Lead Aggravates Viral Disease and Represses the Antiviral Activity of Interferon Inducers

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Lead acetate was administered continuously in the drinking water to CD-1 male mice beginning at 4 weeks of age. An LD_{10-20} of the lytic viruses or 300 plaque-forming units of RLV was inoculated intrapertioneally at 6 weeks of age. Lead increased the response of the mice to all classes of viruses against which it was tested: an RNA picornavirus-encephalomyocarditis (EMCV), a DNA herpesvirus-pseudoribies, an RNA leukemia-virus-Rauscher leukemia (RLV), an RNA arbovirus B-St. Louis encephalitis, and an RNA arbovirus A-western encephalitis. Most studies were performed between lead and EMCV. Increases in EMCV mortality in lead treated mice over controls ranged from $2\times$ at a lead level of $0.004M$ to $7\times$ (100% mortality) at a $0.1M$ lead level. Splenomegaly with spleens 800 to 1100 mg in weight containing high titers of RLV occurred in lead ($0.03M$)-treated mice 3 and 6 weeks after RLV inoculation; spleens or RLV controls were normal in weight (200 mg) and were free of virus. Lead did not reduce the protective effect of mouse interferon (IF) against the lethal action of EMCV, but it did repress the EMCV antiviral effect of poly I/poly C (PIC) and of Newcastle disease virus (NDV) against EMCV mortality. These data indicate several new facts concerning adverse effects lead may have on an animal: (1) lead aggravates viral disease, most likely in part, through reduced IF synthesis; (2) lead represses the anti-EMCV protective effects of both PIC and of NDV, which, in other reports, were shown to induce IF in radioresistant macrophages (PIC) or in radiosensitive lymphocytes (NDV); (3) lead may then be said to repress IF induction in two kinds of cells; (4) however, lead does not inhibit IF action.

Introduction

One of the research programs of the National Institute of Environmental Health Sciences was to screen various classes of chemicals for their adverse or beneficial effects on experimental viral infections. Studies on interactions of this nature appear warranted for several reasons. (a) A direct

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consideration of this research may lead to the fact that elevated environmental chemical levels do in fact aggravate or they may alleviate viral infections. (b) It is known that cigarette smoking aggravates influenzal infection in man (1), and many chemicals have been shown to increase viral infections experimentally (2). (c) Insofar as lead poisoning and viral infection is concerned, possible synergism between lead poisoning and eastern encephalitis viral (EEV) infection was observed "naturally" in a dead calf in Florida wherein EEV was

isolated from the brain, the calf had a viral type of encephalitis histologically, and 20 ppm lead was present in the kidney of the calf. (J. H. Gainer, unpublished observations, Fla. Dept. Agriculture, Kissimmee, Fla, 1964). (d) A viral infection, being a dynamic (and not a static) force within a cell or animal, presents problems diverse from a strictly classical toxicological sense—the host must cope with the background chemical poisoning and then confront itself with the virus that exerts very powerful forces on the basic biochemical machinery of the cell. In contrast, then, to the simple straightforward toxicological approach with one chemical—one host response, here we have the chemical, the host, and the virus, and the virus itself exerts many pressures that just do not exist in the single chemical toxicological study. In addition the chemical may influence viral absorption and replication directly and subsequently may influence the host's defense mechanisms against the virus.

Metallic salts represent a diverse group of naturally occurring biologically active compounds; and certain toxic metals, as well as essential metals, perhaps, are being mobilized by man's activities and therefore are being increased in the immediate environment near to man. Lead has been incriminated as the metal that is being mobilized to the greatest extent by man (3). Metallic salts appeared then to be a logical group of compounds that should be examined for their interactions with viral disease.

The intact mouse rather than tissue cultures was chosen as the test host for interactions between metals and viruses for certain reasons. Although interactions between metals and viruses most certainly will occur in tissue cultures, it is almost impossible to extrapolate tissue culture data to whole animals and to man because of the many differences between a highly artificial and relatively simple tissue culture system and the complex whole animal. The animal has selective absorptive and excretory mechanisms for the chemical that the tissue culture system most likely lacks. The animal

may concentrate the metal in a specific tissue for which the virus has a special affinity, cobalt and the encephalomyocarditis virus (EMCV) both affecting the heart, for example (4). This argument may post disadvantages to certain studies, however; for example, cadmium, which concentrates in the kidney, should ideally be interacted with a virus that infects the kidney and not one that infects the heart or brain, as has been done (5). Certain metals, because of their lack of adequate solubility in tissue culture media (lead, for example), present another barrier to the successful conduct of tissue culture studies. The mouse is relatively inexpensive, and it therefore can be used in sufficient numbers to permit the acquisition of sufficient data for statistical analysis. The mouse has been well characterized for infection studies with several classes of viruses. It has been argued that tissue cultures rather than whole animals should be used, because mechanisms are more easily definable in tissue cultures; this may be the case, but data that follow illustrate that much in terms of mechanisms has been learned in the intact animal.

