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Biologic Effects after a Single Dose of Poly(I):poly(C_{12}U) in Healthy Volunteers

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Poly(I):poly(C_{12}U) (mismatched double-stranded RNA; atvogen), an interferon inducer, is active against human immunodeficiency virus in vitro. To determine the extent and duration of the biologic effects of poly(I):poly(C_{12}U), we administered a single dose of the drug to healthy volunteers in a randomized, double-blind, placebo-controlled 2-week crossover study. We analyzed blood for alpha and gamma interferons, neopterin, 2′,5′-oligoadenylate synthetase, lymphocyte surface markers, lymphocyte proliferation after exposure to soluble antigens and mitogens, and natural killer cell activity. Minimal biologic effects were observed after administration of a single 200-mg dose to four volunteers; therefore, the dose was increased to 600 mg in 10 subjects. Only neopterin levels and symptoms were greater after administration of 600 mg of poly(I):poly(C_{12}U) than after administration of placebo (Wilcoxon signed rank sum test, $P = 0.06$). A definite response in 2′,5′-oligoadenylate synthetase activity, however, was seen in a few subjects. Neither alpha nor gamma interferon was detectable in serum after poly(I):poly(C_{12}U) dosing. The neopterin changes after administration of poly(I):poly(C_{12}U) were similar at both poly(I):poly(C_{12}U) dose levels, with an early decrease at 6 h, a peak at 1 day, and a gradual decrease toward the baseline over the following 3 days. A mild flu-like syndrome occurred in one-half of the subjects following administration of poly(I):poly(C_{12}U) and in only one subject following administration of placebo. This syndrome resolved within 16 h after poly(I):poly(C_{12}U) dosing. We conclude that poly(I):poly(C_{12}U) does not induce measurable levels of interferon and causes only minimal biologic or toxic effects among those parameters measured after administration of a single dose in the 200- to 600-mg dose range in healthy volunteers.

Poly(I):poly(C_{12}U) (mismatched, double-stranded RNA; atvogen) induces an antiviral state in human cells in culture and in animal models against both RNA and DNA viruses (5–7, 9, 12, 21). This is accomplished through the production of interferon and other lymphokines and through augmentation of natural killer cell activity without mitogenic stimulation or significant toxicity in preclinical studies (2, 7, 9, 15, 24). Poly(I):poly(C_{12}U), alone and synergistically with zidovudine, specifically inhibits human immunodeficiency virus (HIV) infection in vitro (13, 14). The drug also restores the antigenic responsiveness of HIV-infected lymphocytes in vitro (11). Interferon, which is induced by poly(I):poly(C_{12}U) in vitro, has been shown to inhibit the production of mature virions from HIV-infected T helper cells and macrophages at several stages of the virus's life cycle, including integration, viral assembly, and release (8).

A double-blind, placebo-controlled study of interferon (average dose, 17×10^6 U) in HIV-infected subjects with greater than 400 CD4 T cells showed increased numbers of CD4-positive cells and decreased p24 antigenemia and HIV-positive cultures after interferon treatment but one-third of these subjects withdrew from that study because of toxicity (10). Clinical studies of poly(I):poly(C_{12}U) have consistently shown low levels of toxicity, especially compared with those achieved with exogenously administered interferon. As many as one-third of subjects, however, have shown side effects temporally associated with the drug's infusion (1a, 3, 4, 18, 20). Furthermore, the anti-HIV effect of poly(I):poly(C_{12}U) in humans remains unproven. The only blinded, placebo-controlled study of poly(I):poly(C_{12}U) (Ampligen) was terminated prematurely after 20 subjects with AIDS-related complex had progressed to AIDS and 12 were found to be on poly(I):poly(C_{12}U) (9a, 19). The failure of that study to show the same efficacy as was suggested by uncontrolled studies of the drug has been attributed to alteration of the poly(I):poly(C_{12}U) when it is stored in plastic bottles (19).

We designed the present study (AIDS Clinical Trials Group protocol 056) to define the time course and spectrum of the biologic effects of a poly(I):poly(C_{12}U). We reasoned that the dose and frequency of a drug to be used in an optimal phase III efficacy study should be rationally designed with the benefit of phase I studies that determined both a biologically active dose and the duration of that biologic effect.

