Cardiotoxic and Lethal Effects of Listeria monocyctogenes Hemolysin

G. CHARLES KINGDON AND C. P. SWORD

Department of Microbiology, The University of Kansas, Lawrence, Kansas 66044

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Cardiotoxic and lethal effects of Listeria monocytogenes hemolysin were studied in CD-1 mice injected with varying doses of hemolysin. Intravenous injection of 100 complete hemolytic units (CHU) caused 100% lethality within 4 to 5 min. Doses ranging from 40 to 50 CHU caused death of approximately 50% of the animals. Adrenergic blocking agents and antihistamine failed to protect mice against lethality and thereby suggested that death was not due to release of vasoactive agents by hemolysin. Plasma levels of creatine phosphokinase increased after intravenous administration of hemolysin and suggested myopathy, possibly of the myocardium. Electrocardiograms from hemolysin-treated mice indicated serious alterations in heart rate and rhythm, suggesting damage to contractile and pacemaker cardiac tissue. In addition, there were indications of increased potassium levels influencing the heart. Presumably, death was due to functional damage to heart muscle and electrical arrest. The cardiotoxic and lethal effects could be prevented by prior incubation of hemolysin with cholesterol, heating, or failure to reactivate the preparation with cysteine.

Harvey and Faber (J. Bacteriol., p. 45–46, 1941) first reported the production of a soluble hemolysin by Listeria monocytogenes. Partial characterization of the physical and chemical properties suggested the lysin may be similar to oxygen-labile hemolysins produced by several other bacterial genera (4, 13; M. Rogul and A. D. Alexander, Bacteriol. Proc., p. 82, 1964). Toxicity tests of Listeria hemolysin have indicated a wide spectrum of toxic activity. Previous reports from this laboratory have described several toxic properties, including solubilization of enzymes from isolated lysosomes, leucocidal activity in vitro and in vivo, and hepatocellular damage (10, 11). In addition, intravenous administration of small amounts of Listeria hemolysin caused a convulsive, rapidly fatal reaction in mice (11). Siddique and Walker (15) reported the lysin caused decreased tonus, amplitude, and frequency of contraction of isolated strips of rabbit ileum. The effect could be modified by the antihistaminic drug pyribenazine but not by α- or β-adrenergic blocking agents, suggesting the possible participation of a vasoactive agent in the toxic reaction.

Oxygen-labile hemolysins produced by a number of organisms have been shown to possess toxic properties under a variety of experimental conditions. Lethality in experimental animals after intravenous injection of streptolysin O has been well documented. The lysin has been shown to be a potent cardiotoxic agent, causing immediate loss of ventricular and auricular contractility in perfused hearts, and respiratory arrest and death after intravenous injection of the lysin (6, 9). The major effect was an irreversible reduction in the force of cardiac contraction, indicating the primary site of action was the heart muscle.

The present study was undertaken to examine the nature of lethality in mice after intravenous administration of Listeria hemolysin. Primary emphasis was placed on evaluation of cardiotoxicity and the possible participation of vasoactive agents in the fatal reaction.

MATERIALS AND METHODS

Hemolysin. L. monocytogenes strain 9-125 was employed for production of hemolysin. Procedures for culture, hemolysin production, purification, and titration have been described previously (10, 11). For use, hemolysin was reactivated by addition of 0.006 M cysteine and incubated at 37 C for 30 min.

Animals. White female mice (CD-1 strain, pathogen-free, 12 to 18 g) were purchased from Charles River Mouse Farms, North Wilmington, Mass.

