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MINI REVIEW

Polyadenylic · Polyuridylic Acid in the Cotreatment of Cancer (42055)

A. M. MICHELSON,* F. LACOUR,† AND J. LACOUR†

*Institut de Biologie Physico-Chimique, 13, rue P. et M. Curie, 75005 Paris, and †Institut Gustave Roussy, 94805 Villejuif Cedex, France

Abstract. The biological properties of polyadenylic · polyuridylic acid (Poly(A) · Poly(U)) are discussed and the application to the treatment of cancer in various animal models is described. Pharmacokinetic properties are presented and the results of clinical application to humans with specific reference to adjuvant treatment of breast cancer are examined in some detail. Given these results and the total lack of toxicity or of secondary effects of Poly(A) · Poly(U) it appears that further development and application of this polynucleotide complex are certainly justified in terms of clinical use in cancer and perhaps in viral pathologies.

Poly(A) · Poly(U) is a double stranded helical ribopolynucleotide complex (for review see (1)) in which each adenine in one strand is hydrogen bonded to an equivalent uracil residue in the complementary strand. The activity of synthetic polynucleotide complexes as adjuvants to the normal immune response was demonstrated about 15 years ago (2–4). In contrast to the acute toxicity shown by Poly(I) · Poly(C) (LD_{50}, 0.5 to 1.0 mg/kg for rabbits, intravenous) no toxic symptoms or excessive pyrogenicity have been observed with Poly(A) · Poly(U). The former complex has nevertheless received much more attention since it is more effective as an inducer of interferon (5).

The cellular targets appear to be multiple and Poly(A) · Poly(U) appears to facilitate the release of secretory proteins by non immunocompetent cells (6) and stimulates the synthesis of hemoglobin in Friend erythroleukemic cells. With respect to immunocompetent cells, the regulator action of the complex is not entirely clear. Effects on macrophages and T lymphocytes in general (T helper, T suppressor, specific and nonspecific T-cytotoxic cells) have been described (7–9) and it is clear that Poly(A) · Poly(U) restores deficient thymic activity. B cells also serve as targets in the adjuvant effects of Poly(A) · Poly(U) *in vivo* (10, 11) and precursor bone marrow stem cells are stimulated (increase in hematopoietic colony forming cells) by Poly(A) · Poly(U) (12, 13) perhaps via T-cell regulatory activity. Other effects of Poly(A) · Poly(U) have been observed in the stimulation of sensitized human leukocytes (14) which suggested that the complex could be useful in the potentiation of deficient human responses to vaccines, particularly for certain viral vaccines, and to immunodeficient diseases in general (15).

Cancer: Animal Models. The earliest use of Poly(A) · Poly(U) with respect to cancer, using the complex as an adjuvant to surgery in spontaneous mammary cancer in mice (16) was described in 1970. The rate of tumor growth was reduced and the mean survival time was significantly increased (17). In contrast with these conditions (treatment coincident with surgery) no effect of Poly(A) · Poly(U) (iv) alone on the developed tumor was observed. However, a significant prophylactic effect on the incidence of spontaneous mammary tumors was observed when the complex was injected (ip) into new born females and the C3H/He mice followed over a 380-day period (18). In another strain (AKR) of mice showing spontaneous leukemia, treatment (sc) with Poly(A) · Poly(U) beginning at 7 weeks of age dramatically prolonged the long-term ($9\frac{1}{2}$ months) survival (19) inciting the authors to suggest that the complex may eventually be used as an anticancer drug in man in view of its extraordinary lack of toxicity and its therapeutic effectiveness in this model, no other therapy being so unequivocal for such a long-term survival.

Poly(A) · Poly(U) (ip complementary to surgery) was also extremely effective in hamsters bearing a highly metastizing transplant-

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able melanoma (T 1196) in that the appearance of early metastases was reduced by 50% as compared with untreated animals or animals treated by surgery alone (17). Decrease in the recurrence of a transplantable syngeneic tumor first removed by surgery by use of the complex has also been reported (20). Antitumor effects were also shown in Rausher MuLV-induced BALB/c mouse ascites (21).

