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Antiviral Activity of an Extract of *Brucella abortus*: Induction of Interferon and Immunopotentiation of Host Resistance[1] (39399)

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The induction of interferon in the virus-infected host has been utilized as one approach to antiviral chemotherapy. The most extensively investigated interferon inducer has been the polyribonucleotide complex, polyinosinic acid:polycytidylic acid [poly-(I:C)]. Although poly(I:C) has been effective in a number of experimental infections in man and animals, important limitations have also been identified. The synthetic nucleotides induce relatively low levels of interferon in humans, and a variety of side effects have been identified in experimental animals (1). One alternative to the use of these synthetic compounds has been the development of natural biological products that have the capability of inducing interferon (2). Youngner and associates (3) have reported that an ether-extracted preparation of *Brucella abortus* (Bru-Pel) induced high levels of circulating interferon in mice, and protected animals against a subsequent lethal challenge of Semliki Forest virus. This report confirms the interferon-inducing capacity of Bru-Pel and extends the study of its potential as an antiviral agent to encephalomyocarditis (EMC) virus and *Herpesvirus hominis* type 2 (HVH-2) infections of mice. In each model, the efficacy of this compound as a biologic inducer is compared with poly(I:C), and its capacity to function as an immunopotentiator in enhancing host resistance to HVH-2 infections is described.

Materials and methods. Bru-Pel. This compound was prepared and supplied through the Antiviral Substances Program of NIH, by Jules Youngner, University of Pittsburgh, Pittsburgh, Pennsylvania. It was received as a lyophilized powder, and reconstituted in phosphate buffered saline (PBS) prior to inoculation into mice. Concentrations as high as 2000 μg per mouse did not produce any apparent toxicity.

Virus strains, media, cell cultures, and interferon assay. The origin and preparation of the MS strain of HVH type 2 and EMC virus pools, the media utilized for the maintenance of L-cell cultures, and the assay for interferon have all been described previously (4).

Statistical evaluation. To compare the final mortality of drug-treated and untreated mice, the data were evaluated by the Fisher Exact test.

Results. Interferon response to Bru-Pel. To determine the kinetics of the serum interferon response to Bru-Pel, groups of six 25-g female Swiss Webster mice were injected intraperitoneally (ip) with concentrations of 1000, 500, or 100 μg per mouse of Bru-Pel and pooled blood obtained at 3, 6, 9, 12, and 24 hr after injection. The capacity of Bru-Pel to induce interferon was compared with mice receiving 100 μg of poly(I:C) ip. The interferon titers obtained are presented in Table I. Peak interferon titers of 3000–5000 units were observed 6 hr after injection of each concentration of Bru-Pel, and persisted through 12 hr. At 24 hr only low levels were detectable. Similar levels of interferon were induced by poly(I:C), with the peak titer occurring at 9 hr after administration.

Hyporeactivity to the induction of interferon. One potential limitation to the use of interferon inducers is the decreasing interferon response observed after repeated injections (4, 5). To determine if Bru-Pel also induces such a state of hyporeactivity, groups of six animals each were given daily

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injections of 100 μg of Bru-Pel for 4 days and bled at 3, 6, 9, 12, and 24 hr after each injection. Interferon titers in serum from 6 to 12 hr in these animals are summarized in Table II. At 6–9 hr after the first injection, 4800 units of interferon were present, while after the second dose of Bru-Pel the peak level was reduced to 600–900 units. After the third and fourth injections, only low levels (50–225 units) of interferon were produced. Although not shown in Table II, the interferon levels in serum obtained at 3 and 24 hr after injection indicated that there was no shift in the peak interferon response.

Antiviral efficacy of Bru-Pel. Based on these data which demonstrate the capcity of this substance to induce interferon *in vivo*, a series of experiments were designed to determine the ability of Bru-Pel to protect mice against three different experimental viral infections: (i) 3-week-old mice infected intranasally with HVH-2; (ii) adult mice infected ip with HVH-2; and (iii) adult mice infected ip with EMC virus. The effect of Bru-Pel or poly(I:C) on the mortality of 3-week-old 10–15-g Swiss Webster mice infected intranasally with HVH-2 was evaluated by treating groups of animals with 500 μg of Bru-Pel or 100 μg of poly(I:C) once daily for 5 days beginning either 6 hr prior to, or immediately after, infection. In a representative experiment (Table III) the PBS-treated virus control animals had a final mortality of 70%. Mice that received therapy with Bru-Pel beginning 6 hr prior to infection had a reduced final mortality while those animals receiving treatment initiated immediately after infection did not. Mice treated with poly(I:C), however, had significantly lower final mortalities whether the treatment was begun prior to or after viral inoculation.