Drug protection studies. Several α- and β-adrenergic blocking agents, guanethidine, and antihistamine

1 Present address: Department of Microbiology, The University of Chicago, Chicago, Ill. 60637.
drugs were used to study protection against a lethal intravenous injection of hemolysin. Mice were pre-
treated with the drugs and challenged with 100 com-
plete hemolytic units (CHU) intravenously. The drugs
and suppliers are as follows: pronethalol, Ayerst
Laboratories, New York, N.Y.; guanethidine, Ciba
Pharmaceutical Co., Summit, N.J.; phenolamine,
Ciba Pharmaceutical Co., Summit, N.J.; pyribenza-
mine, Ciba Pharmaceutical Co., Summit, N.J.; di-
chloroisoproterenol, Eli Lilly and Co., Indianapolis,
Ind.

Creatine phosphokinase (CPK). Plasma values for
CPK were assayed colorimetrically, and enzyme units
were expressed as described by Sigma Technical
Bulletin no. 520 (Sigma Chemical Co., St. Louis, Mo.).
Plasma for each determination was obtained by bleed-
ing from the ophthalmic venous plexus with a hepa-
rinized bleeding tube, and the plasma was separated by
centrifugation.

Electrocardiograms. Mice were anesthetized with
ether and secured in a supine position to a nonconduc-
tive surface by tape at each foot. Electrode placements
were according to the directions of Goldberg et al.
(5). Hypodermic needles (27 gauge) were placed sub-
cutaneously at the base of each extremity, and a single
chest electrode was placed midsternum at the junction
of the fourth costochondral cartilage. Preinjection
recording electrocardiograms were taken prior to intravenous
challenge with 200 to 400 CHU. Post-treatment
tracings were taken until cardiac standstill was evi-
dent. Electrocardiograms were recorded on a Sanborn
Viso-Cardiette Model 500A (Hewlett-Packard, Wal-
tham, Mass.), with a chart speed of 50 mm/sec and a
pen deflection of either 1 cm or 2 cm per 1 mv input.

Blood potassium. Mice were injected intravenously
with 200 CHU and bled from the ophthalmic venous
plexus with heparinized bleeding tubes, and the plasma
was separated by centrifugation. Potassium was de-
termined by use of a flame photometer (Instrumenta-
tion Laboratory, Inc., Watertown, Mass.). Toxic
blood levels of potassium were determined by injecting
various amounts of KCl intravenously and observing
for survival 1 hr after injection.

RESULTS

Lethal effect of hemolysin. Intravenous chal-
lenge with 100 CHU resulted in 100% lethality of
CD-1 mice in 4 to 6 min. To determine that
hemolysin was the lethal agent, mice were chal-
enged with various control preparations of
hemolysin. Table 1 shows that active hemolysin
was lethal, and that lethality was prevented by
prior incubation with cholesterol, by heating, or
by failure to reactivate hemolysin with cysteine.
Lower doses ranging from 50 to 60 CHU usually
killed approximately 50% of the animals with
death generally occurring within 30 min.

Effect of α- and β-adrenergic blocking agents,
guanethidine, and pyribenzamine on lethality. Mice
receiving 100 CHU intravenously died within 4
to 6 min after challenge. Intraperitoneal or intra-
venously administered epinephrine at a dose of

<table>
<thead>
<tr>
<th>Table 1. Effect of hemolysin and various control preparations after injection into mice</th>
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<tbody>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Active hemolysin ................................</td>
</tr>
<tr>
<td>Nonreactivated hemolysin ...................</td>
</tr>
<tr>
<td>Cholesterol-inhibited hemolysin ...</td>
</tr>
<tr>
<td>Heat-inactivated hemolysin* ........</td>
</tr>
<tr>
<td>* Mice received 100 CHU or the equivalent amount of nonreactivated, cholesterol-inhibited, or heat-inactivated hemolysin by the intravenous route.</td>
</tr>
<tr>
<td>† Survivors/mice tested.</td>
</tr>
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<td>‡ Heat treatment, 100 C for 30 min.</td>
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</table>

1 mg/kg of body weight was synergistic rather than ameliorative, suggesting that hemolysin may
cause death by bringing about the release of catecholamine from the adrenal medulla. Adre-
nergic blocking agents were used in an attempt to
protect against lethality due to hemolysin. How-
ever, neither the α- nor β-blocking agents, nor
guanethidine, nor the antihistamine agent pyri-
benzamine had a protective effect against lethal
challenge with 100 CHU of hemolysin (Table 2).

