Modification of Human Leukocyte Interferon Pharmacology with a Monoclonal Antibody ¹

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ABSTRACT

The antitumor and antiviral properties of the interferons have been well established. However, the usefulness of the interferons may be limited, in part, because of rapid clearance from the plasma and degradation by plasma or tissue enzymes. A monoclonal antibody (IFG-252.2) was developed which binds to recombinant DNA-produced human α-interferon (rIFN-αA) without measurably reducing its in vitro antiviral or antiproliferative properties. Pharmacokinetic studies of rIFN-αA-antibody complex in the intact, anesthetized rat showed that rIFN-αA activity cleared from plasma 3-fold slower than found after injection of free rIFN-αA. This resulted in a 15-fold increase in its calculated area under concentration curve compared to that of free rIFN-αA. These studies suggest that interferon bound to a monoclonal antibody may provide a means to prevent the normal clearance and degradation of free interferon and may result in prolonged antitumor and antiviral plasma activity in vivo. Furthermore, it suggests that monoclonal antibodies to various biologically active agents may be used to favorably alter their pharmacokinetics while leaving their biological activity unaltered.

INTRODUCTION

Many therapeutically active agents suffer from shortcomings which limit or complicate their use. Pharmacology studies of some chemotherapeutic agents show that they may be cleared rapidly from the circulation by extensive distribution to tissue compartments, complexed irreversibly with endogenous plasma components, or metabolically inactivated by plasma or tissue enzymes (1–3). All of these dynamic events can severely reduce the amount of biologically active drug available to the tumor. Therapeutically active drug concentrations can be maintained in plasma by sustained release after i.m. administration, frequent i.v. administration, or by continuous infusion. These procedures have been utilized with chemotherapeutic agents such as bleomycin (4), and 1-ß-D-arabinofuranosylcytosine (5) with improved therapeutic activity compared to single-dose regimens.

Alternatively, attempts have been made to increase the plasma half-life of chemotherapeutic agents by chemically linking them to inert, high-molecular-weight proteins (6) and biological matrices (7). In a study by Chu and Whiteley (8), methotrexate was covalently bound to serum albumin. The resulting preparation demonstrated prolonged serum levels and reduced urinary excretion of methotrexate. In a recent study, Kato et al. (9) showed that mitomycin C, conjugated to anti-α-fetoprotein antibody, prolonged plasma levels and has enhanced antitumor activity compared to that of free mitomycin C in a rat hepatoma model in which the tumor produced α-fetoprotein.

The IFNs⁴ are a family of naturally occurring peptide hormones with potent antiproliferative and immunomodulatory properties (10, 11). Clinical studies of the IFNs have demonstrated their antitumor activity against a variety of tumors, such as breast cancer, lymphoma, myeloma, and certain chronic leukemias (12, 13). Pharmacokinetic studies of IFN-γ have shown that IFN antiviral activity is rapidly cleared from the circulation after i.v. administration (t½ = 30 min) (14). Gutterman et al. (14) have shown that the in vivo biological activity of IFN-γ can be markedly changed if the agent is administered by continuous i.v. infusion.

In this investigation, we propose a novel approach using the administration of a specific monoclonal antibody to increase the plasma half-life of biological response modifiers with antitumor activity. To explore this, we have chosen, as a model, recombinant DNA-produced clone A of IFN (rIFN-αA) and an IFN-specific monoclonal antibody (IFG252.2). We show in this study that this antibody binds to rIFN-αA without detectable modification of IFN antiviral or antiproliferative properties in vitro. Moreover, we show that this antibody is capable of increasing the in vivo plasma half-life of IFN resulting in prolonged IFN antiviral and antiproliferative activity in plasma.

MATERIALS AND METHODS

Preparation and Screening of Anti-IFN Murine Monoclonal Antibodies. Monoclonal antibodies to IFN were prepared according to standard procedures. Briefly, spleen cells derived from BALB/c mice which had been immunized with partially purified leukocyte IFN were fused with a myeloma cell line essentially as described by Kohler and Milstein (15). Following fusion, cells were plated into 96-well microtiter plates. Resulting hybridomas were selected, based on the ability of their secreted antibody to bind leukocyte IFN labeled with ¹²⁵I by the method of David et al. (16) in a radioimmunoassay carried out using a semiautomated solid-phase second-antibody assay system (17). Those hybridomas producing antibodies of appropriate specificity were subcloned by limiting dilution to insure monoclonality. The hybridomas were injected i.p. into BALB/c mice, and antibody was purified from the resulting ascitic fluid by sodium sulfate precipitation (18). Antibodies thus prepared were 90 to 95% pure as analyzed by polyacrylamide gel electrophoresis (19).

In Vitro Neutralization of IFN Antiviral Activity by Monoclonal Antibodies. Each of the anti-IFN monoclonal antibodies identified in the above screen was serially diluted into a 96-well microtiter plate. Two IU of rIFN-αA (a gift from Hoffmann-La Roche Inc., Nutley, NJ) were added to

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¹ The abbreviations used are: IFN, interferon; rIFN-αA, recombinant DNA-derived human α-interferon.