The effect of Bru-Pel or poly(I:C) on the mortality of adult mice infected ip with either EMC virus or HVH-2 was examined by treating groups of animals beginning either 6 hr prior to, or immediately after infection with 500 μg of Bru-Pel or 100 μg of poly(I:C) once daily for 10 days. Although treatment with Bru-Pel did reduce the mortality in EMC virus-infected mice (Table IV) therapy with poly(I:C) appeared to be superior [Bru-Pel compared with poly(I:C), $P < 0.01$]. Mortality in the two HVH-2 infected groups of mice treated with Bru-Pel was similar to control animals, while it was significantly reduced in the poly(I:C)-treated groups.

Nonspecific enhancement of resistance. It is recognized that bacterial preparations such as *Corynebacterium parvum (C. parvum)* and BCG have multiple effects on components of the host defense mechanisms. Previous studies from this laboratory indicated that mice pretreated (10–14 days) with a killed suspension of *C. acnes*, *C.*

TABLE I. Interferon Titers in Serum of Adult Mice Injected with Bru-Pel or poly(I:C).

Interferon inducer (μg/mouse)	Interferon titers (units/ml) Hours postinjection				
	3	6	9	12	24
Bru-Pel					
1000	3075	4475	2150	3000	75
500	1100	3850	1075	2325	50
100	400	3225	1050	500	50
Poly(I:C)					
100	3850	3750	5275	3400	150

TABLE II. Interferon Titers in Serum from Mice Given Multiple Injections of Bru-Pel.

Day of Bru-Pel (100 μg) administration	Time of bleeding (hr)	Interferon titer (units/ml)
0	6	4800
	9	4800
	12	1100
0, 1	6	875
	9	600
	12	65
0, 1, 2	6	50
	9	225
	12	50
0, 1, 2, 3	6	50
	9	50

TABLE III. Effect of Bru-Pel on the Mortality of 3-Week-Old Mice Infected Intranasally with HVH-2.

Treatment[a]	Mortality		P value
	Number	Percentage	
PBS	14/20	70	
Bru-Pel, −6 hr	6/20	30	<0.05
Bru-Pel, time 0	11/20	55	NS
Poly(I:C), −6 hr	4/20	20	<0.01
Poly(I:C), time 0	5/17	29	<0.05

[a] Treatment was initiated at either 6 hr prior to (−6 hr), or immediately after (time 0), infection.

TABLE IV. EFFECT OF Bru-Pel ON THE MORTALITY OF ADULT MICE INOCULATED IP WITH EMC VIRUS OR HVH-2.

Treatment[a]	EMC virus mortality			HVH-2 mortality		
	Number	Percentage	*P* value	Number	Percentage	*P* value
PBS	15/15	100		14/15	93	
Bru-Pel, −6 hr	9/15	60	<0.05	12/15	80	NS
Bru-Pel, time 0	10/15	67	<0.05	14/15	93	NS
Poly(I:C), −6 hr	0/15	0	<0.001	2/15	13	<0.001
Poly(I:C), time 0	1/15	7	<0.001	2/14	14	<0.001

[a] Treatment was initiated at either 6 hr prior to (−6 hr), or immediately after (time 0), infection.

parvum, or BCG demonstrated enhanced resistance to infection with HVH-2 (6). These data suggested that Bru-Pel, a cell wall preparation of *Brucella abortus*, might also enhance resistance to viral infections of mice, independent of its capacity to induce interferon. To test this hypothesis, groups of 15 adult mice were treated ip with various concentrations of Bru-Pel 14 days prior to ip challenge with HVH-2. The results of one representative experiment are summarized in Table V. Concentrations of Bru-Pel ranging from 2000 to 125 μg/mouse were all effective in preventing mortality due to HVH-2 infection. In subsequent experiments, dosages of less than 125 μg were inactive.