Creatine phosphokinase. Isolated protein frac-
tions of Listeria have been reported by others to
cause alterations in electrocardiographic tracings
in rabbits (13). When mice dying from hemolysin
treatment were dissected, the heart appeared to
exhibit ventricular standstill. Since CPK is found
in high levels in muscle tissue and increases in the
blood after myocardial or other myopathic dam-
age (17), plasma levels were determined to assess
the possible effect of hemolysin on the myocar-
dium. After injection of hemolysin there was an
18-fold increase in enzyme activity at 90 min fol-
lowed by clearance from the plasma (Fig. 1).

Normal levels were obtained when nonreacti-
vated or cholesterol-inhibited hemolysin was used.
These data suggest hemolysin-induced myopathic damage, possibly to the myocardium.

Electrocardiograms. Increased plasma CPK
values and rapid death of mice after administra-
tion of hemolysin suggested possible damage to
contractile heart muscle, pacemaker muscle fibers,
or both. To further assess the possibility of
cardiotoxicity, a series of electrocardiograms were
obtained on mice receiving lethal amounts of
hemolysin.

Impulses arising in the primary pacemaker, the
sino-auricular node (S-A node), located in the
right atrium, pass through the atrial muscle tissue
causing auricular systole. This depolarization of
the muscle fibers causes a deflection on the elec-
Table 2. Effect of adrenergic blocking agents, guanethidine and antihistamine on hemolysin-induced death in mice

<table>
<thead>
<tr>
<th>Drug</th>
<th>Activity</th>
<th>Dose (mg/kg)</th>
<th>Route of administration</th>
<th>Length of pretreatment</th>
<th>Hemolysin (CHU)</th>
<th>Survival for 1 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S/T*  Per cent  Protec. effect</td>
</tr>
<tr>
<td>Phentolamine</td>
<td>α Blockade</td>
<td>5</td>
<td>Intravenous</td>
<td>20 min</td>
<td>100</td>
<td>10/10 0 0</td>
</tr>
<tr>
<td>Dichloroisoproterinol</td>
<td>β Blockade</td>
<td>10</td>
<td>Subcutaneous</td>
<td>60 min</td>
<td>100</td>
<td>10/10 0 0</td>
</tr>
<tr>
<td>Pronethalol</td>
<td>β Blockade</td>
<td>5</td>
<td>Intravenous</td>
<td>20 min</td>
<td>100</td>
<td>10/10 0 0</td>
</tr>
<tr>
<td>Guanethidine</td>
<td>Blockade, sympathetic release of norepinephrine</td>
<td>1.5b</td>
<td>Oral</td>
<td>2 days</td>
<td>100</td>
<td>10/10 0 0</td>
</tr>
<tr>
<td>Pyribenzamine</td>
<td>Antihistamine</td>
<td>5</td>
<td>Intravenous</td>
<td>60 min</td>
<td>100</td>
<td>10/10 0 0</td>
</tr>
</tbody>
</table>

* Survivors/mice tested.

** Daily dose.

nodal muscle fibers, and thus cause alterations in electrocardiograph tracings.

The normal mouse electrocardiogram showed a pulse rate ranging from 480 to 540 with an average of 510/min (Fig. 2). "P" waves were upright in leads 1, 2, 3, AVF, V, and CF and inverted in leads AVR and AVL. The normal "P-R" interval was 0.06 sec with a "P-P" interval of 0.12 sec. The "Q-T" duration was 0.02 sec with a "T" deflection in lead 2 of 0.075 mv.

