The antiviral activity of Isoprinosine[1]

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A hypothesis predicting that increase in the rate of synthesis of host messenger RNA would be associated with antiviral activity was examined. Biochemically, Isoprinosine or NPT-10381 (methisoprinol) increased the rate of rapid labeling of polyribosomal RNA and protein in uninfected monkey kidney cells in tissue culture. In tissue-culture systems, Isoprinosine exerted antiviral effects against influenza virus, PR-8 and A_2 strains; herpes virus, LU strain; polio virus 3; and adenovirus 10. In in vivo mortality studies, Isoprinosine was observed to exert therapeutic antiviral effects against the A_2 strain of influenza and, as well, against a herpes infection of newborn mice. Experiments were carried out which demonstrated a correlation between the in vivo anti-influenza (PR-8) effects of Isoprinosine and reduction in the virus titer in lungs of infected mice.

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L'hypothèse qu'il y a corrélation entre activité antivirale et l'augmentation du taux de synthèse de l'ARN messager de l'hôte, a été étudiée. Au point de vue biochimique, l'isoprinosine ou NPT-10381 (méthisoprinol) augmentait le taux de marquage rapide d'ARN polyribosomal et de protéine dans des cultures de cellules non-infectées de rein de singe. En culture de cellules, l'isoprinosine montrait des effets antiviraux contre le virus grippal (souches PR-8 et A_2), le virus de l'herpès (souche LU), le poliovirus 3 et l'adénovirus 10. Dans des essais sur la mortalité in vivo, l'isoprinosine montrait des effets thérapeutiques antiviraux contre la souche A_2 du virus grippal, de même qu'elle inhibait une infection herpétique du souriceau nouveau-né. D'autres expériences montraient une corrélation entre l'action in vivo de l'isoprinosine contre le virus grippal (souche PR-8) et la réduction du titre viral dans les poumons de souris infectées.

Introduction

We have observed that the inosine – alkylamino alcohol complex (4, 9), Isoprinosine[2] or NPT-10381 (methisoprinol), induces an increase in the rate of synthesis of mammalian messenger RNA (mRNA) in several tissues in vivo (3, 4), and we have hypothesized that such an action would be associated with antiviral effects (1–3). In developing this hypothesis, we reasoned that a drug priming of mammalian transcriptional activity might occur in the absence of an enhancing effect on virus transcription, and might thereby enhance the capacity of host mRNAs to compete with viral mRNAs for host ribosomes on a mass action basis. Secondly, this might result in an accelerated synthesis of host proteins associated with control of ribosome structure and mRNA selection, consequently enhancing the tendency of ribosomes to express interferon-like effects (6) and translate viral mRNA less effectively than host mRNA.

A series of experiments are reported here which support the antiviral hypothesis. These were done in both tissue culture and mouse systems, using a number of different viruses.

Materials

Viruses

Stock suspension of the PR-8 and A_2 strains of influenza virus were prepared from infected mouse lungs following passage in embryonated hens' eggs and newborn mice. The lungs were homogenized in a Waring blendor (one lung:1.5 ml Hank's balanced salt solution (GIBCO)), filtered through 0.45 μ Millipore filters and titrated for hemagglutinin activity. By this method, a stock virus titer of 2×10^6 hemagglutinating (HA) units/ml was obtained. This stock preparation was separated into aliquots which were frozen for later use. After a single thawing, fresh serial dilutions of stock virus were made for each in vivo experiment.

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[2]Manufactured by Newport Pharmaceuticals International, Inc., Newport Beach, California.

Influenza virus used for tissue-culture studies was adapted by repeated passage on monkey kidney cells, and the titer was determined by hemagglutination techniques. Herpes virus (LU strain) was found to be serologically identical with the small plaque virus of Rapp, and was grown in tissue culture by the method of Rapp and Hsu (8). Polio virus (type 3) and adenovirus (type 10) were grown on the appropriate cell lines, and repassaged for sufficient titer. Herpes-, polio-, and adeno-viruses were titrated by an agar plaque – overlay technique and are expressed in plaque-forming units (pfu)/ml.

Animals

For all in vivo studies, female non-inbred Swiss mice were obtained from A. R. Schmidt Company, Madison, Wisconsin. Size of animal varied according to the individual experiment.