Our double-blind, placebo-controlled crossover study measured interferon, biochemical markers of interferon (2′,5′-oligoadenylate synthetase [2,5AS] and neopterin), T-cell subsets, lymphocyte proliferative responses, natural

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killer cell activity, and clinical effects over 1-week periods following administration of a single dose of poly(I):poly(C_{12}U) and placebo. The use of subjects as their own controls in this blinded crossover fashion significantly improved the statistical strength of the study and clearly delineated the baseline variability for the outcomes measured, thereby preventing false attribution of immunologic or symptomatic effects to the study drug.

MATERIALS AND METHODS

Subjects. Thirteen healthy nonsmoking men between 20 and 46 years of age (median, 30 years), of various races (seven black, five white, one Hispanic), and weighing between 66 and 111 kg (median, 73 kg) participated in the study. Their good health was ascertained by medical history, physical examination, and a laboratory screening panel. No subject had taken any medication or experienced any illness (including allergies or flu-like symptoms) for at least 2 weeks prior to the study, nor had alcohol been ingested for at least 48 h before the study. All subjects gave written informed consent, and the study was approved by our institutional review board. One patient participated in both the 200- and 600-mg phases of the study, but participation in the two phases was separated by 15 months.

All subjects were admitted to the hospital about 20 h before drug or placebo administration, and baseline screening tests were repeated (day 0). Subjects were confined to the Clinical Research Center of the Johns Hopkins Hospital for the 12-day duration of the study. An absolute fast was maintained for 10 h before and 2 h after drug or placebo infusions on days 1 and 8. Each volunteer received a doubly blinded single dose of study drug (200 mg in subjects 1 to 4 or 600 mg in subjects 10 to 19) or placebo (normal saline) by intravenous infusion over 30 min (subjects 1 to 4) or 60 min (subjects 10 to 19) on days 1 and 8. The schedule of infusions [poly(I):poly(C_{12}U) on day 1 and placebo on day 8 or placebo on day 1 and poly(I):poly(C_{12}U) on day 8] was determined by a random sequence generated and maintained by the Investigational Drug Service of the Johns Hopkins Hospital. All personnel who cared for the subjects, performed assays, and analyzed data were blinded until all clinical and laboratory data for all subjects at each dose had been completed and recorded. Blood for immunologic assays was collected at 0, 2, 6, 10, 24, 32, 48, 56, 72, and 96 h after administration of each dose. Blood for toxicity monitoring was collected in the mornings of days 0, 3, 5, 10, and 12. Vital signs, temperature, and clinical symptoms were recorded every 2 h on the dosing days until 16 h postinfusion (approximately midnight) and then during each work shift until the next dose or the end of the study on day 12.

Drug preparation. The study drug was manufactured and characterized under the control of the Department of Biophysics, Johns Hopkins University School of Public Health, and with approval of the U.S. Food and Drug Administration (Bureau of Biologics Investigational New Drug 2795) for clinical investigation. The drug was supplied to the manufacturing pharmacy of the Johns Hopkins Hospital after single-strand synthesis (NATA, Inc., Baltimore, Md.), reannealing, placement in vials, and lyophilization (Bell-More Labs, Inc., Hampstead, Md.). This same process, recorded in our Drug Master File, which is on file with the U.S. Food and Drug Administration, was used to prepare the drug for use in the preclinical and clinical cancer studies cited previously (2, 5, 6, 9, 11, 15, 20, 21, 24). The same lot of drug used in the antiviral animal model and the in vitro HIV studies cited previously (2, 11, 15) was also used in the current study. Individual 200-mg (80 ml) and 600-mg (400 ml) unit doses were prepared in two batches from two lots of the drug by the manufacturing pharmacy by solubilizing the lyophilized compound, heating the batch to 65°C for 18 to 24 h, cooling to 37°C over 1 h, placing aliquots into glass bottles with opaque brown coverings, labeling, and freezing at −20°C. Normal saline placebo unit doses were prepared in identical bottles, labeled, and frozen. Prior to infusion pump administration, the patient- and week-specific bottle was heated and cooled as described above. A small (0.5 ml) aliquot for poly(I):poly(C_{12}U) assay was collected from the intravenous line immediately proximal to the patient near the end of the infusion. Samples were assayed for melting temperature, double strandedness, and molecular weight and were found to be consistent with the original material assayed prior to lyophilization. On the basis of the poly(I):poly(C_{12}U) concentration of the aliquots taken during infusion, the median calculated dose infused was 632 mg (range, 592 to 664 mg) or 316 ng/m^2 (range, 255 to 371 ng/m^2) for the 600-mg dose recipients.