Listeria hemolysin caused serious alterations of heart rhythm in mice receiving a lethal concentration intravenously. All mice showed slowing of the heart rate and various classes and degrees of arrhythmias, frequently suggesting conduction disturbance in several areas of the heart. Figure 3A shows diminished but upright "P" waves and dropping of occasional beats suggesting intermittent 2° sino-auricular block and interference with impulse conduction to atrial muscle fibers. In addition the "T" wave became more prominent, a possible indication of potassium increase.

Sino-auricular block (2°) with nodal rhythm (Fig. 3B) suggested damage to the primary pacemaker, and movement of pacemaker site to the A-V node. Broad, retrograde "P" waves with occasional notching and dropped beats 2 and 8 and the upright "P" wave preceding beat 13 suggested that the S-A block may be intermittent with possible A-V conduction disturbance and various degrees of A-V block. The increased "T" wave, "RS-T" upward displacement, and slightly longer "Q-T" duration (0.04 sec) were suggestive of potassium increase. Marked slowing of the heart rate and broad infrequent "P" wave activity with pause and slowing suggested 1° S-A
block (Fig. 3C). A-V conduction disturbance and varying degrees of A-V block were indicated by abnormally prolonged “P-R” intervals and “P” waves not followed by the “QRS” complex. The “QRS” complex became broad with an increased “T” wave. Broad “P” waves not followed by the “QRS” complex and a prolonged “P-R” interval indicated complete or almost complete A-V block (Fig. 3D). The markedly slower ventricular rate and wide, bizarre “QRS” complexes were suggestive of complete atrial-ventricular dissociation with an idioventricular pacemaker focus below the bifurcation of the common bundle. The “T” wave became broad and tall. Extensive damage to the S-A nodal fibers and auricular muscle tissue was indicated by the absence of atrial activity (Fig. 4A). Broad, bizarre “QRS” complexes suggested pacemaker activity at an idiocentral focus. Marked slowing and fusion of the “QRS-T” complex were suggestive of the dying heart (Fig. 4B, 4C, 4D). Cardiac standstill was the terminal event (Fig. 4E). Electrocardiograms from control animals injected with phosphate-buffered saline containing 0.006 M cysteine or nonreactivated hemolysin showed no abnormalities.

**Plasma potassium.** Potassium intoxication of increasing severity is associated with positive spiking of the “T” deflection, widening of the “QRS” complex, incidental displacements of the
"RS-T" junction, a diminution or loss of the "P" deflection, and, finally, deterioration of "QRST" into large diphasic complexes (1). Electrocardiograph tracings (Fig. 3 and 4) depicted several or all of the alterations associated with increased serum potassium levels. To establish whether changes in potassium levels might be responsible for alterations in the tracings, direct measurements of plasma potassium in hemolysin-treated mice were done (Table 3). Normal mice showed an average potassium level of 7.9 meq per liter with a range of 7.6 to 8.2 meq per liter. Mice receiving nonreactivated hemolysin had a slightly lower potassium level with an average of 7.4 meq per liter and a range of 7.2 to 8.2 meq per liter. Treated mice showed an increased potassium level with an average of 8.9 meq per liter, and a range of 8.2 to 9.6 meq per liter after receiving 200 CHU of hemolysin. There was essentially no difference between normal potassium levels and nonreactivated hemolysin control animals. There was a significant difference at the 99% confidence level between normal and hemolysin-treated mice. However, intravenous injection of KCl to raise the blood potassium level to 20 meq per liter was not lethal for normal mice.

**DISCUSSION**

It is doubtful that the previously reported toxic features (10, 11) would result in the rapidly fatal reaction observed in mice after intravenous administration of 100 CHU or more of hemolysin. It is also not likely that sudden release of large amounts of vasoactive materials is the cause of lethality, although release has been reported for other toxins (18). Schayer (14) found that bacterial endotoxins produce a considerable increase in the tissue activity of histidine decarboxylase and speculated that, during the later phase of endotoxin shock, there was an increased production of histamine. Gilbert (3) showed that antihistamines counteracted the immediate hypertensive effects of endotoxin in cats, whereas antiserotonins and adrenergic blocking drugs were not as effective. Studies by Siddique and Walker (15) on the effect of *Listeria* hemolysin on the
TABLE 3. 