Drug

Isoprinosine or NPT-10381 (the acetamidobenzoate salt of an inosine – dimethylamino isopropanol complex, mole ratio 1:3) (4, 9) was obtained from Newport Pharmaceuticals International, Newport Beach, California.

For in vivo studies, dose varied according to the experiment between 300 and 500 mg/kg per day. Here, it is important to be aware of the relative nontoxicity of Isoprinosine. Oral LD_{50} in mouse, rat, and cat is greater than 5 g/kg (5). Intraperitoneally, toxic signs do not appear until a dose of 3 g/kg is reached (Lynes, personal communication).

For tissue-culture studies, the drug was used at a concentration of 10 μg/ml. At this and higher concentrations, Isoprinosine exhibited no cytotoxicity on any of the cell lines used. In previous studies, we determined that concentrations up to 200 μg/ml did not cause the tissue-cultured cells to appear abnormal. Treated cells showed no signs of pyknosis; the cells multiplied in a normal manner as observed under the microscope, without rounding of cell cytoplasm and without appearance of multinucleated forms (Gordon and Brown, unpublished observations).

Cell Lines

Influenza strains and polio virus were grown and passed on monkey kidney cells; herpes virus on human amnion cells; adenovirus on human epithelium, as described by Merchant *et al.* (7). These cell lines had been cloned and tested for mycoplasma before use in these studies.

Tissue-Culture Growth Medium

Tissue-culture medium consisted of Eagle's minimum essential medium with Earle's salts, L-glutamine, and nonessential amino acids, but without sodium bicarbonate (F-15, GIBCO). To this was added sterile 10% fetal calf serum, with no antibiotics.

Methods

Influenza Mortality Study in vivo

Effects of Isoprinosine on the mortality of 12–14 g mice infected with influenza virus (A_2 strain) were evaluated by varying both virus titer and the day on which drug treatment was initiated. Mice were inoculated intranasally with 0.1 ml of undiluted and four serial 10-fold dilutions of stock virus, forming five separate virus dilution groups of 80 mice each. After inoculation, the mice of each dilution category were randomly distributed between a control and three different treatment groups of 20 mice each. Treated groups received 500 mg/kg per day Isoprinosine intraperitoneally, with treatment initiated on day 0 (3 h after virus inoculation), day 1 or day 2, respectively. Mortality statistics were collected at day 16.

Herpes Mortality Study in vivo

Effects of Isoprinosine were examined on a herpesvirus infection (LU strain) in newborn mice. One-day-old mice were each given an intraperitoneal inoculation of 0.5 ml of a dilution of virus suspension (1×10^4 pfu/ml) previously determined to be sufficient to kill 90–100% of such mice. Inoculated animals were then randomly distributed between an untreated group, a saline-treated group, and a drug-treated group of 30 mice each. Intraperitoneal drug (400 mg/kg per day Isoprinosine) and saline injections began at day 1 and continued to day 20, when survival was evaluated.

Antiviral Effect of Isoprinosine in Tissue Culture

The following viruses were selected for study: influenza virus, PR-8 and A_2 strains; polio virus, type 3; herpes virus, LU strain; and adenovirus, type 10. For each virus strain, growth was evaluated in 10 control and 10 experimental 30-ml T-flasks, each containing 5 ml growth medium. After checking for toxicity and sterility, Isoprinosine was added to the culture-incubation medium of the experimental flasks on day 0.

An assessment of influenza virus titer was made at zero time and 72 h by hemagglutination techniques. The polio-, herpes-, and adeno-viruses were titrated at zero time and 72 h by the plaque-count technique. The 72-h experimental period was found to lie well within the logarithmic growth phase of the viruses studied, and was chosen arbitrarily to serve as an initial methodology for assessing drug effect on virus growth in tissue culture.

Drug Effect on the Weight and Influenza Virus Content of Mouse Lungs Examined by in vitro Titration

Two experiments were carried out in which the relationship of antiviral effects to Isoprinosine reduction in lung virus content was evaluated.

In the first experiment, the PR-8 virus filtrate was diluted so that it contained about 2×10^2 HA units/ml. On day 0, 1500 mice weighing 26–28 g were each given an intranasal inoculation of 0.1 ml. In these mature mice, this dosage of virus produced mild to moderate influenza with only 5–10% death. After virus inoculation, the mice were randomized and divided into two groups (a control and a treatment group). As a control series, another 1500 mice not given virus were randomly divided into a control and a treatment group. Both groups of treated animals received 300 mg/kg per day Isoprinosine throughout the course of the experiment.