Adjuvant treatment by Poly(A)·Poly(U) with chemotherapy has also been studied in view of the highly potent immunostimulant effects of the complex on both humoral and cell-mediated immune responses. Highly significant tumor inhibition was observed in C3H/He mice bearing established grafted subcutaneous mammary tumors when alternate weekly administrations of cyclophosphamide (ip) and Poly(A)·Poly(U) (iv) were applied for 8 consecutive weeks (22). Average tumor diameter on Day 63 was one-third of that of mice receiving cyclophosphamide alone and a significant decrease in mortality, an increase in median survival time, and a higher rate of complete tumor regression was also observed. Use of alternate (not coincident) treatment with Poly(A)·Poly(U) and cyclophosphamide thus showed a very marked improvement over chemotherapy alone. Again, treatment with Poly(A)·Poly(U) alone (started at Day 21, i.e., fully developed tumors) showed no significant effects on survival. However it was noted that the *in vivo* cytotoxicity of splenic mononuclear cells (using a YAC-1 lymphoma cell line as target) was significantly ($P < 0.001$) increased at 48 hr after iv inoculation of Poly(A)·Poly(U) whereas ip injection of cyclophosphamide significantly decreased cytotoxic activity. The increased cytotoxic activity due to Poly(A)·Poly(U) was even more pronounced in the case of animals receiving alternate chemotherapy and polynucleotide treatments particularly at Day 44 (mice now 10 weeks old) when augmented activity was still observed in mice receiving the combined treatment whereas those treated with Poly(A)·Poly(U) alone showed a low level of splenic nonadherent mononuclear (N.K.) cell cytotoxicity.

Mechanisms. The biological mediator properties of Poly(A)·Poly(U) involve various activities which lead to a synergistic tumor inhibition, whether coupled with surgery or chemotherapy. Thus the number of T lymphocytes is increased by Poly(A)·Poly(U) (whereas cyclophosphamide depresses suppressor T cells for delayed-type hypersensitivity) and specific T-cell-mediated immune response is increased, as is the natural killer cell activity against residual tumor cells (22). At the level of biochemical events, it has been shown that plasma protein kinase (p 67 K kinase) activities in mice are enhanced after treatment with Poly(A)·Poly(U) using doses of 0.4 to 0.6 mg/kg at 6 to 24 hr after injection (23). Oligo 2–5 A synthetase levels are also enhanced in mice spleen and lungs after treatment with Poly(A)·Poly(U) (24). Increase of both of these enzymes is evidence of the presence and action of interferon. However, at the dose levels used, corresponding to clinical levels, no interferon could be detected.

Nevertheless, at higher doses of Poly(A)·Poly(U) (2.0 to 10 mg/kg) low levels of acid stable interferon were detected in mice (23). The kinetics show that maximum amounts are detectable between 6 and 9 hr after administration. It may be noted that the enhancement of protein kinase activity follows the kinetics of interferon production with maximum levels some 15 hr after the peak of circulating interferon. The acid stability of this interferon suggests that it is type I i.e., α (leukocyte)- or β (fibroblast)-interferon. It is neutralized by anti-mouse α- and β-interferon, but not by anti-mouse γ-interferon antibodies.

In view of this interferon induction as evidenced by increased levels of protein kinase and oligo A synthetase as well as direct measures of interferon at higher Poly(A)·Poly(U) doses it is perhaps useful to summarize the effects of interferon on cells (25). An antiviral state is created in which the replication of a large spectrum of both RNA and DNA viruses is inhibited, alterations in certain cell functions are observed and the multiplication of both tumor cells and normal cells of different species is inhibited. Modifications in the immune response and alterations of the cell surface are observed and interferon may also change the expression of erythroid differentiation.