Interferon. Alpha and gamma interferons were measured by ^{125}I-labeled monoclonal antibody radioimmunoassays in commercially available kits (Celltech, Ltd., Berkshire, United Kingdom, and Centocor, Inc., Malvern, Pa., respectively). The limit of detection of each assay was 62.5 and 0.1 U/ml for alpha and gamma interferons, respectively. Serum was assayed at 0, 2, 6, 10, 24, 48, 72, and 96 h after administration of each dose.

Neopterin. For the assay of neopterin in serum, we used a ^{125}I-labeled double-antibody radioimmunoassay (Henning, Berlin, Germany) with a 0.9-ng/ml limit of sensitivity. Samples were assayed at 0, 2, 6, 10, 24, 32, 48, 56, 72, and 96 h after administration of each dose. Results were expressed as area under the concentration-time curve (AUC) at 1, 2, and 4 days for comparison between drug and placebo.

PBMC preparation. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, N.J.) density gradient centrifugation and were delivered immediately to the laboratory that performed the 2,5AS, lymphocyte proliferation, and natural killer cell assays.

2,5AS activity. 2,5AS activity was assayed by the method of Schattner et al. (17), with the modifications described by Witter et al. (23). After Nonidet P-40 lysis, the 2,5AS in the cell extracts was bound to poly(I · C)-agarose beads and activated with poly(I · C). The [^{32}P]ATP was incorporated into newly synthesized 2′,5′-adenylate oligomers, and the terminal phosphates were cleaved with alkaline phosphatase. The (A2′p)$_n$5′A oligoadenylate cores were finally eluted over an alumina column. Incorporation of ^{32}P into the eluate was determined in a gamma scintillation counter. Results were expressed as picomoles of (A2′p)$_n$5′A cores per 10^5 cells per hour. Samples were drawn at 0, 2, 6, 10, 24, 32, 48, 56, 72, and 96 h after administration of each dose. For drug-placebo comparisons, the AUC for 1, 2, and 4 days after each infusion was used.

Lymphocyte phenotypic markers. Blood for surface marker assays was collected in EDTA blood tubes. After staining with monoclonal antibodies stained with fluorescein isothiocyanate or phycoerythrin (CD3, CD4, and CD8; Becton Dickinson, San Jose, Calif.), erythrocytes were lysed with ammonium chloride and samples were run on a two-color flow cytometer (Coulter EPICS-C). Blood drawn simultaneously was assayed in the Clinical Laboratory of the Johns Hopkins Hospital for complete blood cell count with auto-

mated differential (STKR Hematology Analyzer; Coulter Electronics, Hialeah, Fla.).

Lymphocyte proliferation (LP) assay. PBMCs were incubated with pokeweed mitogen (final concentration, 5 μg/ml; Sigma, St Louis, Mo.), phytohemagglutinin (final concentration, 2 μg/ml; Sigma), concanavalin A (final concentration, 5 μg/ml; Sigma), tetanus toxoid (1:250 dilution; Lederle, Poughkeepsie, N.Y.) and cytomegalovirus antigen (1:1,000 dilution; Whitaker Bioproducts, Walkersville, Md.) in triplicate wells at 10^5 cells per well in 96-well plates at 37°C. On day 2 for phytohemagglutinin and concanavalin A and on day 4 for pokeweed mitogen, tetanus toxoid, and cytomegalovirus antigen, cells were pulsed with [^{3}H]thymidine and were harvested 18 to 22 h after the pulse. Incorporation of [^{3}H]thymdine was determined on a beta scintillation counter. Results were expressed as lymphocyte stimulation index (LSI), which was equal to the mitogen counts per minute/control counts per minute. PBMCs were collected at 0 and 24 h after each dose for this assay.

