Modified Polyriboinosinic-Polyribocytidylic Acid Complex: Sustained Interferonemia and Its Physiological Associates in Humans

KEITH J. CHAMPNEY,† DONALD P. LEVINE,‡ HILTON B. LEVY,† AND A. MARTIN LERNER‡

Hutzel Hospital Medical Unit, Department of Medicine, Wayne State University School of Medicine, Detroit, Michigan 48201,† the Veterans Administration Medical Center, Allen Park, Michigan 48101, and the Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205‡

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Fourteen patients with severe viral illnesses were given intravenous infusions of a modified interferon inducer, polyriboinosinic-polyribocytidylic acid—poly-L-lysine complexed with carboxymethylcellulose [poly(I:C-LC)], during a phase 1 clinical trial. The first eight patients received 0.15 to 0.30 mg of poly(I:C-LC) per kg of body weight daily for 5 consecutive days, and another received two courses separated by 1 week. A second group of five patients was given single intravenous infusions of 0.10 to 0.15 mg of poly(I:C-LC) per kg. Interferon was detectable in the serum 8 to 16 h after injection. Titers ranged from 15 to 800 U/ml and varied directly with the dose of poly(I:C-LC). Interferonemias persisted for 12 to 48 h. In patients receiving 5-day courses of poly(I:C-LC), lower levels of serum interferon (0 to 160 U/ml) occurred on days 2 through 5, characteristic of a hyporesponsiveness state. An exception was a 69-year-old patient with disseminated varicella-zoster, multiple myeloma, and renal insufficiency whose serum contained 3,150 U of interferon per ml on day 3 of 0.3 mg of poly(I:C-LC) per kg. Fever (39 to 40.5°C, rectally; 13 of the 14 patients) peaked 3 to 8 h after completion of infusions. Other toxic effects included lymphopenia (10 of the 14 patients), hypotensive episodes (7 of the 14 patients), and minor elevations of serum glutamicoxalacetic transaminase and lactic dehydrogenase.

When used prophylactically in respiratory infections due to influenza virus or rhinoviruses, intranasal exogenous human leukocyte-derived interferon decreased the symptoms and frequency of virus shedding and led to a decreased antibody production (25, 28). Likewise, interferon administered parenterally suppressed hepatitis B virus and Dane particle markers, inhibited viruria transiently in cytomegalovirus infections, and shortened the duration of pain while facilitating crust formation in adults with herpes zoster (2, 11, 12, 15, 24). Human interferon has also been given prophylactically to patients with malignancies to attempt to protect against acute infections (1, 30).

The difficulty of extensive controlled clinical testing with exogenous interferon in humans is great because to treat one patient many 500-ml units of whole blood must be processed (18). Therefore, endogenous induction of interferon has been explored. Polyinosinic-polycytidylic acid [poly(I:C)] is an effective antiviral agent in rodents (4, 18, 32). Unfortunately, poly(I:C) is rapidly hydrolyzed by nucleolytic enzymes in primate sera, resulting in poor interferon induction (17, 26). Recently, one of us (H.B.L.) prepared a soluble complex of poly(I:C) with poly-L-lysine and carboxymethyl cellulose [poly(I:C-LC)] which is 5 to 10 times more resistant to primate serum than the parent poly(I:C) and induces significant levels of serum interferon in monkeys and chimpanzees under conditions in which poly(I:C) itself induces no interferon (23). Poly(I:C-LC) has been shown to be effective in the prophylaxis of simian hemorrhagic fever, rabies, yellow fever, and hepatitis in nonhuman primates (22). No toxic effects due to poly(I:C-LC) were observed. The purpose of this initial phase 1 clinical trial was to determine dose responses and the possible toxic effects of poly(I:C-LC) in humans with virus infections (33).
MATERIALS AND METHODS

Administration of poly(I:C-LC). Poly(I:C-LC) was prepared and stored (4°C) as before (23). Immediately before use, poly(I:C-LC) was added to 250 ml of 5% glucose in water or, later in the study, to physiological saline. Solutions of poly(I:C-LC) (0.15 to 0.30 mg/kg) were given intravenously over 90 min daily for 5 consecutive days to eight patients. One patient received two 5-day courses, the first at 0.15 mg/kg, and 6 days later another series of 0.20 mg/kg. Finally, an additional five patients received single infusions of 0.10 to 0.15 mg of poly(I:C-LC) per kg.