On day 0, and on each day following through day 11, 50 mice from each of the above four groups were sacrificed and their lungs removed and weighed, forming a separate lung pool of 100 lungs for each group. By this means, the effects of influenza infection and drug effects thereon were examined against the growth curve for lung weights of treated and untreated normal mice.

Lung pools from the untreated and drug-treated virus-inoculated animals were then processed separately for the evaluation of virus titer. Lung homogenates were prepared, 5% by weight, in Earle's balanced salt solution. A series of 10-fold dilutions was prepared, added to a mouse-embryo cell-culture system and, on day 3, evaluated for virus titer by both hemagglutination and hemadsorption, and observed for cytopathic effect. Data presented are results for hemagglutination studies; other parameters gave similar results.

Drug Effect on Lung-Virus Content Examined by in vivo Titration

In the second experiment, decreasing concentrations of virus were given to different groups of mice, beginning with a concentration four orders of magnitude greater than the concentration used in the lung-weight experiment. Sixty 12- to 14-g mice were inoculated with 0.1 ml per virus-dilution category. After inoculation of the virus, the mice which received each virus dilution were randomly divided into three groups, with treatment given intraperitoneally once per day. One group received saline, while the other two groups received 500 mg/kg Isoprinosine with treatment initiated on day 0 and day 1, respectively. The 10 sickest animals from that drug-treated group manifesting the most apparent treatment effects, as determined by mortality at 7 days, were sacrificed to provide virus from drug-treated animals for the next phase of the study; the 10 surviving control animals from the same virus-dilution category were sacrificed to provide virus from the untreated animals.

To determine the degree of viral infectivity found in treated and untreated lungs, a characteristic presumably related to the relative number of infectious units present, five serial dilutions of homogenates of lung material obtained from these two sources were inoculated intranasally into normal recipient mice (12–14 g). For each virus source, 50 mice were inoculated per virus-dilution category. Morbidity and mortality were evaluated on a double-blind basis after 7, 14, 21, and 28 days. Our method for ranking animal morbidity is shown in Table 1. It should be noted that only those mice suffering from serious illness (ranks 3 and 4) are reported; animals with these levels of illness ultimately succumb.

TABLE 1
Morbidity index

Rank	Description
0	Normal mouse evincing no weight loss, coat disturbance, or breathing difficulty.
1	Animal with minimal signs.
2	Animal with weight loss greater than 15%, some sneezing and coat disturbance, but no marked disturbance of respiratory excursions.
3	Animal with weight loss of 20% or greater, ruffled fur, and a measure of breathing difficulty, but no moribund unsteadiness of locomotion.
4	Moribund animal evincing a weight loss of more than 25% of starting weight, ruffled fur, exaggerated respiratory excursions, and typical unsteadiness.

Biochemical Studies in Tissue Culture

The effects of Isoprinosine on the incorporation of RNA and protein precursors into the polyribosomes of monkey kidney cells were studied in uninfected and PR-8 influenza virus-infected systems.

The first experiment, carried out on the uninfected system, used sixteen 500-ml culture flasks, eight of which received 10 μg Isoprinosine/ml growth medium. Cultures were incubated for 48 h. Drug-containing and control media were made up to 1 μcurie/ml orotic acid – ^{3}H and 1 μcurie/ml ^{14}C – amino acid mixture 45 min before cell harvest. Cells were harvested by standard techniques and, after trypsinization, were pooled into two groups according to treatment. Polyribosomes were distributed along the sucrose-density gradient by ultracentrifugation as described previously (3), and examined for ultraviolet absorbance at 254 mμ by an ISCO automatic fractionator and UV monitor and by a Gilford 2400 spectrophotometer.

The protein content and acid-insoluble radioactivity of all fractions were evaluated by standard Lowry and double-label radioisotope techniques, respectively (3). The data are expressed as the specific activity of the drug-treated sample as a percent of the uninfected control. The relative specific activities so obtained, using the optical density at 254 mμ, did not differ significantly from those generated by the protein analysis.

The following week, an identical experiment was carried out on a PR-8 influenza virus-infected system where, simultaneously with addition of the drug to the eight experimental flasks, all 16 flasks received about 1.5×10^3 HA units/ml of the virus. Data are expressed as the specific activity of the drug-treated infected sample as percent of the infected control.