Natural killer cell activity. Effector cells (the subjects' PBMCs) and target cells (K562 and U937 cells labeled with ^{51}Cr) were incubated in 96-well plates at 50:1, 25:1, 12.5:1, and 6.25:1 effector cell:target cell ratios. Control wells for spontaneous and maximum ^{51}Cr release were also used. After 16 h of incubation at 37°C, the supernatants were removed from each well and ^{51}Cr release was measured in a scintillation counter. The percentage of isotope released was calculated as follows: percent release = (counts per minute released from cells during incubation/total counts per minute incorporated into cells) × 100. The percent specific cytotoxicity of the target cells was calculated as the difference in percent ^{51}Cr release between the experimental samples and media controls. Results are expressed as lytic units (LU), which is the number of effector cells that caused 30% specific lysis of target cells. This was calculated by linear regression (program supplied by Frederick Cancer Research Center, National Cancer Institute, Frederick, Md.) with extrapolation to 30% lysis (16). Samples were collected at 0 and 24 h after each dose.

Symptom score. The occurrence of clinical symptoms was solicited from the volunteers in a nondirected manner at intervals after dosing and were scored as mild (score, 1), moderate (score, 2), or severe (score, 3) on a score sheet of common interferon-related symptoms.

Statistical analysis. The area under the parameter-time curve, calculated by using the trapezoidal rule method, was the unit of analysis for parameters in which three or more observations in a week were used. Lymphoproliferation and natural killer cell assays, which were done only at 0 and 24 h after the administration of each dose, were described by absolute values of stimulation indices or total LU, respectively. Ratios of absolute values (AUC, LSI, total LU) were chosen to quantify the poly(I):poly(C_{12}U)–placebo relation. In this crossover design, we tested for and found, in three parameters, period effects (neopterin, 0- to 1-day AUC, phytohemagglutinin lymphocyte response) and treatment-by-period interactions (cytomegalovirus lymphocyte response) that were statistically significant (1). Accordingly, the significance of any conclusions based on these three parameters could be due to factors of the study design other than drug intervention. Descriptive statistics are summarized by the median (lower quantile, upper quartile). The Wilcoxon signed rank sum test compared poly(I):poly(C_{12}U) responses with placebo responses. Coefficients of variation were used to summarize parameter variabilities at the baseline. The time course of neopterin after poly(I):poly(C_{12}U) was based on the time effects calculated from a median polish in which the median effect, patient effects, and residual effects were removed (22).

RESULTS

No consistent difference between the poly(I):poly(C_{12}U) response and the placebo response was observed for any immunologic parameter in the first four subjects who received the 200-mg dose (data not shown). The decision was then made to advance to a single 600-mg dose in the hope of achieving some measurable response so that the time course and the immunologic breadth of response to poly(I):poly(C_{12}U) could be observed. The ratio of the poly(I):poly(C_{12}U) (600 mg)-to-placebo response for all measurable parameters is displayed in side-by-side box plot summaries of all patients in Fig. 1. Alpha and gamma interferons are not shown in Fig. 1 because the values of these parameters for all subjects were below the detection limits of the assays. For the poly(I):poly(C_{12}U) response, only neopterin levels in serum (AUC for days 0 to 1, $P = 0.09$; AUC for days 0 to 2, $P = 0.06$) and the 16-h symptom score ($P = 0.06$) were greater than those for the placebo response. A majority of the subjects had 2,5AS and T-cell subset poly(I):poly(C_{12}U)/placebo ratios greater than and less than 1, respectively, but the poly(I):poly(C_{12}U)–placebo differences were not statistically significant at even the 10% level. Subjects' lymphoproliferative and natural killer cell activity responses were balanced around a drug/placebo ratio of 1, indicating no response. The distribution of neopterin and 2,5AS AUC ratios in Fig. 1 was the result of clear individual responses to poly(I):poly(C_{12}U) in only three (neopterin) or four (2,5AS) subjects (on the basis of blinded visual inspection of parameter-time curves). In the remaining subjects, poly(I):poly(C_{12}U) and placebo responses could not be differentiated visually (individual data not shown).

Having shown that the drug/placebo ratio was not significantly different from 1.0 for all but neopterin and symptom scores, the drug/placebo ratio that could confidently be excluded (power, 80%) was determined for all other parameter groups: 2,5AS, 1.40 to 1.7; T-cell subsets, 1.6 to 1.8; LP, 1.7 to 1.8; natural killer cell activity, 1.6 to 1.8; leukocytes and percent lymphocytes, 1.1. Only in the case of the cytomegalovirus antigen-induced proliferative response of lymphocytes (ratio, 2.4) could a doubling of the baseline level not be excluded.