Patients. All patients receiving poly(I:C-LC) had severe viral illnesses, e.g., St. Louis encephalitis (SLE; five patients), disseminated varicella-zoster (four patients), and eczema herpeticum, disseminated herpes simplex virus, type 2 infection, herpes simplex virus encephalitis, Jakob-Creutzfeldt disease (presumed viral), and virus hepatitis, type B (one patient each). Patients receiving poly(I:C-LC) were closely observed in an intensive care unit. After each infusion, blood pressures, pulses, and rectal temperatures were taken each hour for 24 h, or longer if vital signs had not returned to normal. Water intake and output were recorded. Complete blood counts, platelets, prothrombin time, partial thromboplastin time, blood urea nitrogen, creatinine, bilirubin, alkaline phosphatase, serum glutamic oxaloacetic transaminase, lactic dehydrogenase, electrolytes, glucose, and urine were tested daily for 10 days in patients receiving 5-day courses of poly(I:C-LC), and for 5 days in others who had single infusions of the interferon inducer. Blood was also taken periodically for assays of interferon and antibodies. Serum was promptly separated and stored at 4°C until use.

During this study (late summer 1975), we saw 13 patients with epidemic SLE (6). We compared clinical courses of four patients with SLE who received poly(I:C-LC) with those of nine others treated supportively.

Each patient with SLE was examined for neurological findings daily. State of consciousness, orientation, memory, affect, irritability, photophobia, seizures, frontal lobe signs, cranial nerve palsies, localized weakness, tremor, rigidity, coordination, Babinski sign, nuchal rigidity, and papilledema were surveyed. A neurological score based upon a single point for each finding was calculated for all patients with SLE during each of the first 10 days of hospitalization.

Virological studies. In 10 patients, SLE was confirmed by fourfold or greater rises in complement fixing or hemagglutinating inhibiting antibodies (HIA) between acute and convalescent phase sera. Poly(I:C-LC) was given after informed consent of the patient, with approval of the U.S. Food and Drug Administration and the Human Investigation Committee of Wayne State University. In two patients, SLE was highly probable on the basis of a single titer of HIA antibodies ≥1:80. Tests for HIA to SLE were done in the laboratory of the Michigan State Department of Public Health, Lansing, Mich. Finally, SLE virus was isolated from the brain of a 69-year-old woman at autopsy. This virus produced characteristic cytopathic effects in Vero cell tissue cultures, was inactivated by ether, hemagglutinated 1-day-old chicken erythrocytes at pH 6.8, and was neutralized by mouse ascitic fluid containing antibodies to group B arthropod-borne virus (prepared by the Yale University Arbovirus Research Unit, New Haven, Conn.). With 10^2 50% tissue culture infective doses of SLE virus, the minimal inhibitory concentration of human leukocyte-derived interferon in Vero renal tissue cultures was 2.6 U/ml (21).

After collection, sera were stored (4°C), frozen (−20°C) within 24 h, and thawed once at assay. In earlier studies in primates, it has been shown that when poly(I:C-LC) is given intravenously it is removed from the circulation, probably by the mononuclear phagocytic system, within 10 min. Isolated human sera here were tested for residual poly(I:C-LC) in nonhuman tissue cultures. No residual poly(I:C-LC) remained in serum. Interferon levels in sera of patients were measured in human foreskin fibroblast tissue cultures, using vesicular stomatitis virus as challenge virus as previously described (23, 27). Titer was expressed as reference units after adjustment to the human reference interferon standard (National Institute of Allergy and Infectious Diseases catalog no. GO23901537).