Recently it has been shown that treatment

of HeLa tumor-bearing athymic nude mice with mouse interferon (α and β) resulted in enhanced activities of oligo 2–5 A synthetase and protein kinase in the spleen and lung (i.e., mouse cells) but not in the human HeLa cells (26). The two activities were increased in the tumor cells only after treatment of the mice with human fibroblastic (β) interferon, only very slight effects in the spleen and the lung being observed. When HeLa tumor-bearing mice were injected with Poly(A)·Poly(U) both the protein kinase and oligo A synthetase levels were increased in both the tumor cells and the different tissues since both mouse and human interferons were produced under these conditions. The results indicate a direct action of interferon on homologous tumor cells and that tumor cells in an organism may themselves produce their own interferon and respond to it. This suggests a possible advantage of an interferon inducer such as Poly(A)·Poly(U) over interferon in the treatment of different tumors since the type of interferon used may not necessarily be the one which interacts with tumor cells. It may also be concluded that the overall action of interferon on tumor cells in an organism cannot be uniquely attributed to action on the host defensive mechanism and that there is also a direct interaction with the tumor cells (26).

In humans after intravenous injection of Poly(A)·Poly(U) (30–60 mg, i.e., 0.5 to 1.0 mg/kg) results very similar to those seen in mice have been observed. Thus while no circulating interferon is detected (levels too low), enhanced levels of protein kinase (p 72 K in humans) are observed in the plasma (platelets). The level of this kinase in 16 different patients treated with 30 mg Poly(A)·Poly(U) (0.4 to 0.6 mg/kg) was increased in 14 cases compared to the level before treatment (24), this level varying from one patient to another (as with normal subjects). The kinetics of the enhancement observed in each case varied and in some the maximum was at 7 to 9 hr after injection whereas in others higher levels of kinase were observed at 24 hr probably reflecting differences in the response of each patient.

While Poly(A)·Poly(U) is no doubt a poor inducer of interferon compared with other inducers it is possible that the amount of interferon due to this complex is sufficient for a meaningful physiological response. The optimal level of circulating interferon is unknown and there may be a saturation point with respect to full biological action. Thus C3H mice injected with 200 μg of Poly(I)·Poly(C) produce 10-fold higher levels than with an injection of the same amount of Poly(A)·Poly(U). However, the levels of two interferon-mediated enzymes, protein kinase and oligo A synthetase in the spleen are identical. Thus higher levels of circulating interferon may not necessarily lead to a better response and could indeed give rise to undesirable secondary effects.

The level of oligo 2–5 A synthetase in peripheral blood lymphocytes of patients treated with a single iv injection of 60 mg Poly(A)·Poly(U) was also increased at 24 and 48 hr after injection. (Patients treated with interferon show an increase in both the protein kinase and the oligo A synthetase.) Mean values of the enzyme in 48 patients (breast cancer) were 2.97 ± 1.40 nmole AMP/10^6 lymphocytes and in 46 controls 2.0 ± 0.97 nmole units, a significant ($P < 0.001$) difference. After treatment with Poly(A)·Poly(U) the values in the patients were increased to 5.31 ± 3.12 at 24 hr and to 5.13 ± 3.79 at 48 hr. The kinetics of enhancement were again variable in individual patients ranging from 24 to 48 hr, some patients showing very little response (14.6%) or no response (6.2%). However a significant increse of 1 to >4 units was observed in 79.2% of the cases (27).

A single treatment with 60 mg Poly(A)·Poly(U) also enhanced natural killer cell (NK) cytotoxicity significantly against human myeloid K 562 target cells, 24 and 48 hr after injection in 40 patients. The increase is principally observed in patients with low NK activity before treatment. Average cytotoxicities were 38.7% before treatment, 42.2% at 24 hr and 47.8% at 48 hr after injection, with values of $P < 0.01$ and <0.001, respectively. This enhancement may be due to induction of interferon despite the lack of detection, since by analogy with mice the level would be too low to be detectable in an *in vitro* assay, especially since circulating interferon has a very short half-life. It may be noted that NK cytotoxicity is not modified

or is depressed when patients are treated with high doses of recombinant leukocyte interferon (100 to 400 $\times$ 10^6 units per week) whereas lower doses (60 $\times$ 10^6 units per week) did increase levels of NK activity (28), that is a better boosting effect is observed with low doses of interferon compared with higher doses (29). This again suggests certain advantages of Poly(A)·Poly(U) compared with nonoptimized interferon therapy in which both the dose and the type of interferon may have a different mode of action on natural killer cell activity and it is clear that cancer patients at different stages of the disease and of different etiology may respond differently to interferon. These problems are considerably reduced in the case of Poly(A)·Poly(U).