This paper describes in mice (a) the interactions between lead treatment and five different viral infections, (b) interactions between lead treatment and interferon action, and (c) interactions between lead treatment and the antiviral actions of the interferon inducers poly I/poly C and Newcastle disease virus.

Methods

Metals, Mice, Viruses

A screening program was begun in which the salts of metals were interacted with a few viruses in CD-1 male mice (Charles River Breeding Farms, Wilmington, Mass.). Metals were administered continuously in demineralized drinking water, beginning with mice at 4 weeks of age. A safe concentration of a metal was determined as the maximum concentration of the metal the mice could tolerate without appreciable reduction in liquid consumption or appreciable

loss in weight. Viruses used were common laboratory strains; the viruses were inoculated intraperitoneally (IP) at 6 weeks of age. Death rate was the parameter used in the lytic viral infections; splenomegaly was followed in the Rauscher viral infections. Lead acetate was found to be the least toxic of the metallic salts tested; however, lead was found to result in a large magnitude of synergism of the viral disease response. In fact, of several metals tested, $NaAsO_2$, $CdSO_4$, $CoSO_4$, $HgCl_2$, $NiSO_4$, and lead acetate, lead acetate was the most potent "synergizer" of viral infections (4–6).

Interferon Studies

To attempt to delinate modes of action of the lead-enhanced virus-induced disease, interferon (IF) was considered to be involved in that IF is one of the first lines of defense an animal has against a viral infection (7). Virus titers and IF levels were examined sequentially in brain and spleen of mice infected with the encephalomyocarditis virus (EMCV). Preliminary assays of the titers of virus and of IF in spleens and brains of virus-control mice and of lead-treated EMCV-infected mice killed or dying 2 to 4 days after viral inoculation revealed high titers of virus and of IF in tissues from both groups of mice. Lack of sufficient time prevented careful study of these two parameters to determine if in fact the lead-treated, virus-infected mice had lower IF titers and higher virus titers than did the the nontreated virus-infected control mice. Nevertheless, lead-treated EMCV infected mice did contain high titers of EMCV, indicating that these mice were in fact dying of a virus-induced encephalitis.

Earlier studies had shown that arsenicals repressed the antiviral activity of the IF inducer poly I/poly C for EMCV mortality (8). Poly I/poly C has been shown to protect mice against EMCV mortality, the mechanism of action being through the synthesis and action of IF (9). Recently it was reported that poly I/poly C induced IF synthesis in large radio-resistant macrophages whereas Newcastle disease virus (NDV),

which will also induce the synthesis of IF, induced it in radiosensitive lymphocytes (10, 11). The effect of lead acetate on the antiviral activity of poly I/poly C and of NDV was determined. The method of procedure was simply that mice were laden with the lead as in the straight virus procedures. Poly I/poly C (Biopolymers, Chagrin Falls, Ohio, lot no. 12) or NDV was administered IP, and 2 or 24 hr later, respectively, an LD_{95} of virus was administered. A commerical lot of NDV (La Sota strain, B1 type vaccine, American Scientific Laboratories, Madison, WI) was used. Deaths were tallied and differences were compared. The interferon inducers protected against EMCV mortality whereas the lead-treated mice were not protected by either IF inducer from dying with EMCV infection. Lead was then given at various times with regard to the poly I/poly C to determine certain time sequences of lead repression of IF synthesis. Lead was also tested against preformed mouse interferon *in vitro* and *in vivo* to determine if it would influence the antiviral activity of the interferon.

Results

Lead and Viral Disease

Table 1 shows that lead acetate exaggerated the response of mice to all viruses against which it was tested. Although the differences between virus controls and lead treated were not statistically significant in the case of the St. Louis encephalitis and the western encephalitis virus, there was a higher per cent morality in the lead-treated mice than there was in the controls in all cases. The concentration of lead used with the pseudorabies virus and with the two encephalitis viruses was not as high as in the case of EMCV. Spleens in the lead-treated, Rauscher virus-infected mice were very large; and high titers of virus were present in lead-treated Rauscher virus-infected spleens (up to 10^6 PFU/gram); virus was not recovered from virus-control spleens.