Because there appeared to be a real effect of poly(I):poly(C_{12}U) on neopterin levels, the time course was analyzed more carefully. The magnitude and duration of serum neopterin levels was similar for both the 200- and 600-mg poly(I):poly(C_{12}U) doses, with an early 0.5- to 1-ng/ml decrease at 6 h, a 1.5- to 2-ng/ml peak at 24 h, and a gradual tapering over several more days (Fig. 2). In the four patients with 2,5AS elevations after poly(I):poly(C_{12}U), the time course was similar to that of neopterin, with variable peaks from 24 to 32 h (data not shown).

Symptom scores were greater after poly(I):poly(C_{12}U) administration than after placebo administration (Fig. 1), and 83% of the recorded symptoms occurred in the week following poly(I):poly(C_{12}U) dosing. Of the symptoms that occurred following poly(I):poly(C_{12}U) dosing, 67% occurred in the first 4 h and 98% occurred within the first 16 h. Although the difference in symptoms was statistically significant—greater on the day following poly(I):poly(C_{12}U) dosing compared with that on the day following placebo dosing—the symptoms were mild in nature (Table 1). A flu-like syndrome

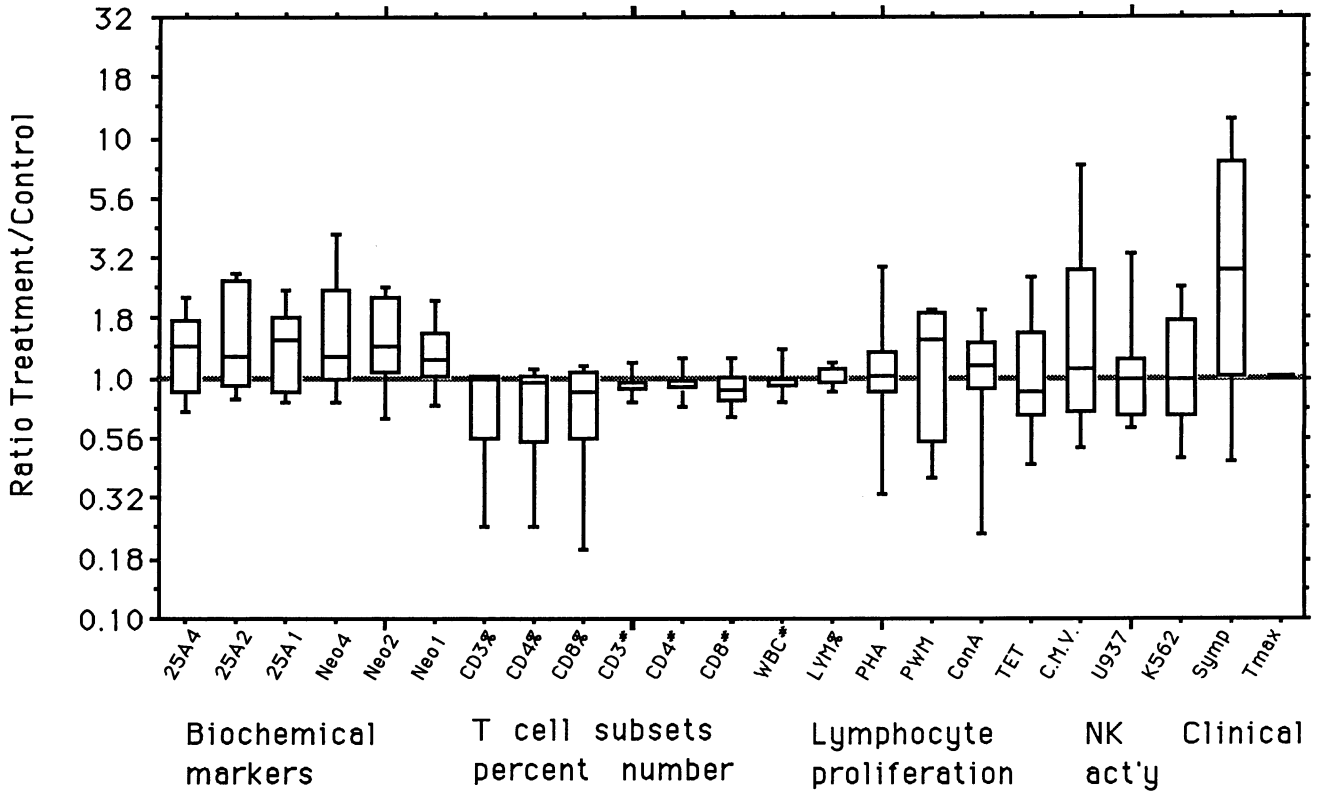

FIG. 1. Side-by-side box plots of immunologic and clinical parameters. The values expressed are ratios of post-poly(I):poly(C_{12}U) response to post-placebo response (logarithmic y-axis scale). A ratio of 1:0 reflects no difference between poly(I):poly(C_{12}U) and placebo responses. The parameters included are 2',5'-oligoadenylate synthetase cummulative AUC for 4-, 2-, and 1-day periods after dosing (25A4, 25A2, and 25A1, respectively); serum neopterin AUC for 4-, 2-, and 1-day periods (Neo4, Neo2, and Neo1, respectively); T-cell subsets as both the percentage of lymphocytes (CD3%, CD4%, and CD8%) and the absolute number of T-cell subsets (CD3#, CD4#, CD8#); leukocyte 2-day AUC (WBC); percent lymphocyte 2-day AUC (LYM%); lymphocyte proliferation in response to phytohemagglutinin (PHA), pokeweed mitogen (PWM), concanavalin A (ConA), tetanus toxoid (TT), and cytomegalovirus (C.M.V.); natural killer (NK) cell activity with U937 and K562 target cell lines; symptom score at 16 h (Symp); and maximum temperature recorded within 4 days after dosing (Tmax). The box plots describe the median (central bar), the middle two quartiles (box), and the 90% limits of the data (bars above and below the box).