Poly(A)·Poly(U) appears to interact with many cell populations. Its biological activity could therefore be exerted at different levels. Indeed in patients, 24 hr after a single iv injection of 30 mg of this complex, it was observed that the mean percentage of E. Rosette forming cells was significantly ($P = 0.001$) higher than that found before the injection (60.8% versus 51%), confirming observations on T lymphocytes in mice (30).

Pharmacokinetics and Distribution. Few *in vitro* studies have been reported on the cellular properties of Poly(A)·Poly(U) and until recently *in vivo* organ distribution and pharmacokinetics were totally unexplored. It has been shown that the complex penetrates Ehrlich ascites tumor cells without separation of the two strands (31), and that uptake by mouse ascites tumor cells and human lymphocytes in culture occurs (32), the material migrating rapidly into the cell nucleus and remaining intact for 2 hr. There is also evidence than 80% of thymocytes bind Poly(A)·Poly(U) on the cell membrane *in vitro* (33).

The first pharmacokinetic studies of Poly(A)·Poly(U) in animals, including lifetimes and intracellular as well as organ distribution, were reported (34) in 1985. Prevailing conceptions were highly suggestive of rapid metabolic destruction after injection due to nuclease activities. However, Poly(A)·Poly(U) showed, in fact, an unexpected metabolic stability.

Rabbits were injected with ^{51}Cr-labeled Poly(A)·Poly(U) and the metabolic fate and stability were studied using scintigraphic techniques and subcellular fractionation. The complex has a very long lifetime (measured in days) in both liver and spleen, the major locations of concentration in the animal, with about nine times more per gram of tissue in the spleen compared with that in the liver; though given the relative sizes of the two organs, the major fraction of injected material is localized in the liver. Direct determination of the radioactivity in the liver and in the spleen 90 min after injection of ^{51}Cr-labeled Poly(A)·Poly(U) showed that 61.8% of the input radioactivity was incorporated in the liver, and 8.5% of the total radioactivity was located in the spleen. Calculated decay times to 50 and 25% of maximum radioactivity in the liver were 4 days and 9 days, respectively, for Poly(A)·Poly(U). In spleen cells the material is about equally divided between nuclei and cytoplasm. The polymer is slowly degraded to shorter molecules in these cells but even 1 week after injection, significant amounts of apparently intact polynucleotide complex can be detected in both cellular fractions. Sucrose gradient sedimentation profiles indicated that the complex remained undegraded in spleen cells compared to the profile of intact 16 to 18*s* Poly(A)·Poly(U). This result is not altogether unexpected since an *in vitro* study in tumor lymphocytes had shown earlier than Poly(A)·Poly[^{3}H]U remains intact for at least $2\frac{1}{2}$ hr (30). The study conducted *in vivo* showed that shorter fragments of Poly(A)·Poly(U) appear 24 hr after inoculation and the amount increased over 7 days. Nevertheless, even after 1 week a nonnegligible quantity remains undegraded in the cytoplasm and in the nuclei of spleen cells (34).

This somewhat surprising *in vivo* metabolic stability of Poly(A)·Poly(U) may well have a bearing on the biological activities of this polynucleotide complex. However, the relevance of the long lasting persistance of undegraded Poly(A)·Poly(U) in the liver and the spleen to antitumor effects observed both in experimental animal models and in humans remains to be determined.

Human Clinical Trials of Poly(A)·Poly(U) in Cancer. The absence of any toxic or undesirable secondary effects confers a special interest to pharmacological studies of this complex. In addition, Poly(A)·Poly(U) can be easily prepared in industrial quantities, by methods which routinely give a product with the same biological and physical properties. However, up till the present time only one serious clinical trial has been reported in detail. This trial was conducted at the Institut Gustave Roussy, Villejuif, one of the largest cancer hospitals in France under the direction of J. Lacour, beginning in 1972. By the end of 1979, 300 patients with primary operable breast cancer had been included (35–38).