Table 1. Effect of lead acetate on virus mortality and on Rauscher virus splenomegaly in mice.[a]

| | | No. dead/no. inoculated and per cent mortality | | |
Virus	Concentration of lead used, M	Demineralized water control mice	Lead acetate-treated mice	P
Encephalomyocarditis	0.01 and 0.004 [b]	0/31, 0%	7/32, 22%	<0.05
	0.05	6/41, 15%	9/21, 43%	0.04
	0.10	6/41, 15%	11/22, 86%	<0.01
Pseudorabies	0.0003 [b]	4/34, 12%	6/15, 40%	<0.05
	0.003 [b]	6/34, 12%	4/14, 29%	0.06
St. Louis encephalitis	0.03 [b]	5/23, 22%	7/20, 35%	N.S.
Western encephalitis	0.015 and 0.03 [b]	2/20, 10%	5/39, 13%	N.S.
Rauscher leukemia	0.03	204±31, 3WPI; 142±12, 6WPI;	858± 96, 3WPI 1048±374, 3WPI	<0.01

[a] Mice were treated with lead acetate beginning at 4 weeks of age; at 6 weeks they were infected with viruses. Mortality patterns were followed in the first 4 viruses; spleen sizes and splenic virus content were observed in the Rauscher leukemia infections.

[b] Unpublished findings; other data from Gainer (5, 16).

[c] Spleen weights in mg with standard deviations; WPI denotes weeks after virus inoculation.

Table 2. Effect of lead acetate on the antiviral activity of poly I/poly C and on Newcastle disease virus on EMCV mortality.[a]

| | Virus mortality | | |
Interferon inducer	Lead acetate	Demineralized water controls	P
Poly I/poly C	12/19, 63% [b]	1/20, 5%	0.001
Newcastle disease virus (NDV)	10/15, 67% [c]	20/74, 27%	0.008

[a] Lead acetate was begun at 4 weeks of age. At 6 weeks poly I/poly C and NDV were given IP, followed 2 and 24 hr later, respectively, with virus. Data are from Gainer (12).

[b] Lead acetate, 0.03M.

[c] Lead acetate, 0.003M.

Lead and Interferon Inducers

The interactions of lead acetate with poly I/poly C and with NDV in their antiviral protective activities against EMCV mortality are presented in Table 2. The lead level in the poly I/poly C study was tenfold that in the NDV study, and the poly I/poly C alone protected against EMCV mortality to a greater magnitude than did the NDV; it is not possible to make direct comparisons between lead and poly I/poly C and lead and NDV. Chessboard experiments to work out these interactions in intricate detail were not performed.

Three concentrations of lead acetate, 0.003, 0.01, and 0.03M, were interacted with 0.2 mg/kg of poly I/poly C. The 0.03M lead exerted as high a reversal activity against poly I/poly C, 9/10, $P = 0.01$, as did the ten-fold concentration of lead, 0.03M, 7/9, P = 0.05; poly I/poly C control mortality was 5/17, 29%. The intermediate lead concentration of 0.01M resulted in a mortality of 7/10, $P = 0.07$ (12).

Temporal Relationships of Lead and Poly I/Poly C

Table 3 presents the results of temporal relationships between the administration of lead acetate and poly I/poly C. Lead given

Table 3. Influence of the time of administration of lead on the antiviral activity of poly I/poly C in mice.[a]

Lead treatment	Mortality	P
1 hr before poly I/poly C	11/20, 55%	NS
4 hr after poly I/poly C	5/16, 31%	0.05
24 hr after poly I/poly C	3/20, 15%	0.01
Virus controls, no lead, no poly I/poly C	27/41, 66%	—

[a] Six-week-old mice were used. Lead acetate, 100 mg/kg, was given IP at times before and after Poly I/Poly C, 0.3 mg/kg, IP. EMCV was given IP 1 hr after poly I/poly C. Data are from Gainer (12).

1 hr before poly I/poly C repressed nearly all of poly I/poly C's antiviral activity. When given 4 hr after poly I/poly C, lead still reversed the protective effect to some extent; but 24 hr after poly I/poly C, lead did not have any influence.

Effect of Lead on Interferon Action

Mouse interferon was prepared by injecting poly I/poly C, 7.5 mg/kg, IP into 8 week old C3H male mice (Jackson Labs., Bar Harbor, Maine). The animals were exsanguinated 4–5 hr later. Plasma from heparinized blood was pooled with triturated spleens and lungs, this mixture comprised the IF preparation. Plasmas, spleens, and lungs were collected from normal untreated mice and served as control preparations. Lead acetate when administered prior to the administration of IF did not influence the protective activity of the interferon (Table 4). This lack of effect of the lead on IF action compares favorably with in vitro studies in which lead at 10^{-4} and 10^{-5} M, when added to IF preparations, did not inhibit the antiviral activity of IF against the vesicular stomatitis virus (12). In the in vitro studies the difficulty was encountered that lead is quite insoluble in tissue culture mediums; it is conjectured that, because of insolubility of the lead acetate in tissue cultures, it was probably not possible to achieve sufficiently high concentrations to test adequately the effects of lead on interferon action.