with several hours of multiple symptoms (fatigue, feverishness or chills, myalgia or arthralgia, mild headache) was seen in half the patients after administration of the 600-mg poly(I):poly(C_{12}U) dose. One subject experienced this syndrome after placebo dosing. The maximum temperature recorded for 13 of 14 subjects occurred within 16 h after poly(I):poly(C_{12}U) dosing. Only one patient's temperature, however, went as high as 38°C. We observed reversible aspartate aminotransferase and alanine aminotransferase elevations above normal (greater than two times the baseline value or the upper limit of laboratory normal, whichever was greater) in 3 of 14 subjects after poly(I):poly(C_{12}U) dosing (1 subject after the 200-mg dose and 2 subjects after the 600-mg dose) and in 1 of 14 subjects after placebo dosing. Two other subjects had milder alanine aminotransferase elevations less than two times normal after poly(I):poly(C_{12}U) dosing. One patient had aspartate aminotransferase (four times normal) and alanine aminotransferase (eight times normal) elevations that began 3 days before the poly(I):poly(C_{12}U) infusion.

From 3 to 16 serial placebo measurements prior to drug infusion were recorded for all parameters in 7 of 14 subjects, yielding valuable baseline variability data. The coefficient of variation of baseline values (Table 2) was less than 20% for a few of the immunologic parameters measured, including T-cell subsets and neopterin. The coefficient of variation was much greater, ranging from 20 to 44% for all cellular assays (except for tetanus toxoid at 123%) and 2,5AS. This variation over time was fairly comparable to chemistry and hemoglobin values in which the coefficient of variation ranged from 1 to 34%.

DISCUSSION

We detected a small but measurable immunologic response to a single 600-mg dose of poly(I):poly(C_{12}U) that lasted several days in our healthy volunteers. This response was based on (i) median drug/placebo ratios greater than 1.0 for neopterin AUC on days 0 to 2 (statistically significant at the 6% level), (ii) neopterin time-effect curves with nonzero magnitudes and similar biphasic shapes at both doses studied, and (iii) clear 2,5AS and neopterin responses in some subjects, determined on the basis of blinded visual inspection of the individual subjects' parameter-time curves. The use of placebo observations minimized our erroneous attribution of fluctuations of any measurement, immunologic or symptomatic, to the effect of the drug alone rather than to random baseline fluctuations. The individual's variability for each parameter, defined by repeated baseline measurements, should be useful for sample size planning in future