RESULTS

Interferon in serum. When testing for interferon in serum, in each case, blood was taken before beginning treatment with poly(I:C-LC) (Fig. 1). These specimens contained no detectable interferon. Similar specimens were taken from patients with SLE who were treated supportively. Only 1 of 18 of these sera had a detectable interferon titer (32 U/ml). On the other hand, poly(I:C-LC) induced significant levels of serum interferon. The only exception was a 62-year-old man with bronchogenic carcinoma and disseminated varicella-zoster infection who had no interferon response, despite five or six daily doses of 0.20 mg of poly(I:C-LC) per kg. Otherwise, on day 1 of the poly(I:C-LC) infusions, interferon titers ranged from <50 to 800 U/ml with higher doses of the inducer generally producing the greater and more prolonged response. Peaks occurred 8 to 16 h after infusions. Mean peak levels were 70, 250, and 675 U/ml in patients receiving 0.1, 0.2, and 0.3 mg of poly(I:C-LC) per kg, respectively. After the first and second infusions, these interferon levels persisted for 12 to 48 h, but beginning with day 3 of the 5-day schedules, lower levels were found.

In striking contrast to this usual response, a 69-year-old woman with multiple myeloma, chronic renal insufficiency, and disseminated varicella-zoster experienced successively rising interferon titers with each of three infusions of 0.30 mg of poly(I:C-LC) per kg. Titers were 320 U/ml (12 h after dosing), 610 U/ml (12 h after dosing) and 3,150 U/ml (16 h after dosing) on days 1, 2, and 3, respectively. The 65-year-old
Clinical findings (fever, hypotension). Within 3 to 8 h after receiving poly(I:C-LC), fever developed. The mean peak temperature was 39 to 40.5°C but, rarely, recordings up to 41°C were observed. Temperatures returned to their previous values within 4 to 6 h. The single patient with no interferon response remained afebrile (Fig. 2). Fever preceded interferonemias,
supporting the distinctiveness of interferon and endogenous pyrogens. As with interferon titers, the highest temperatures appeared on the first 3 days of treatment. Temperatures were suppressed by acetaminophen and hypothermic blankets.

Hypotension was the major toxic effect of poly(I:C·LC) and occurred in 7 of the 14 treated patients (Table 1). A hypotensive episode was considered to occur when systolic blood pressures fell ≥30 mm Hg, and the systolic reading reached ≥90 mm Hg. Falls in diastolic pressure accompanied systolic drops, but lower diastolic values were less easily heard and were sometimes not recorded. With doses >0.15 mg of poly ILC/LC per kg, hypotension appeared 10 times in seven patients. Pressures fell 2 to 20 h after infusions and were often brief, lasting as little as 15 min. These episodes had no apparent relationship to fever, interferon titer, or day of treatment. Central venous pressures were low, and blood pressure usually responded to intravenous fluids. Blood pressures are known to be labile in patients with encephalitis, and this was the diagnosis in five of the seven patients experiencing hypotension. Each of the patients with encephalitis recovered from hypotension.

Two patients died during the course of this study; a 14-year-old girl with Hodgkin’s disease and disseminated varicella-zoster with myocarditis and pneumonia. (At autopsy, varicella-zoster virus was isolated from the heart and lung in human foreskin fibroblast tissue cultures.) This illness was terminal at the time poly(I:C·LC) was initially given, and the cardiovascular collapse and death of the patient cannot be attributed to poly(I:C·LC). The 69-year-old woman with multiple myeloma, renal insufficiency, and disseminated varicella-zoster was also very seriously ill at the time poly(I:C·LC) was given. Whether poly(I:C·LC) contributed to her illness cannot be stated, but it may be that poly(I:C·LC) should be administered with special caution to patients with compromise of their mononuclear phagocytic function (e.g., liver and spleen), lymphoma, multiple myeloma, and renal insufficiency. This possibility will have to be considered in further studies.