Discussion

Data presented show that lead poisoning aggravated the response of mice to the five viruses against which it was tested. Four viruses are of the RNA type, and one, the pseudorabies, is a DNA virus. The concentrations of lead used were not the same with every virus, so that one is uncertain that the magnitude of virus stimulation from virus to virus is really comparable. In addition, the dose of virus used is hardly comparable from one virus to the next, the number of virus particles per infectious unit being of considerable consequence as to whether or not a virus disease process may be exaggerated by a chemical poisoning. Nonetheless, responses to all viruses in the mice were exaggerated by the lead treatment, although not significantly so in the case of the two encephalitis viruses. It is possible that the lead had not reached strong virus-enhancing levels in the brains, where it would most likely have significant effect with the encephalitis viruses. It is suggested that the lead is attacking a common biochemical parameter in the infectious process (such as interferon), rather than a specific biochemical pathway related to one particular virus only. Although the determinations of virus titers and IF levels were not satisfactory and did not delineate particular relationships between the two entities, other interrelationships between lead poisoning and IF formation and action did lead to very significant results. Lead poisoning did not inhibit interferon action either in vitro or in vivo. This finding would suggest that for IF to be active another protein is not formed to account for the antiviral action; this contrasts with earlier findings in which it is reported that IF results in synthesis of a secondary protein that actually provides for the antiviral action (7). In the case of lead poisoning, the interferon is fully active in its presence; this suggests that if the lead inhibits RNA synthesis, as has been reported (13), it is not in-

Table 4. Effect of lead acetate on the antiviral activity of interferon against encephalomyocarditis virus mortality in mice.*

Treatment			Mortality, no. dead/	
0 hr	2 hr	4 hr	total	P
Lead acetate, 100 mg/kg	Interferon	Virus	11/20	0.01
Saline	Interferon	Virus	11/20	0.01
Saline	Normal mouse tissues (controls)	Virus	20/21	—

* Five-week-old mice were injected three times each IP as stated. Mortality was followed. Data are from Gainer (12).

hibiting whatever RNA synthesis is occurring as a primary effect of interferon action.

Lead poisoning inhibited the anti-EMCV protective effect of poly I/poly C, presumably in large macrophages, and of Newcastle disease virus, presumably in small lymphocytes. These findings suggest strongly but do not confirm unequivocally that the mechanism of lead enhancement of viral disease is through depressed interferon synthesis. Since the lymphocytes are radiosensitive, one might predict that lead poisoning would add or would synergize with radiation-induced injury to further intensify viral disease responses.

Interactions of lead poisoning with Rauscher virus infection affords other points of discussion in addition to probable effects of lead poisoning on the defense mechanisms. Lead poisoning causes a myriad of pathological effects, one of the most noteable of which is the induction of anemia (14) through an inhibition of δ-aminolevulinic acid dehydratase and heme synthetase which are critical for hemoglobin synthesis. Ebert et al. (15) illustrated recently that in RLV infection, reduced δ-aminolevulinic acid synthetase activity was associated with the development of anemia and splenomegaly. Thus Rauscher virus infection and lead poisoning may be acting synergistically through inhibition of similar enzymes involved in hemoglobin synthesis.

Sequential studies on the plasmas and tissues in the lead-poisoned animals after virus infection for such parameters as antibody, leukocyte counts, interferon, and virus titers would clarify several issues concerning mechanisms involved in these interactions. The temporal relationships found here between lead and poly I/poly C indicates clearly that lead disrupts interferon synthesis and not interferon action.

Summary

The administration of lead acetate in the drinking water aggravated the response of mice to five viruses against which it was tested: encephalomyocarditis (EMCV), pseudorabies, Rauscher leukemia, St. Louis encephalitis, and western encephalitis. On treatment with 0.1M lead, EMCV mortality increased sevenfold (to 100% mortality) and spleens of mice treated with 0.03M lead and infected with the Rauscher leukemia virus weighed 800–1100 mg and contained nearly 10^6 PFU of virus per gram 3 and 6 weeks after virus inoculation; Rauscher virus-infected control spleens were normal in weight and did not contain any virus.

Lead did not inhibit interferon (IF) action in mouse embryo cultures in its inhibition of plaque formation by the vesicular stomatitis virus, and it did not inhibit IF action in that it did not reduce the anti-EMCV protective effect of IF against EMCV mortality in the intact mouse. However lead did repress the EMCV antiviral effect of poly I/poly C (PIC) and of Newcastle disease virus (NDV) against EMCV mortality. These data indicate several new facts concerning adverse effects lead may have with respect to a viral infection: (1) lead aggravates viral disease, most likely in part through reduced IF synthesis, as a result of reduced RNA synthesis; (2) lead represses the anti-EMCV protective effects of both PIC and of NDV, which, in other reports, were shown to induce IF in radioresistant macrophages (PIC) and in radiosensitive lymphocytes (NDV); lead may then be said to have the capacity to repress IF synthesis in at least two kinds of cells; (3) however, lead does not inhibit IF action.

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