To determine the time interval necessary for induction of this nonspecific resistance, adult mice were given Bru-Pel 1, 4, 7, 10, and 14 days prior to ip inoculation of HVH-2. Control animals that received PBS 14 days prior to infection had a final mortality of 87% (Table VI). Animals receiving Bru-Pel 1 day prior to infection had a reduced final mortality presumably reflecting the action of interferon since no protection was observed when Bru-Pel was administered 4 days prior to infection. A significant degree of protection was observed, however, in mice receiving Bru-Pel 7, 10, and 14 days prior to infection as evidenced by final mortalities of 47, 33, and 13%, respectively. In these experiments both Bru-Pel and HVH-2 were administered by the ip route. This nonspecific enhancement of host resistance by Bru-Pel to HVH-2 infection may be the result of activation of macrophages within the peritoneal cavity. If this is the mechanism of protection one might expect that the effect would be compartmentalized, that is, confined to the peritoneal cavity. The next series of experiments were designed to determine whether this enhancement of host resistance to HVH-2 infection would occur when the virus was inoculated by a different route than the Bru-Pel. Groups of 2-week-old mice were initially injected ip with either PBS, killed *C. acnes*, or 1000 μg of Bru-Pel. Seven days later the mice were infected with HVH type 2 by either the intranasal or the ip route. The results from one experiment are tabulated in Table VII. There was no reduction of mortality in those animals pretreated with either *C. acnes* or Bru-Pel and

TABLE V. NONSPECIFIC RESISTANCE TO HVH-2 INFECTION IN ADULT MICE INDUCED BY VARYING CONCENTRATIONS OF Bru-Pel.

Bru-Pel[a] (μg/mouse)	Mortality		
	Number	Percentage	*P* value
None (PBS)	12/15	80	
2000	2/15	13	<0.001
1000	4/15	27	<0.01
500	1/15	7	<0.001
250	1/15	7	<0.001
125	2/15	13	<0.001

[a] Treatment ip 14 days prior to HVH-2 inoculation.

TABLE VI. NONSPECIFIC RESISTANCE TO HVH-2 INFECTION IN ADULT MICE INDUCED BY Bru-Pel ADMINISTERED AT VARIOUS TIMES PRIOR TO VIRAL CHALLENGE.

Treatment[a] (days)	Mortality		
	Number	Percentage	*P* value
PBS, −14	13/15	87	
Bru-Pel, −1	4/15	27	<0.01
Bru-Pel, −4	12/15	80	NS
Bru-Pel, −7	7/15	47	0.05
Bru-Pel, −10	5/15	33	<0.01
Bru-Pel, −14	2/15	13	<0.001

[a] Treatment with 2000 μg was initiated 1–14 days prior to infection with HVH-2.

TABLE VII. NONSPECIFIC RESISTANCE TO HVH-2 INFECTION IN 3-WEEK-OLD MICE INDUCED BY BRU-PEL.

Treatment −7 days[a]	HVH-2 IN[b] Mortality			HVH-2 IP[c] Mortality		
	Number	Percentage	*P* value	Number	Percentage	*P* value
PBS	25/38	66		18/39	46	
C. acnes	21/31	68	NS	5/39	13	<0.01
Bru-Pel	21/32	66	NS	3/33	9	<0.001

[a] Seven days prior to infection with HVH-2.
[b] HVH inoculated intranasally.
[c] HVH inoculated intraperitoneally.

then infected intranasally. In contrast, those animals pretreated ip with either compound and then infected ip had a significantly lower mortality than control mice. These data suggest that both bacterial preparations were effective locally in the peritoneal cavity and that neither preparation was active against HVH-2 infection initiated by the intranasal route.

Discussion. The kinetics of the interferon response to Bru-Pel is similar to that induced by viruses and synthetic nucleotides, rather than the more rapid production of interferon usually induced by bacterial products such as endotoxin. The response of mice to bacterial lipopolysaccharides is characterized by the induction of only a few hundred units of interferon with peak serum levels occurring at 1–3 hr after injection (2), whereas Bru-Pel induced several thousand units with a peak at 6–9 hr. The development of hyporeactivity on the part of the host to produce interferon after multiple injections of Bru-Pel is similar to that induced by poly(I:C) and tilorone hydrochloride (4, 5). This progressive decrease in interferon production, however, may not necessarily mean loss of antiviral activity since Stringfellow *et al.* (7) have reported that multiple doses of poly(I:C) were more effective than a single dose in protecting mice against an EMC virus infection.