Laboratory findings (lymphopenia, increased hepatic enzymes). Transient absolute lymphopenias (<1,000 cells per mm<sup>3</sup>) developed after the first infusion of poly(I:C·LC) in 10 of the 14 patients. Lymphopenia persisted for 2 to 5 days before returning to normal (Fig. 3). These peripheral lymphopenias could well have been due, at least in part, to concurrent viral diseases. We did not determine whether lymphopenias were deficiencies of thymus- or marrow-derived cells, or both. Transient leukocytoses (>10,000 cells per mm<sup>3</sup>), along with “shifts to the left” (>5% juveniles), followed in several patients. No other hematological alterations were noted.

Elevations of hepatic enzymes are diagnostic in virus hepatitis and are usual, but of lesser severity, in patients with SLE (29). Therefore, rises in serum glutamic oxalacetic transaminase and lactate dehydrogenase were attributed to poly(I:C·LC) only in patients with no other ob-

<table>
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<th>Patient no.</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Poly(I:C·LC), dosage (mg/kg per day)</th>
<th>Day of treatment</th>
<th>Hypotension</th>
<th>Onset [h. after poly(I:C·LC) infusion]</th>
<th>Duration (h)</th>
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<td>F</td>
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<td>1</td>
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<td>16</td>
<td>4</td>
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<td>2</td>
<td>36</td>
<td>F</td>
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<td>18</td>
<td>0.5</td>
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<td>4(a)</td>
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<td>F</td>
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<td>0.2</td>
<td>1</td>
<td></td>
<td>10</td>
<td>2</td>
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<tr>
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<td>88</td>
<td>F</td>
<td>St. Louis encephalitis</td>
<td>0.2</td>
<td>1</td>
<td></td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>43</td>
<td>M</td>
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<td>1</td>
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<td>2</td>
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<tr>
<td>6(a)</td>
<td>69</td>
<td>F</td>
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<td>3</td>
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<td>5</td>
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<tr>
<td>6(b)</td>
<td>69</td>
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<td>6(c)</td>
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<td>Disseminated varicella-zoster</td>
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<td>15</td>
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</table>

* Systolic blood pressure ≥90 mm; fall ≥30 mm. F, Female; M, male.
INTERFERONEMIA WITH POLY(I:C-LC) IN HUMANS

Fig. 3. Daily absolute lymphocyte counts per mm$^3$ (●) and their means (○) are shown in patients receiving 5-day courses of 0.15 to 0.3 mg of intravenous poly(I:C-LC).

vious cause of hepatic derangement. The latter are seen in Fig. 4. Elevations of hepatic enzymes were dose related, mild to moderate in severity (≤200 U/ml), increased with repeated infusions, and returned to normal within several days after poly(I:C-LC) was stopped. Alkaline phosphatase, serum bilirubin, renal function, and clotting test results remained normal. At autopsy, the patient who died of multiple myeloma had no hepatic abnormalities.

Neurological scores in patients with SLE. In poly(I:C-LC) and symptomatically treated patients, neurological scores decreased during hospitalization (Fig. 5). Patients receiving poly(I:C-LC) were sicker (higher neurological score) and older [mean age of the poly(I:C-LC)-treated group, 63.4 years; mean age of “control,” 52.6 years]. Likewise, the average duration of symptoms before admission was 4.5 days in the poly(I:C-LC) group, but control patients were admitted to the hospital on day 1 of illness. Neither a beneficial nor deleterious effect for poly(I:C-LC) was evident and, of course, this phase 1 study was not designed to assess clinical efficacy.