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plasma potassiuma (meq/liter)</th>
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<tbody>
<tr>
<td>Normal (nontreated)</td>
<td>7.9</td>
</tr>
<tr>
<td>Nonreactivated hemolysin (200 CHU)</td>
<td>7.4</td>
</tr>
<tr>
<td>Active hemolysin (200 CHU)</td>
<td>8.9</td>
</tr>
</tbody>
</table>

* Values represent the mean of determinations from 15 animals.

isolated ileum of rabbits indicated that the antihistaminic agent, pyribenzamine, protected against the effects of hemolysin, whereas phenoxybenzamine, an α-adrenergic blocking agent, and pronethalol, a β-adrenergic blocking agent, were not protective. Failure to block the lethal action of hemolysin by blockade of the α- and β-adrenergic receptor sites and antihistamine treatment in the present study probably preclude participation of vasoactive materials in hemolysin-mediated death.

The most probable cause of rapid death is both a direct and indirect toxic injury to contractile and pacemaker myocardial tissue. Large increases in plasma creatine phosphokinase levels are suggestive of myocardial damage and are used clinically as a reliable index of heart injury (8). Todd (16) reported that large doses of streptolysin O were lethal for mice when given by the intravenous route. A single application of Clostridium septicum delta toxin to isolated frog heart caused an immediate decrease in systole and diastole (2).
After 20 min, the ventricle had stopped beating midway between the systole and diastole, while the auricles continued to beat for some time, eventually becoming distended with fluid. Streptolysin O and pneumolysin were also cardiotoxic, causing complete systolic contracture of the ventricle. Kellner et al. (9) observed immediate loss of ventricular and auricular contractility when streptolysin O was added to isolated, perfused hearts of guinea pigs, rabbits, and mice. The major effect of the lysis was an irreversible reduction in the force of cardiac contraction, suggesting that the primary site of action was the heart muscle. Streptolysin O was cardiotoxic after intravenous injection into rabbits (6). The animals developed a series of motor convulsions with the head in extreme extension. Respiratory arrest and death usually followed within 5 min. Electrocardiograms showed ventricular fibrillation and standstill. The heart showed small focal inflammatory lesions in the ventricles, with accumulations of polymorphonuclear leukocytes. Halpem and Rahman (7) demonstrated that mice receiving massive doses of streptolysin O intravenously showed electrocardiogram alterations including A-V block and dissociation with eventual ventricular standstill and auricular fibrillation. Electrocardiograms of mice dying from Listeria hemolysin showed very significant alterations in pacemaker site, rate, rhythm, and conformation of the various deflections. In most instances, the changes were sufficient to indicate serious damage to the heart muscle. Whether this damage was a direct effect of hemolysin on myocardium or a secondary effect of increased blood potassium has not been ascertained. The ratio of potassium to the heart muscle. Whether this damage was a result of increased potassium level in the plasma (possibly released from the cytoplasm of myocardial or other tissue damaged by hemolysin) would lead to a reduction in the excitability and contractility of cardiac fibers (1). It is, however, not likely that all cardiotoxicity is the result of increased potassium levels, since intravenous injection of potassium ion to raise blood levels to 20 meq per liter did not cause death in normal animals. It seems more probable that hemolysin directly damages both pacemaker and contractile cardiac fibers and that a secondary or possibly synergistic effect is caused by the increased potassium levels. Presumably, death is due to functional damage to cardiac muscle and electrical arrest of the heart. However, other factors resulting from damage to the reticuloendothelial system or hepatocytes described previously (11) may also play additional roles in the lethal reaction.

ACKNOWLEDGMENTS

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LITERATURE CITED