Experiments on uninfected and infected systems were carried out on alternate weeks. Each system was evaluated a total of 8 times.

Results

Influenza Mortality Study in vivo

The effect of Isoprinosine on influenza mortality is shown in Table 2. Under certain conditions, an Isoprinosine suppression of influenza

TABLE 2
Effect of Isoprinosine on influenza mortality in mice

Virus[a] dilution	Dead/inoculated[b]			
		Drug[c] initiated on		
	Control	Day 0	Day 1	Day 2
10^0	20/20	20/20	9/20	8/20
10^{-1}	20/20	20/20	7/20	8/20
10^{-2}	15/20	8/20	5/20	6/20
10^{-3}	3/20	3/20	3/20	3/20
10^{-4}	1/20	1/20	1/20	1/20

[a]Influenza virus, A_2 strain; titer of 10^0 dilution was 2×10^6 HA units/ml, inoculated intranasally.
[b]Mortality statistics collected at day 16.
[c]Five hundred milligrams per kilogram per day Isoprinosine, intraperitoneally.

mortality is highly significant, with protection falling between $\frac{1}{3}$ and $\frac{2}{3}$ of the infected population. The data give evidence that, at the level of drug dose used, the specific experimental conditions relating to virus titer and day of treatment initiation determine the degree to which these beneficial treatment effects are seen. Over many experiments, we observed that delaying treatment initiation until 1 to 4 days post virus inoculation usually enhanced the intensity of antiviral effect, and sometimes was necessary for a treatment effect to be manifested.

TABLE 3

Antiviral effects of Isoprinosine in tissue culture

Virus	Treatment[a]	Virus titer Day 0	Virus titer Day 2[b]
Polio Type 3	Control	1.3×10^5	1.0×10^7
	Isoprinosine	" "	1.5×10^4
Adenovirus Type 10	Control	1.5×10^3	4.1×10^4
	Isoprinosine	" "	1.0×10^3
Influenza A_2	Control	1.2×10^2	1.1×10^4
	Isoprinosine	" "	3.4×10^2
Influenza PR-8	Control	1.5×10^3	3.4×10^4
	Isoprinosine	" "	3.4×10^3
Herpes LU	Control	1.5×10^3	3.4×10^4
	Isoprinosine	" "	3.4×10^2

[a]Drug concentration = 10 μg Isoprinosine/ml medium.
[b]Each figure represents the average count of 10 flasks.

Herpes Mortality Study in vivo

The results on studies of newborn mice infected with herpes virus are (expressed as dead/inoculated) untreated, 29/30; saline-injected, 29/30; Isoprinosine-treated, 13/30. Thus, as is shown, drug effect is highly significant ($P < 0.01$).

Antiviral Effect of Isoprinosine in Tissue Culture

The data given in Table 3 reveal 1 to 3 log differences between virus titer of control and treated cultures at the end of 3 days of growth. Each number represents the average of 10 culture tubes. Such an experiment was repeated three times, with essentially identical results. Since a hemagglutination technique was used for the titration of influenza virus, the results indicate that the drug suppressed the synthesis of influenza antigen (protein).

Drug Effect on the Weight and Influenza-Virus Content of Mouse Lungs Examined by in vitro Titration

The effect of influenza-virus infection on lung weights, the minimizing of this effect by drug treatment, and the action of Isoprinosine on normal lungs are shown in Fig. 1. These data establish that progressive virus infection increases lung weight, presumably through the initiation of pulmonary inflammation, and that