Both Bru-Pel and poly(I:C) were more effective against EMC virus than they were against HVH-2 infection in adult mice. This difference may be due to the fact that EMC virus is two- to fourfold more sensitive to the action of interferon than is HVH-2 (4). Additionally, both inducers were more effective when HVH-2 was inoculated by the ip rather than the intranasal route. We have previously reported data which suggest that after intranasal inoculation, HVH-2 probably spreads from the nasopharynx directly to the CNS (8) and thus may be less amenable to systemic therapy. This concept is supported by the evidence that chemotherapy with a number of antiviral agents has not been successful in preventing mortality due to HVH-2 infections initiated by the intranasal route, but was effective when the ip route of viral inoculation was used (4, 8–10).

In all three experimental viral infections utilized in this study, Bru-Pel did not appear to be as effective as poly(I:C). The reason for the diminished protective efficacy of Bru-Pel as an interferon inducer is not completely explained, particularly since the levels of interferon induced by a single dose of Bru-Pel are similar to those seen after a single dose of poly(I:C) and the degree of hyporeactivity associated with multiple doses of either drug is similar. These data suggest that the amount of interferon stimulated by interferon inducers should not be the only parameter measured to determine potential antiviral efficacy of such compounds. The data further suggest that host resistance mechanisms other than interferon may be stimulated by interferon inducers in the treatment of viral infections. Although the data presented suggest that Bru-Pel is less effective than poly(I:C) as an interferon inducer in protecting mice against viral infections, a number of factors should be considered in evaluating the potential of this substance for use in the treatment of viral diseases of man. In contrast to poly(I:C), it is possible that Bru-Pel will be an effective interferon inducer in humans and have little or no toxicity. Although preliminary evaluation suggests that Bru-Pel is considerably less toxic to mice than poly(I:C), detailed studies have not been carried out.

The enhancement of host resistance to HVH-2 infection when Bru-Pel was administered 10–14 days prior to viral inoculation

suggests that this compound may have more than one mechanism of action. In support of this concept is the report of DeClercq and De Somer (11), who showed that administration of live *Brucella abortus* to rabbits 1 week prior to vaccinia virus challenge, inhibited lesion development in the absence of detectable circulating interferon. The results presented in this paper suggest that Bru-Pel, in addition to its action as an interferon inducer, can also protect mice from viral infection by nonspecific induction of resistance, presumably through the activation of cells of the reticuloendothelial system. Two other interferon inducers, poly(I:C) and tilorone hydrochloride, both failed to induce resistance to HVH-2 infection in mice when tested under similar conditions. The active component of Bru-Pel responsible for nonspecific enhanced resistance is not known. It does not appear to be mediated by endotoxin, however, since *E. coli* 0111.B4 lipopolysaccharide (Difco), or a glycolipid preparation from *Salmonella minnesota* failed to induce enhanced resistance to HVH-2 infection of mice (unpublished results). The stimulation of an exudative reaction of cells in the peritoneal cavity does not provide an adequate explanation either, since three inducers of an inflammatory reaction (peptone, glycogen, and thioglycollate) used in our laboratory have failed to increase nonspecific resistance to HVH-2 infection in mice using pretreatment regimens, even though all stimulated a cellular reaction in the peritoneal cavity.

Summary. These data provide evidence that (i) the peak level and duration of the serum interferon response induced in mice by Bru-Pel is similar to that induced by some viruses and poly(I:C); (ii) the induction of interferon is markedly reduced after multiple injections of Bru-Pel; (iii) when administered as an interferon inducer, Bru-Pel does not appear to be as effective as poly(I:C) in protecting mice infected with either encephalomyocarditis virus or *Herpesvirus hominis* type 2; and (iv) when given 10–14 days prior to virus inoculation, Bru-Pel is effective as a nonspecific enhancer of host resistance to viral infection. These data suggest that Bru-Pel, in addition to its use as an interferon inducer, may have a role as a nonspecific potentiator of host resistance similar to that which has been reported for other bacterial agents such as *C. parvum* and BCG.

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