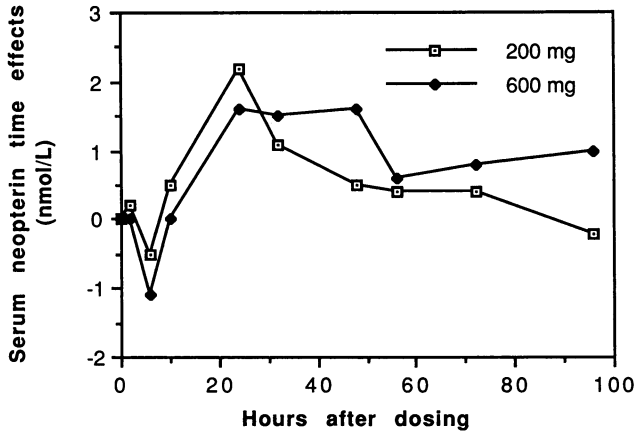

FIG. 2. Median neopterin response in serum over time (time effect) after administration of poly(I):poly(C_{12}U), by dose. The time effects shown are calculated values that reflect the median changes in neopterin over time after baseline values (4.1 nmol/liter), individual patient effects, and random error are removed (data not shown).

studies of the immunologic response to immunomodulating drugs.

Our conclusions may be limited because we evaluated "healthy" populations, because of the assays that we used (limited by their sometimes significant variability), and because only a single 600-mg dose was used. Healthy volunteers were chosen to maximize the sensitivity of the immunologic assays by reducing the background "noise." The study subjects had no clinically evident allergic, viral, or other illnesses and were on no medications. These requirements were chosen so that we could select an immunologically "quiet" population to minimize the inherent baseline variability of the tests chosen to measure an immunologic response to poly(I):poly(C_{12}U). Immunologically impaired HIV-infected individuals may have a greater range of responses, because some parameters which are abnormal at the baseline may normalize after drug administration, but this remains to be proven. For several of the measures that we chose, it is known that the baseline values are far higher and more variable in HIV-infected subjects than in our volunteers. Preclinical studies, nevertheless, demonstrated the immunologic effects of poly(I):poly(C_{12}U) in immunologically healthy animals (2, 7). We reasoned that an immunologically ill population was best studied after a measurable immunomodulating dose with a well-described time course of biologic effect had been characterized in healthy volunteers.

The immunologic parameters we chose represent a broad spectrum of functions, many of which have been augmented by poly(I):poly(C_{12}U) in preclinical studies in normal animals. The timing of measurements was also very broad for most measures chosen, at least daily for 2 to 4 days, but was limited to a single measurement at 24 h for the cellular assays; the blood volume was the limiting factor. Our best prediction, which was based on other studies and which proved to be true for neopterin and 2,5AS activity, was for the peak effects at 1 day after dosing. The use of subjects as their own controls minimized the variability of results. Extended control periods provided well-defined baseline variability. Even though we chose a small number of subjects, we had sufficient power to exclude what we considered to be clinically important (50 to 75%) differences between the effects of the study drug and the placebo, even for cellular responses. For example, a small (3×10^6 U) dose of interferon consistently doubles the 4 day 2,5AS AUC beyond the baseline in healthy volunteers (23), an effect greater than what we observed in the present study.

Given the wide variety of subjects' responses in the present study, a 600-mg dose may simply be too low on some individuals' dose-response curves to generate a measurable response. Multiple doses of a drug with an immunologic

TABLE 1. Number of patients with symptoms within 16 h after receiving 600 mg of poly(I):poly(C_{12}U)[a]

Symptom	No. of patients	
	Poly(I):poly(C_{12}U)[b]	Placebo[c]
Fatigue	5	2
Chills	5	1
Headache	4	1
Cold arm during infusion	4	1
Feverishness	3	1
Lightheadedness	2	0
Flushing	1	0
Myalgia	1	0
Mental confusion	1	0
Palpitations	1	0
Nervousness	0	1
Flu-like syndrome[d]	5	1
Any symptom	9	4

[a] Some 97% of all symptoms were experienced within 16 h of receiving 600 mg of poly(I):poly(C_{12}U) (n = 10 subjects).
[b] Excludes one patient with flushing after receiving 200 mg of poly(I):poly(C_{12}U); all other symptoms are after administration of 600 mg of poly(I):poly(C_{12}U).
[c] Excludes one patient with headache after placebo (200-mg phase); other symptoms are for subjects that received placebo (600-mg phase).
[d] Two or more of the following symptoms: fatigue, fever or chills, myalgia or arthralgia, or headache.

