and phosphodiesterases; all are believed to play a role in interferon's cellular and antiviral effects. The antiproliferative effects of interferons are seen in normal, tumor, and transformed cells in vitro.

To make clinically useful conclusions for several reasons, including 1) the rapidly changing understanding of immnosurveillance mechanisms; 2) the difficulty of obtaining reproducible clinical and laboratory results; and 3) the difficulty of separating effects of anesthetic agents themselves from the multiplicity of other factors influencing the patient perioperatively.

Despite these limitations, there is reasonable evidence from prior research that many elements of immunity are depressed in postoperative patients. Postoperative patients show decreased numbers of B-lymphocytes, T-lymphocytes, and natural killer (NK) cells. Proliferation of B-lymphocytes and T-lymphocytes is impaired postoperatively. The killing abilities of T-lymphocytes, of NK cells, and of monocytes of nonself antigen (including tumor cells) also are impaired postoperatively.

In an effort to clarify the role of anesthetic agents themselves in this apparent postoperative immune compromise, new in vitro techniques have been developed. In vitro halothane-exposed T-lymphocytes have shown decreased proliferative ability and decreased ability to release IFN-γ, in findings that reinforce clinical observations. In vitro halothane-exposed mononuclear phagocytes and polymorphonuclear granulocytes also have shown decreased cytotoxicity, as they do in vivo. In contrast, other parameters of B-lymphocyte and monocyte function seem unaffected by anesthetic agents in vitro.

![Graph](image-url)
HALOTHANE POTENTIATES THE ANTITUMOR ACTIVITY OF IFN-γ

The study detailed in this report evaluated the effect of halothane on the tumor cytostatic and tumoricidal activities of IFN-γ. Our results indicate that the known baseline tumor cell cytostatic and tumoricidal activities of IFN-γ can be augmented by coexposure of tumor cells to halothane at concentrations as low as 1% after long-term exposure. Maximal activity was observed at the 2% halothane concentrations for prolonged time periods (72 h). Nearly identical augmentation of IFN-γ antitumor activity was observed when the known calmodulin inhibitor TFP was used rather than halothane.

The effect of halothane on IFN-γ appears to be similar to that of TFP. Although many distinct intracellular metabolic mechanisms may explain this cytokotic mimicry, it is possible that the effects of halothane in this system are related to the activity of calmodulin. Local anesthetics are known to inhibit various Ca²⁺ and calmodulin-dependent pathways. Inhalational anesthetics also are believed to affect Ca²⁺ and calmodulin-related events, such as smooth and skeletal muscle contraction, as described in a recent review. Since calmodulin activity is integrally related to protein kinase-C activity, which in turn is required for the function of IFN-γ, this intracellular mechanism seems worthy of further exploration.

It is important to emphasize that halothane appears to mimic the action of TFP only when administered for prolonged time periods that exceed normal clinical uses of this agent. However, if subsequent research finds that halothane indeed inhibits calmodulin, this drug may become an important substrate for future research in the area of intracellular second messenger systems; in addition, halothane exposure may act synergistically to produce toxicity in patients receiving other calmodulin-inhibiting agents. The observations of this study also may provide new insights into the mechanisms of action of potent inhalational anesthetic agents. The observation that in vitro halothane augments IFN-γ-induced cytotoxicity against the tumor cell line HT-29 should be borne in mind and further explored, particularly in research subjects receiving IFN-γ who must undergo surgical procedures. In addition, newer anesthetic agents also should be screened in this regard.

References

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