Patient selection was based upon the following criteria; (a) the tumor was an infiltrating carcinoma of medium size, 2–7 cm at its greatest diameter, without skin involvement and without complete fixation to the greater pectoral muscle; (b) there were no palpable nodes, or nodes were palpable but movable; and (c) there was no detectable metastasis. According to the TNM classification, these patients corresponded to category T_2 and part of T_3, N_0N_1, and M_0. Patients were considered ineligible if they (a) had small tumors, (b) had a large-size tumor that had been treated at the Institute by preoperative radiotherapy, (c) were over 65 years of age, (d) had received an immunosuppressive treatment within the 3 months preceding their admission, (e) had other neoplasms, or (f) were poor surgical risks.

The locoregional treatment was the same for all the women. This conventional treatment consisted of a modified radical or extended mastectomy without complementary treatment when the axillary nodes were negative for the presence of cancer. Patients with cancer-positive nodes received complementary cobalt therapy (4500 rad were delivered to the chest wall, axilla, supraclavicular nodes, and internal mammary chain) and radiotherapeutic castration if they still had menses or had entered menopause within the past 2 years. The patients were randomized into two groups, one group receiving Poly(A)·Poly(U) given intravenously at the rate of one injection of 30 mg per week for 6 weeks beginning 8 days after surgery, and a control group receiving a placebo on the same schedule.

Patients were evaluated for the incidence of locoregional recurrences and for distant metastases, and the overall and relapse-free survival were determined. They were given a complete clinical examination every 3 months during the first 3 years, every 6 months during the following 2 years and then once a year. Chest and pelvic X rays were routinely performed once a year, more often and associated with other investigations when clinical examination revealed abnormal symptoms. All statistical analyses employed two-tailed tests to assess the significance of differences.

The 5-year results (36) indicated that Poly(A)·Poly(U) produced a significant increase in the overall actuarial 5-year survival rate ($P < 0.05$) which was 72% for conventional treated patients and 82% for those supplemented with Poly(A)·Poly(U). Of the former group 31% had metastases compared with 22% for the latter. Relapse-free survival curves, despite a pronounced difference, were not significant ($P = 0.11$ by the log rank test) the actuarial 5-year survival being 56 and 72%, respectively. In the case of node-positive patients (102 controls, 103 treated with Poly(A)·Poly(U) who have a higher risk of metastasis the overall survival was 65 and 76% ($P = 0.07$), respectively, while relapse-free survival after 5 years showed 47% in the first group and 71% in the Poly(A)·Poly(U) group ($P < 0.03$). The results suggested that the treatment was statistically significant particularly in cases when three or less nodes were involved, and that Poly(A)·Poly(U) may suppress microscopic distant metastases or at least delay the development of distant foci by reducing the number of neoplastic cells. It may also be noted that no adverse effects were observed and the quality of life of the patients was not deteriorated.

Updated results of the trial were analyzed in January 1983 with a mean follow-up time of 87 months and entirely confirmed the adjuvant action of Poly(A)·Poly(U) (38). In the whole series of patients, the overall survival curves are significantly different ($P < 0.05$). The actuarial 8-year survival rate was $71 \pm 4\%$ in the Poly(A)·Poly(U) group

and 57 ± 5% in the controls; the total percentage of deaths was 25% (39 cases) and 35% (51 cases), respectively. There is a slight but insignificant difference in the relapse-free survival curves between the two groups. The 8-year actuarial relapse-free survival rate was 57 ± 4% in the group receiving Poly(A)·Poly(U) and 52 ± 5% in the controls.

In the case of node-negative patients no significant differences in overall or relapse-free survival curves were observed. However, a separate analysis of the 205 N^+ patients showed 29% of deaths in the Poly(A)·Poly(U) group (30 cases) and 44% (45 cases) in the control (conventional treatment) group. The overall survival curves differ significantly ($P < 0.02$) showing an 8-year actuarial survival rate of 46% in the controls and 67% in the Poly(A)·Poly(U) group and at 8 years 42% of patients in the control group and 56% in the Poly(A)·Poly(U) group were relapse free, but this was not statistically significant.