TABLE 2. Coefficient of variation of predrug datum points

Assay	Baseline median[a]	Median coefficient of variation[a]
2,5AS	151 pmol/10^5 cells/h	42.2
Neopterin	4.1 ng/ml	18.6
CD3	78%	1.0
CD4	46%	2.0
CD8	31%	5.1
CD3	1,473 cells/mm^3	12.4
CD4	808 cells/mm^3	12.8
CD8	617 cells/mm^3	13.5
Leukocytes	6,033 cells/mm^3	9.9
Lymphocyte	34%	9.9
LP phytohemagglutinin	44 LSI	27.9
LP pokeweed mitogen	20.1 LSI	44.1
LP concanavalin A	49 LSI	26.1
LP tetanus toxoid	8.5 LSI	122.9
LP cytomegalovirus	0.8 LSI	20.1
Natural killer cell U937	322 LU	32.7
Natural killer cell K562	163 LU	35.6
Sodium	139 meq/liter	1.0
Creatinine	0.9 mg/dl	5.5
Aspartate aminotransferase	18 U/liter	10.5
Alanine aminotransferase	20 U/liter	34.0
Hemoglobin	13.8 g/dl	2.2
Temperature	36.5°C	1.0

[a] Median value from among each individual patient's median or coefficient of variation.

response below our limits of detection could have a steady-state effect in a higher and more easily detectable range than was achieved with our single-dose study.

A surprising finding was the absence of detectable alpha or gamma interferon after administration of a drug that is an interferon inducer in vitro (6). Despite this, neopterin (produced primarily by macrophages in response to gamma interferon) seems to have a consistent elevation (evidenced by its elevated drug/placebo ratio and nonzero biphasic course) that was marked in some subjects and that lasted for 3 to 4 days after a single poly(I):poly(C_{12}U) dose. The level of 2,5AS (induced by alpha interferon) was also clearly elevated in a few subjects for several days. Greater and more consistent responses among subjects, possibly at a higher dose, will be necessary to clarify whether these immunologic changes are mediated by a mechanism other than interferon or whether our assays were more sensitive for biochemical markers of interferon than for interferon itself. On the basis of the results of studies in healthy animals, we expected to see elevations of natural killer cell activity after poly(I):poly(C_{12}U) administration (2, 14). We failed to demonstrate this, and the data from our study have the statistical power to exclude a 60 to 80% difference. We also failed to show increased lymphocyte proliferation or changes in T-cell subsets after poly(I):poly(C_{12}U) dosing, but our expectations of these parameters were less clear because of insufficient preclinical data.

In addition to the weak immunologic effect of poly(I):poly(C_{12}U) at the doses used in the present study, the drug has some toxicity and is extremely awkward to prepare and administer. These 200- and 600-mg doses of poly(I):poly(C_{12}U) were well tolerated in our healthy volunteers. Symptoms were mild and transient and would not have interfered with the volunteers' daily activities. Only one temperature as high as 38°C was recorded. Reversible serum transaminase elevations after a single poly(I):poly(C_{12}U) dose, however, outnumbered the elevations after placebo. Regarding awkward drug preparation, we used two water bath steps and infused a relatively large volume (400 ml) over 1 h (1.5 mg/ml) in our 600-mg recipients. The drug can be prepared in a higher concentration (2.5 mg/ml), but more rapid infusion has been complicated by more frequent infusion-related reactions in HIV-infected subjects (1a). The large volume itself is prohibitive for other than intravenous administration.

In the only placebo-controlled study of this drug in HIV-infected subjects (300 mg twice weekly) (9a), no clinical effect was demonstrated, although this was attributed to a problem with the drug preparation (19). In an open-label, positive-controlled escalating-dose study of this drug in subjects in an early stage of HIV infection, subjects were given higher doses, up to 570 mg/m^2 twice weekly for up to 9 to 24 weeks (1a). That study suggested lower rates of $CD4^+$ T-cell decline after the administration of high doses compared with the rates after the administration of low doses of the drug. Occasional infusion-related symptoms occurred in one-quarter of the subjects, but it did not warrant discontinuation of the study. We studied similar doses in immunologically healthy and immunologically less variable subjects in whom more subtle differences should have been detectable. Our findings of low levels of immunologic modulation only in some subjects suggest that the poly(I):poly(C_{12}U) dose that induces a consistent and measurable biologic response has yet to be identified. Further studies are needed, therefore, to define the optimal dose and dosing interval for this mismatched double-stranded RNA for large-scale studies to assess its clinical efficacy.

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