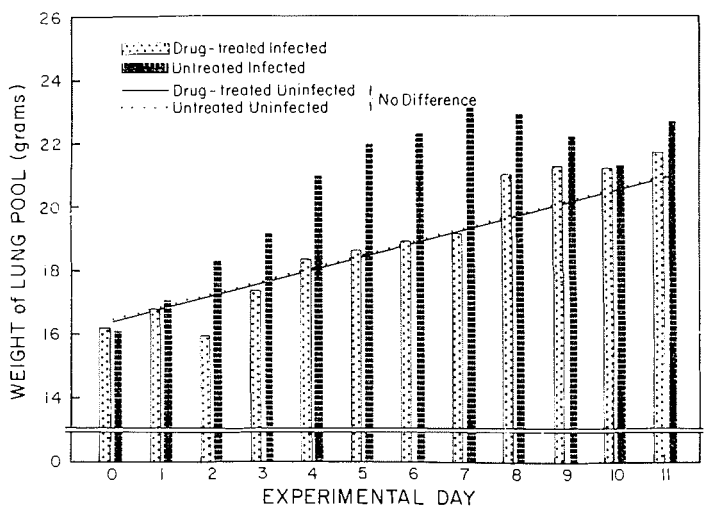

FIG. 1. Female mice, 26–28 g, were inoculated intranasally with 2×10^2 HA units/ml of PR-8 influenza virus. Each animal was injected intraperitoneally once daily with 500 mg/kg Isoprinosine or with saline. Each day for 12 days, 50 mice from each group were sacrificed and lungs pooled and weighed. The same procedures were followed with uninoculated, untreated mice (dotted line) and uninoculated, drug-treated mice (solid line).

treatment with Isoprinosine suppresses this increase. It is clear that the drug, by itself, does not affect the weight of uninfected lungs, while it has a marked effect on the weight of lungs from animals infected with influenza. Isoprinosine was found to abolish the lung-weight increase associated with influenza-virus infection between experimental days 2 and 9, the average drug effect on lung weight between infected control and infected drug-treated animals being -12% ($P < 0.01$, matched pair comparisons). The maximum drug reduction of -17% occurred on day 7. These results have been consistently reproduced in our laboratories.

Influenza antigen titers from the lungs of untreated and drug-treated virus-inoculated mice are shown in Fig. 2. Between day 1 and day 8, titer of virus hemagglutinin in lungs of drug-treated animals is less than in the control by from 10- to 10 000-fold, according to the experimental day. These data indicate that, in association with suppression of lung-weight increase, Isoprinosine markedly suppressed lung-viral titer.

Drug Effect on Lung-Virus Content Examined by in vivo Titration

The effect of Isoprinosine on 7-day mortality of mice given PR-8 influenza virus was most striking in the delayed treatment group inoculated with 0.1 ml of a virus dilution of 10^{-2}. In this group, 10 of 20 control animals died, while all of the treated animals survived (although three did manifest symptoms of serious illness).

Passage of lung inocula prepared from such surviving control and drug-treated mice into recipient mice resulted in a pattern of morbidity and cumulative mortality that revealed a drug suppression of influenza-virus infectivity. Combining cumulative mortality and morbidity as defined above at 21 days into a single index, we generate the following 50% end-point titers for serious illness and death. The virus dilution from control source producing this was 1×10^{-5}; from drug-treated source, 3×10^{-3} ($P < 0.001$). This indicates an apparent drug reduction in virus titer in the drug-treated donor animals of about $2\frac{1}{3}$ logs. Similar data could be obtained from observations made after 7, 14, and 28 days.

Biochemical Studies in Tissue Culture

In uninfected monkey kidney cells, Isoprinosine enhanced the incorporation of radioactive orotic acid and radioactive amino acids into polyribosomes without significantly changing acid-soluble isotope or free amino acid levels. One of the significant characteristics of the drug effect in uninfected cells was that the enhancement of incorporation of RNA precursors and the enhancement of the incorporation of amino acids were exerted in a synchronous fashion across the gradient (Fig. 3). In those polyribosome fractions where the incorporation of RNA precursors was strikingly enhanced, the

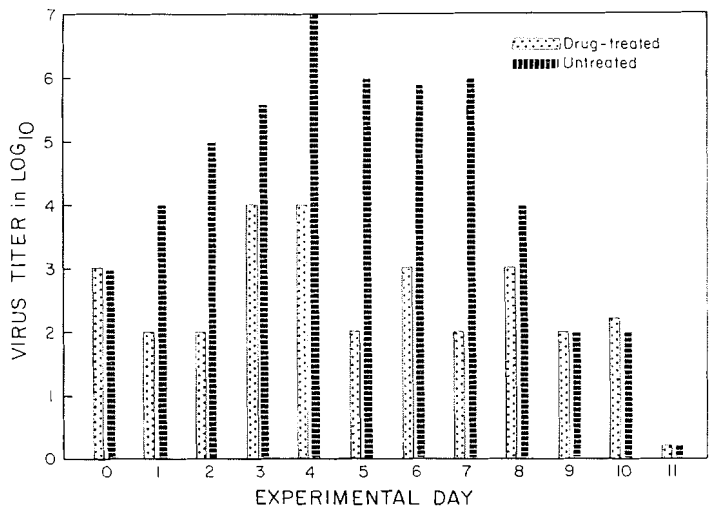

FIG. 2. Hemagglutination titers obtained from the infected lung pools whose weights are shown in Fig. 1.