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to each well, and the plates were incubated for 60 min at 37°C. A standard cytopathic effect assay for antiviral activity was performed in each test well solution using MDBK cells and VSV virus as described previously (20). This procedure allowed us to differentiate between neutralizing and nonneutralizing anti-IFN monoclonal antibodies. To establish that the antibodies actually formed immune complexes with IFN, 5 nonneutralizing antibodies selected by the above screening procedure were tested in an immunoabsorption assay as follows. In a 1.5-ml plastic centrifuge tube, each monoclonal antibody was added to 2 IU of rIFN-αA and allowed to incubate for 1 h at 37°C. Sheep anti-mouse immunoglobulin (North Valley Farms, Inc., Hopedale, MA) bound to Sepharose was added to each reaction mixture and allowed to incubate with shaking for 3 h at 37°C. Each tube was centrifuged at 13,000 × g for 1 min, and an aliquot of the supernatant fraction was analyzed for antiviral activity as described above.

In Vitro Effect of Monoclonal Antibodies on the Antiproliferative Activity of IFN. Human lymphoblastoid cells (Daudi) were grown in minimal essential medium supplemented with 10% fetal calf serum. Either rIFN-αA (100 IU/ml) alone, monoclonal antibody IFG-252.2 (3.8 μg/ml) alone, or rIFN-αA with monoclonal antibody IFG-252.2 (incubated for 1 h at 37°C) was added to cells growing in 25-ml flasks. Each experiment was done in triplicate. At 24, 48, and 72 h after addition, triplicate aliquots were removed from each flask, the cells were counted in a Coulter Counter, and cell viability was determined by trypan blue dye exclusion.

In Vivo Pharmacokinetics of Human rIFN-αA and rIFN-αA-Monoclonal Antibody. To compare the in vivo pharmacology of free IFN to that of IFN-monoclonal antibody complex, 250- to 260-g Fischer rats were lightly anesthetized with sodium thiopental. Plastic cannulae were surgically inserted into both the femoral vein and the femoral artery. A bolus dose of either rIFN-αA (7600 IU, in 0.5 ml of 0.9% NaCl solution (saline)) or rIFN-αA (7600 IU) and monoclonal antibody 252.2 (preincubated for 1 h at 37°C) was added to cells growing in 25-ml flasks. Each experiment was done in triplicate. At 24, 48, and 72 h after addition, triplicate aliquots were removed from each flask, the cells were counted in a Coulter Counter, and cell viability was determined by trypan blue dye exclusion.

RESULTS

Preparation and Screening of Anti-IFN Murine Monoclonal Antibodies. As shown in Table 1, 24 monoclonal antibodies with IFN binding activity were screened for ability to neutralize IFN antiviral activity. Fourteen of 24 (58%) of the antibodies tested significantly reduced (>70%) the antiviral activity of 2 IU of rIFN-αA, surpassing or equal to a neutralizing titer of 1:102,400. Eight antibodies had little antiviral neutralizing activity. To establish that the antibodies formed immune complexes with IFN, 5 nonneutralizing antibodies were tested for the ability to adsorb antiviral activity to sheep anti-mouse IgG-Sepharose beads (Table 2). One antibody, IFG-252.2, showed sufficient reactivity with IFN to remove 80% of its antiviral activity in the immunoabsorption assay. On the basis of this failure to neutralize the antiviral activity of IFN and subsequent demonstration of reactivity with IFN, this antibody was chosen for further investigation.

In Vitro Antiproliferative Effects of IFN:Monoclonal Antibody Complex. To determine whether the antiproliferative properties of IFN are intact in the presence of IFG-252.2, human lymphoblastoid (Daudi) cells were exposed to IFN alone, monoclonal antibody alone, or IFN complexed with monoclonal antibody. As shown in Chart 1, the monoclonal antibody alone had no significant effect on the growth of Daudi cells. Both IFN alone and IFN complexed to monoclonal antibody, however, reduced the cell number to 30% of control by 72 h after addition. There-

<table>
<thead>
<tr>
<th>Antibody code</th>
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<tbody>
<tr>
<td>M2G</td>
<td>4000</td>
</tr>
<tr>
<td>IFB</td>
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<tr>
<td>IFE</td>
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<td>IFG-252.2</td>
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</tr>
<tr>
<td>IFG-635</td>
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Table 1

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<tr>
<th>Antibody</th>
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<tbody>
<tr>
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<td>95</td>
</tr>
<tr>
<td>IFE</td>
<td>100</td>
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<td>94</td>
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<tr>
<td>IFG-252.2</td>
<td>100</td>
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Chart 1. Antiproliferative activity of IFN alone, monoclonal antibody IFG-252.2 (3.8 μg/ml) alone, or IFN plus IFG-252.2 against human lymphoblastoid (Daudi) cells in culture.
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![Graph showing plasma clearance of IFN antiviral activity in intact anesthetized rats.](Image)