When one to three nodes are invaded the difference between the two groups is highly significant with respect to both overall survival (80% for Poly(A)·Poly(U) and 59% for the controls, $P < 0.005$) and for relapse-free survival (68 and 46%, respectively, $P < 0.05$). When four or more nodes are involved differences are not statistically significant.

The results observed after 3 more years of follow-up confirm those published in 1980 and indicate that Poly(A)·Poly(U) has an inhibitory effect on human operable breast cancer. It is obvious that the significant beneficial effect observed in the entire series of patients is due to the increased survival in node-positive patients, for whom a general adjuvant treatment is indicated because of the high risk of metastases, numerically the most important group. The differences between the overall survival curves are more significant ($P = 0.02$) whereas relapse-free survival curves no longer show the significant differences they did in 1980. One possible explanation might be that Poly(A)·Poly(U) postponed the onset of metastases. The best results were observed in patients with one to three nodes invaded, with both overall and relapse-free survival significantly improved. It may be noted that similar observations in patients receiving chemotherapy have been made (39). Thus adjuvant therapies seem to be more effective in breast cancer of medium severity, having only a limited number of invaded nodes.

Poly(A)·Poly(U) is an effective form of adjuvant therapy in breast cancer and may well be as effective as chemotherapy. Unfortunately it is impossible to repeat the trial with Poly(A)·Poly(U) in breast cancer on a large scale since no oncologist would agree to keep a control group of N^+ patients without any general complementary treatment. In order to confirm indirectly the effect of the complex and to compare strictly with chemotherapy using cyclophosphamide, methotrexate, fluorouracil (C.M.F.) a new trial has been designed. This controlled randomized study, undertaken in the frame of "Federation Nationale des Centres de Lutte Contre le Cancer" is applied to N^+ breast cancer first treated by mastectomy plus axillary dissection. The patients are randomly allocated into two groups. The first receives cobalt therapy and Poly(A)·Poly(U) and the second, chemotherapy with C.M.F. Eleven French cancer centers are engaged in this trial and within 2 years 300 cases have been included.

Two other multicentric trials using Poly(A)·Poly(U) were more recently undertaken in tumors for which adjuvant chemotherapy failed, melanoma and colorectal carcinoma. Both compare surgery alone versus surgery plus the complex. The first one is being done in the framework of the "W.H.O." group on melanomas" run by U. Veronesi, Milan, the second at the Institut Gustave Roussy with the collaboration of several gastrointestinal surgical services in Paris.

Another trial involving 200 patients has begun in Korea for the treatment of cancer of the stomach, particularly frequent in Korea, the mortality of these cases being highest among cancers (31.7%). The patients (stages I and II) will be randomized in two groups, one treated by surgery plus chemotherapy and the other with Poly(A)·Poly(U) as adjuvant, using six weekly injections (iv) of 100 mg of the complex, beginning 10–20 days after surgical intervention.

Phase I Studies. Preclinical studies showed that no toxicity and no lethal dose for Poly(A)·Poly(U) could be established in mice or in rats. No teratogenic effects have been observed in pregnant mice injected intrave-

nously with 1 mg of Poly(A)·Poly(U), i.e., approximately 40 mg per kilogram equivalent to 2.0 g for a human. Levels for treatment of humans range from 30 to 100 mg per injection and phase I studies were initiated at the Institut Gustave Roussy, Villejuif, to determine the tolerance to Poly(A)·Poly(U) using increasing amounts per injection of 90, 180, 300, and 450 mg and to quantify the immunological response by estimation of specific markers. The material was administered by slow iv injection or by perfusion for the highest doses. Patients had received no chemotherapy treatment for 2 to 3 weeks before hand. Apart from general clinical observations, specific examination of platelets, blood urea, creatine, ionogram, and hepatic parameters were followed as well as natural killer cell activity, oligo A synthetase, protein (K 72) kinase, and interferon levels. The results may be briefly summarized in that up to the highest level used (injection of 450 mg) no biological anomalies were observed and tolerance was excellent. No toxicity could be demonstrated and subsequent treatment of several patients by chemotherapy or radiotherapy showed no increase in the toxicity of these two treatments (40). However, as with any treatment, it cannot be excluded that Poly(A)·Poly(U) may have a long-term effect not as yet detected (after 8 years in patients included in the clinical trial).

