Histopathology and Pathogenesis of Listeriosis Caused by
Listeria monocytogenes Isolated from Raw Milk in Mice

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Abstract: Pathogenicity and histopathology of Listeria monocytogenes isolates (11) from raw milk in mice was
done. Swiss albino mice (18-22 g) were used as experimental animals. All the isolates were pathogenic and
causd mortality within 2 to 6 days after intraperitoneal injection. Reisolation of the microorganism from
different organs was done. Pathological changes in the liver, spleen, brain, kidneys and intestines were
recorded.

Key words: Histopathology, listeria, listeriosis

INTRODUCTION

Listeriosis is one of the most important foodborne
diseases that different regulatory agencies throughout
the world have been dealing with for the last few years. The
causal agent of this illness is Listeria monocytogenes, a
pathogen widely distributed in the natural environment
(Fenlon et al., 1996, Moshtaghi et al., 2004) and
consequently present in many animal and plant food
products (Biliard, 2000). It is well established that the
ingestion of contaminated food products causes a range
of symptomatic manifestations (including septicaemia and
meningitis), with an approximate 20% case-fatality rate
(Rouquette and Berche, 1996), that increases up to 75% in
high-risk groups, such as pregnant women, neonates and
immunocompromised adults. In the last few years,
several outbreaks of listeriosis due to consumption of
contaminated food products have been reported
(Anonymous, 1999). The extended distribution of
Listeria monocytogenes in the environment, combined with the
specific growth conditions of the pathogen, appear to be
the main cause of its high prevalence in different kinds of
food products. On the other hand, when outbreaks of
human listeriosis occur, it is important for clinicians to be
able to verify the pathogenicity of associated isolates,
particularly isolates from the suspected vehicle of
infection. Hemolytic activity correlates with the
pathogenicity of L. monocytogenes (Groves and
Welshimer, 1977; Skala et al., 1982). However, hemolysis
is frequently weak and hemolysis tests often yield
ambiguous results (Domínguez Rodríguez et al., 1986).

The main objective of this study was to understand
the pathogenicity and histopathology of milk isolates of
Listeria monocytogenes.

MATERIALS AND METHODS

Eleven isolates of Listeria monocytogenes from raw
milk that collected from private dairy farms in Shahrekord
city (Iran) from May 2004 to December 2005, were
examined for their pathogenicity and histopathological
changes in mice.

Initially, pathogenicity was determined by a
modification of the method of Ralovich (1984). For each
isolate, five 18-22 g Swiss albino mice were given 0.1 mL
of the suspension, containing 10⁶ cells, intraperitoneally
(i.p.). A group of 5 mice was inoculated with 0.1 mL
peptone water to act as control. Strains that killed three or
more mice were considered to be pathogenic. The mice
were provided with food and water ad lib and were
observed daily for clinical signs and mortality up to
12 days and deaths were recorded. Strains that killed three
or more mice were considered to be pathogenic. The mice,
which survived, were sacrificed after 12 days.

The mice were examined by necropsy for gross
changes. Portions of the liver, spleen, intestine and brain
were collected aseptically in sterile vials and reisolation of
the test organism from these was done by direct plating
on PALCAM agar. Sections were stained by eosine and
haematoxyline staining method and Taylor’s method
(Taylor, 1966).
RESULTS AND DISCUSSION

All the isolates of *L. monocytogenes* were serotype 4b which is associated with the majority of cases of human listeriosis and were pathogenic to mice and led to mortality. The mortality started two days post-inoculation and continued up to 6 days, after which the mice survived the infection. Grey and Killinger (1966) and Pine et al. (1990) have also reported up to 6 days after inoculation, but our results were not in agreement with the results of Low and Donachie (1997) that had been reported serotype 4b strains are not pathogenic in mice. However, of the 35 mice inoculated with serotype 4b strains, 51% (18 mice) 2 days, 5.7% (2 mice) 3 days, 2.9% (1 mouse) 4 days after inoculation had died and mortality was recorded 5.7% (2 mice) on day 6.

Microbiological examination of all the dead mice led to recovery of *L. monocytogenes* in large numbers and in pure cultures. Presence of the organisms in small numbers was also recorded in the organs of the mice, which survived the infection. Between days 5 and 7 postinfection, *L. monocytogenes* bacteria start to disappear from mouse organs until their complete clearance as a result of gamma interferon (IFN-γ)-mediated macrophage activation and the induction of an acquired immune response primarily mediated by CD8 lymphocytes, which together destroy *Listeria*-infected cells (Mielke et al., 1988; Kaufman, 1993; Jose et al., 2001). The above course of events is accelerated in immune animals, resulting in rapid elimination of *L. monocytogenes* from the liver. This is probably the most common outcome of *L. monocytogenes* infection in humans and animals in normal conditions, given the potentially high frequency of exposure to the pathogen via contaminated food and the relatively rare occurrence of clinical disease.

Different organs of the mice in control group did not show any gross and histopathological changes. In the infected group, grossly there were a number of small, white necrotic foci in the liver and spleen. At places, the liver was congested. The number of necrotic foci was more in mice that died 2 or 3 days post-inoculation. Similar findings have been reported by Mandel and Cheers (1980) and Mainou-Flower et al. (1988).

Histopathological examination of the liver revealed congestion and pyo granulomas consisting of necrosed hepatic cells, macrophages and neutrophiles (Fig. 1). There was lymphocyte depletion and necrosis of lymphocytes in white pulp of spleen of the infected mice (Fig. 2). Brain tissue showed microabscesses and necrosis of neurons, which was characterized by satellosis and
neurophagia (Fig. 3). In kidneys, histopathological changes were characterized by the presence of focal interstitial nephritis, focal areas of neutrophils and lymphocytes in interstitial tissue and atrophy of tubules. Besides these, hyaline casts in tubules and cloudy swelling were recorded. Sections of the intestine showed characteristics of enteritis. The lesions in the liver, kidney, spleen and intestine showed the presence of the microorganisms, which stained blue to blue-black by Taylor's stain. The lesions recorded in mice were similar to those reported by earlier workers (Grey and Killinger, 1966; Mandel and Cheers, 1980; Mainou-Flower et al., 1988).

CONCLUSIONS

The findings suggested that *L. monocytogenes* isolates, which are commonly known to saprophytic existence in the environment, are potential pathogens. These may pose serious threat to animal and human health.

ACKNOWLEDGMENTS

This study was supported by the Directory Research of Shahrekord University, Iran.

REFERENCES