incorporation of amino acids was enhanced to an almost identical degree. Similarly, in those polyribosome fractions where incorporation enhancement was absent, both categories of isotope were almost identically affected. The large changes seen were changes in rate of turnover and were not accompanied by changes in absolute amount of RNA, ultraviolet absorbance, or Lowry-reactive material (amino acids). Thus, polyribosomal RNA and protein synchronously increase in specific activity from 25% to 250%, according to the polyribosome size category.

In the presence of PR-8 influenza-virus infection, Isoprinosine, as compared to the infected control, was always found to induce a selective depression of RNA label incorporation into polyribosomes in two broad bands, one in the light and the other in the heavy polyribosome size category, as is shown in Fig. 4. In contrast, amino acid incorporation in the light polyribosome zone (dimer, trimer, tetramer) was not depressed, but was increased; while, in the heavy polyribosome zone, it was decreased to a lesser degree than the rate of incorporation of RNA precursors.

The drug effects described for orotic acid and amino acid incorporation into polyribosomes in the presence of PR-8 infection were observed in eight of eight experiments (Fig. 4). In the light polysome region, a drug-induced moderate increase in amino acid incorporation (averaging +19% at peak) was always associated with a drug-induced depression in orotic acid incorporation (which averaged −30% at trough). Similarly, in the heavy polyribosome region from PR-8 infected samples, the drug always induced a depression of RNA-precursor incorporation into polyribosomes (which averaged −33% at trough) which was always more profound than the lesser reduction in amino acid incorporation (−15%) found for these polyribosomes.

The significant drug effect on acid-insoluble incorporation of RNA precursors and amino acids into polyribosomes is to be contrasted with an absence of significant drug effect on acid-soluble cytoplasmic tritium radioactivity and on the average specific activity of intracellular free amino acids. Cytoplasmic tritium counts, in the control and drug-treated samples, averaged respectively 28 204 and 26 200 dis-

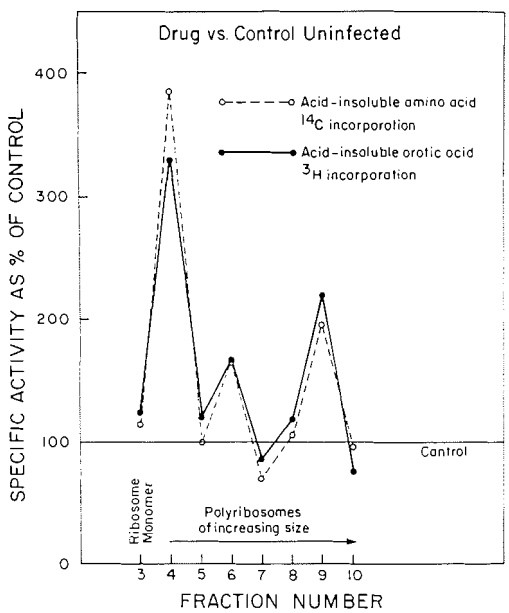

FIG. 3. Effect of Isoprinosine on the incorporation of radioactive rapidly labeled RNA and of radioactive amino acids into polyribosomes of uninfected monkey kidney cells in tissue culture. Shown are dpm/unit optical density (254 mμ) for drug-treated polyribosomes as percent of control.

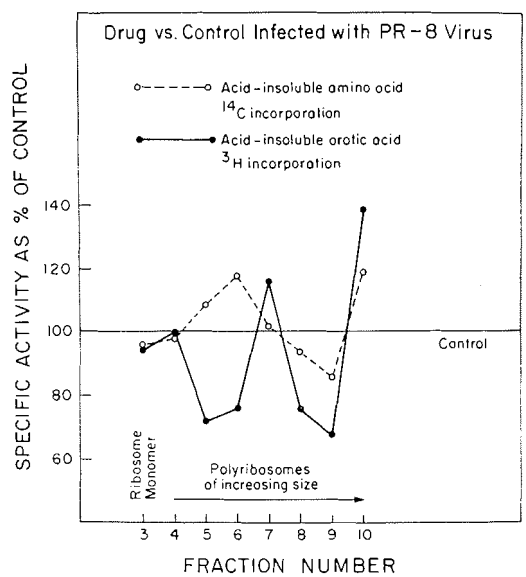

FIG. 4. Same as Fig. 3 except that, simultaneously with addition of Isoprinosine to experimental flasks, all flasks were infected with about 1.5×10^3 HA units/ml of PR-8 influenza virus. Shown are dpm/unit optical density (254 mμ) for polyribosomes of drug-treated infected cells as percent of infected control.