**Discussion**

Favorable modification of the pharmacology of antitumor agents is a cornerstone in the development of new and less toxic agents for therapeutic use. Prolongation of plasma half-life, protection against enzymatic degradation, improving the disposition of drug to target sites, and reducing the distribution of drug to sensitive toxicity sites are all of major therapeutic concern. Several investigators have attempted to modify the pharmacology of a variety of chemotherapeutic agents, such as daunorubicin (21) and methotrexate (22), by covalently linking them to high-molecular-weight carriers. This allows continuous release of the agents from the complex, thus providing sustained plasma drug levels and prolonged plasma half-lives. Monoclonal antibodies may be uniquely suited to this same purpose, since the data in this study demonstrate the novel use of a specific monoclonal antibody to prolong the in vivo plasma half-life of rIFN-αA biological activity. This approach may also be applicable to other therapeutically active agents. Studies are now under way to determine whether these changes in the pharmacology of rIFN-αA can be translated into corresponding favorable alterations in the in vivo antiproliferative and antiviral activity of rIFN-αA.

The current study shows that, through our selection procedure, a monoclonal antibody which binds to rIFN-αA without measurably compromising its antiviral or antiproliferative activity can be successfully selected. In vivo administration of the antibody in combination with rIFN-αA substantially prolonged the biological activity of rIFN-αA in plasma, resulting in a 17-fold increase in the calculated area under concentration curve compared to the same dose of free rIFN-αA. In addition, the calculated apparent volume of distribution decreased from 17.2 ml to 11.9 ml for rIFN-αA alone and rIFN-αA:antibody complex, respectively. These data suggest a redistribution of rIFN-αA from the tissue compartment to the central compartment. The reason for the prolonged biological activity of rIFN-αA:antibody complex in plasma compared to that of free rIFN-αA is not clear but may be due to a variety of factors, including reduced tissue disposition and urinary excretion due to the increased size of the complex, or may be due, in part, to protection of IFN from serum proteolytic activity.

The use of a specific antibody to prolong the biological activity of rIFN-αA is in marked contrast to classical antibody therapy in which the primary function of the immunoglobulin is both to block the biological activity of the antigen and to facilitate its removal from the body. A recent study (23) has shown that F(ab)₉ fragments of a monoclonal antibody reactive with digoxin can inactivate this agent when administered to a patient suffering from digoxin overdose. From our studies, it is clear that, by appropriate selection, specific antibodies may be used to either prolong or reduce the plasma concentration of biologically active therapeutic agents.

Our method of modifying IFN may provide a distinct advantage over chemically coupling it to a carrier agent. Several studies have suggested that IFN may require internalization and metabolism to exert its biological effect (24, 25). Covalent coupling of

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**Pharmacokinetic summary**

<table>
<thead>
<tr>
<th>Agent</th>
<th>IFN (dose)</th>
<th>C × t</th>
<th>Vₐ</th>
<th>V₉</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN</td>
<td>7,600</td>
<td>4.50</td>
<td>59.8</td>
<td>17.2</td>
</tr>
<tr>
<td>IFN + monoclonal antibody 252.2</td>
<td>7,600</td>
<td>1.37</td>
<td>187.8</td>
<td>11.9</td>
</tr>
</tbody>
</table>

*Vₐ, volume of distribution; C × t, area under concentration curve.*

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IFN may affect internalization and, therefore, may modify the biological activity of the complex. The use of a simple antigen:antibody interaction may well eliminate this problem. The affinity of rIFN-αA for its cellular receptor (26) appears to be greater than the affinity of IFG-252.2 for rIFN-αA (data not shown). Thus, it is possible that the unimpaired biological activity observed for this complex may be due, in part, to rIFN-αA exchange from the antibody to its higher-affinity cellular receptor.

For the IFNs such as IFN-γ and for other biological response modifiers requiring prolonged contact time with target cells to elicit a biological response (27), the current approach may be of particular importance, since it provides prolonged plasma levels of a biologically active agent. In addition, in contrast to utilizing chemical coupling of IFN to carrier molecules which has been reported previously (28), this method of noncovalent coupling of IFN requires no special conditions for formation of the IFN:carrier complex and results in no apparent loss of biological activity.

This new use of specific monoclonal antibodies to favorably alter the pharmacokinetics of selected drugs or biological agents has potential utility beyond the IFN system. A library of monoclonal antibodies reactive with other therapeutically important biological agents such as the interleukins, new genetically engineered IFNs, traditional chemotherapeutic agents, or various growth factors could be prepared. The concomitant administration of these biological response modifiers with the appropriate antibody could greatly enhance their therapeutic efficacy by increasing their in vivo plasma half-life and altering their biological distribution to provide maximal therapeutic advantage.

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