DISCUSSION

Poly(I:C-LC) induces high titers of interferon when given intravenously to monkeys, chimpanzees (23), and as shown here, to humans. Inter-
feron titers in humans were somewhat lower than those seen in some primates, but higher doses of poly(I:C-LC) were used in the latter cases. In these studies of patients with virus infections, the intravenous doses of 0.2 to 0.3 mg of poly(I:C-LC) per kg were most active. The titers of interferon we observed are significantly higher than those obtained with unmodified poly(I:C) (12) and compare favorably with levels of interferon found in sera of patients treated with several million units per day of exogenous interferon (15). It would be most useful if further work might lessen the effects on interferon inductions of the hyporeactive period, but nevertheless, these findings are encouraging. Caution is required because of the hypotensive episodes that seven of our patients experienced. However, in parallel studies at the National Cancer Institute, A. Levine and one of us (H.B.L.) have noted serious hypotensive responses less frequently than reported here (A. Levine, and H. B. Levy, personal communication). It may be that patients with malignancies other than multiple myeloma may be less responsive to the side effects we have noted.

In one report, stimulated leukocyte cultures from patients with solid tumors produce 2 to 16 times less interferon than similar cells from normals (8), and one patient here with carcinoma of the lung had no rise in interferon titer or fever.

Hyporeactivity did not develop in a 69-year-old woman with multiple myeloma and renal insufficiency. The highest interferon titer (and fever) we note appeared on day 3 of her treatment. In vitro malignant plasma cells from patients with multiple myeloma may spontaneously produce interferon (13), a property not shared by normal bone marrow lymphocytes. Malignant plasma cells may not produce (or respond to) a control protein normally terminating interferon synthesis. This control protein has been postulated as a possible molecular cause of the hyporeactive state (9, 13, 18). About 10% of exogenously administered interferon is excreted via the kidney (18), and since interferon titers in this patient returned to zero after the first and second doses of poly(I:C-LC), it is not likely that renal failure contributed to the unusual interferon titers on day 3 here. In experimental animals, tolerance to synthetic interferon inducers lasts about 1 week (9, 18), and this is consistent with our patient with SLE who again responded normally to a second 5-day course of poly(I:C-LC) after a recovery period of 6 days.

Toxic effects of poly(I:C-LC) were fever, hypotension, lymphopenia, and increases in serum glutamic oxalacetic transaminase and lactic dehydrogenase. These toxic effects lessened as did interferon titers on days 3 to 5 of the administration of poly(I:C-LC). Such amelioration of the effects of interferon inducers has been defined as a "hyporeactive state." After poly(I:C-LC) was stopped, all abnormalities disappeared.

Hypotension, fever, lymphopenia, interferonemia, and tolerance (hyporeactivity) of poly(I:C-LC) are reminiscent of endotoxemia in rabbits. Tolerance to bacterial endotoxin has been ascribed to a facilitated capacity of the mononuclear phagocytic system to clear this macromolecule from the blood (3, 34). Facilitated clearance of poly(I:C-LC) is an alternate explanation for the induction of the hyporeactive state. It is unclear which of the toxic effects may be due to poly(I:C-LC) or, in turn, the induced interferon. Indeed, exogenous interferon in humans has caused fever, leukopenia, hypotension, and falls in platelet and reticulocyte counts (5, 10, 15, 19, 31).

Finally, no evident benefit resulted from the use of poly(I:C-LC) in these patients. However, it is important to note that serum interferon diffuses very poorly into the cerebrospinal fluid and brain (5, 7, 10, 16). Although the hypotensive effects of the present preparation of poly(I:C-LC) are a cause for concern, they may be amenable to pharmacological management. A companion paper in which poly(I:C-LC) is infused intravenously in rabbits addresses this possibility (B. G. Gatmaitan, R. C. Legaspi, H. B. Levy, and A. M. Lerner, submitted for publication.) The high titers of serum interferon obtained in this study and in the other studies alluded to above encourage further work with this and other further possible modifications of poly(I:C-LC).
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LITERATURE CITED