Specifications of the Poly(A)·Poly(U) used in Clinical Trials. The Poly(A)·Poly(U) used to treat humans up till the present date is covered by authorization No. 3927 (24 February 1982) of the French Ministery of Health, this visa permitting use in Hospitals under strict medical control. It is prepared by polymerization of ADP to give Poly(A) and of UDP to give Poly(U), using polynucleotide phosphorylase isolated and purified from nonpathogenic bacteria, followed by formation and purification of the double stranded helical complex obtained on mixing stoichometrically the two homopolynucleotides (Michelson, unpublished results). The material is available in bulk and the purity (absence of nucleases) is such that these solutions can be stored for moe than $2\frac{1}{2}$ years at +4°C without detectable degradation or loss of biological activity.

Biophysical properties of the material used since 1975 may be summarized briefly as follows. The thermal hyperchromicity (20–70°C) is 51–58%, with a dissociation temperature (T_m) of 59–62°C. The λ max at neutral pH is 257–258 nm and the ratio of absorbance 280 nm/260 nm is 0.45 ± 0.03. The absorption at 260 nm of a solution at 1 mg/ml is 15–18 optical density units. Sedimentation constant ($s_{w,20}$) is >7.5 and the material has a minimal molecular weight of 270,000 (average molecular weight is about 500,000 but the product is poly disperse), using the relationship:

$$\text{mol wt} = \left[\frac{S_{w,20}}{0.0882}\right]^{2.82}.$$

It is thus clear that this is not an extremely high molecular weight Poly(A)·Poly(U) (up to 20×10^6) nor is it extremely small. This avoids practical problems related to excessive viscosity and facilitates sterile filtration and intravenous injection of solutions up to 5 mg/ml.

Using standardized techniques preparations are highly reproducible, the material can be easily prepared in kilogram quantities, it is nonpyrogenic (at the doses used), and is as active with respect to biological tests as very high molecular weight material.

Conclusions. Given the accessibility of Poly(A)·Poly(U), its total lack of toxicity, and the clinical results already obtained, it is clear that further clinical studies of this helical double stranded polynucleotide complex are fully justified. Various problems remain to be explored and a comparison with Poly(I)·Poly(C) (within the toxicity limits of this polynucleotide, a toxicity which has not yet been explained) and even with interferon could be extremely useful provided that this comparison is made in the same conditions (as an adjuvant) and on the same kind of solid tumor, before it has reached the disseminated stage. While Poly(I)·Poly(C) is indeed a much more potent inducer of interferon it may be that other biological effects specific to Poly(A)·Poly(U) play at least as important a role in inhibiting cancer processes. Perhaps chemotherapy can be replaced or at least aided by "soft" biochemical approaches in which stimulation of the inherent natural defenses of the patient is efficient in the absence of secondary effects which at best

are highly undesirable from the patient's viewpoint. The results of the different trials, mainly in breast cancer, will certainly provide information about the respective values of Poly(A)·Poly(U) and chemotherapy as adjuvant. It is obvious that if efficacity is similar, preference will be given to treatment with Poly(A)·Poly(U) since it is short, nontoxic, and well accepted by the patients. Various possibilities have not as yet been developed. For example, Poly(A)·Poly(U) could be used in combination with chemotherapy either as an adjuvant in operable cancer or even in treatment of metastatic forms. Some indications that this could be valuable are given by the results obtained in animal models (22).

In addition the possible anti-viral properties cannot be neglected. Intramuscular injection of 10 mg Poly(A)·Poly(U) per week (at 5 mg/ml saline) has been employed for the treatment of Herpes simplex in at present a small series (five cases) with success. This trial will be extended to a large scale in the near future. Two cases of Kawasaki disease in infants have also been treated in Japan with positive results.

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