integrations per minute (dpm)/g wet weight packed cells. The free amino acid specific activities for the control and treated groups, respectively, were 1.2 and 1.3 μcuries/μmole tyrosine equivalent.

The 45-min labelling period used directs the RNA label, for the most part, into mRNA. Our results, then, establish an infection-dependent drug effect on the functioning of polyribosomes.

Discussion

In support of our hypothesis on the significance to viral infection of a drug priming of host transcription, it is apparent that Isoprinosine exerts significant antiviral effects in vivo and in vitro. For influenza A strains, effects in vivo are seen as a suppression of mouse mortality and, at lower levels of virus infectivity, as a suppression of mouse morbidity and lung influenza antigen content. It is apparent from biochemical effects of Isoprinosine that the drug acts on host mechanisms; not surprisingly, its antiviral effect is broad-spectrum in nature.

In the biochemical experiments reported, the drug enhanced the specific activity of RNA in polyribosomes from uninfected cultures without altering the acid-soluble radioactivity. However, to firmly establish the drug effect as including an enhancement of transcription, new studies on the pool size and specific activity of pyrimidine-precursor metabolites are now being carried out for this system. The drug effect on amino acid incorporation in the monkey kidney cell system is unambiguous. Since acid-soluble amino acids did not change in average specific activity, while nascent proteins did, it would seem highly probable that Isoprinosine effects a real increase in the average rate of protein synthesis in the uninfected monkey kidney cell system.

It was noted that, in the presence of influenza-virus infection, we did not achieve an enhancement of the incorporation of RNA precursors and amino acids into polyribosomes. Instead, we obtained bands of depression of the incorporation of RNA precursors, not associated with parallel decreases in the average synthesis of protein (Fig. 4). This drug effect may reflect a selective exclusion of viral mRNA from polyribosomes. At present, we can propose that the effect is associated with a predicted consequence of such exclusion: a significant reduction in virus-directed protein synthesis, as measured by reduced PR-8 influenza hemagglutinin titer.

We shall now examine in greater detail our experiments concerned with the measurement of lung weights and lung-virus antigen content in influenza-infected mice. In these experiments, we used an experimental influenza system which employs relatively low levels of virus and which focuses on a morbidity parameter rather than on mortality. This experimental approach minimized the number of spontaneous deaths and rendered ill mice available for sacrifice as needed. The virus titer data of the treated animals from experimental days 1 and 2 reveal virus concentrations 10-fold less than on day 0, which suggests that the drug exerted a specific antiviral effect against virus particles already present. The results obtained were paralleled by those observed for the experiment examining lung-virus content by in vivo titration, where virus infectivity was much higher. Therefore, we conclude that the drug suppression of virus antigen titer is effective over a range of infecting virus concentrations, the limits of which have not yet been determined.

Data for the lung-weight study give additional evidence for the antiviral activity of Isoprinosine and indicate that, in the absence of significant death, lung weights become a reliable measure of disease progression or disease suppression by the drug. Evidence for this has also been obtained by Lynes (published in ref. 3). Because change in lung weight does, in fact, correlate with change in lung-virus content, we offer this parameter as a new means of assaying the efficacy of virus-limiting agents.

Analysis of lung-virus titers in vivo by various hemagglutination techniques used to detect the presence of an influenza antigen revealed that Isoprinosine suppresses the apparent virus concentration by one to four orders of magnitude, according to which of the experimental days one examines. This degree of suppression is greater than that reported in the tissue-culture systems, where the drug characteristically produced an influenza-virus suppression which lay between one and three orders of magnitude. While the reasons for the greater in vivo effect are still being investigated, we attribute at least some of the difference to methods of drug administration used. In tissue-culture systems, the drug was added only once to the growth medium, in

which we found it to be relatively rapidly destroyed; infected mice received drug injections daily throughout the course of an experiment.

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