Pathological Effect of Lactobacilli on Rat Liver and Kidney

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Abstract

Three Lactobacillus isolates (viz. L. bulgaricus, L. plantarum and L. acidophilus) were isolated from yogurt, vinegar and vagina, respectively, and injected (1.5 X 10^8 CFU/ml), intraperitoneally, in rats. Histopathological sections showed that all these isolates were able to cause signs of damage to the liver and kidneys of rat. These signs included infiltration of inflammatory cells and necrosis in liver. While the kidney showed signs of haemorrhage and congestion.

Introduction

Lactobacilli belong to a diverse group of bacteria that are gram-positive, facultatively anaerobic, non-spore forming, and produce lactic acid as a major product of carbohydrate fermentation. These bacteria are ubiquitous in the environment and propagate in ecological niches (Van Pijkeren and O’Toole, 2009). In humans they colonize the oral cavity, gastrointestinal tract, and vagina (Axelsson, 2004). In general, lactobacilli are currently proposed as probiotic agents in several dietary products (Martínez-Cañavate et al., 2009; Hoffmann et al., 2008).

In blood cultures, they are usually considered as contaminants, but in recent years they have been recognized as causal infectious agents of endocarditis, urinary tract infections, meningitis, intra-abdominal infections and bacteraemia (Tommasi et al., 2008). Also Land and his coworkers reported that the molecular DNA fingerprinting analysis showed that the Lactobacillus strain isolated from blood samples was indistinguishable from the probiotic strain ingested by the patients. This report indicates that invasive disease can be associated with probiotic lactobacilli (Land, et al., 2005).

Slover and Danziger (2007) stated that more research is needed to expand our basic understanding of the conditions under which lactobacilli cause infection, especially with the mounting interest in using Lactobacillus sp. products as “natural forms” of disease treatment.

Accordingly, the present study aimed, for the first time, to evaluate the histopathological effect of lactobacilli when they get entrance to the circulation via ulcers or wounds in mouth, intestine or vagina.

Materials and Methods

Laboratory animals

Twelve female rats weighted 185 – 220 grams are distributed into four groups (A through D), three animals per group. Each animal was put in a single cage; however, all animals were fed the same food and water.
Isolation and identification

To isolate lactobacilli, three different samples of vinegar, yoghurt (were supplied from local markets) and vagina swab. One milliliter of each sample was spreaded onto De Mann-Rogosa-Sharpe agar (MRSA) (pH 5.5, Himedia, India) plates and incubated at 37 °C for 48 h under anaerobic conditions. The lactobacilli were initially identified by their ability to grow on the selective MRSA. Purified cultures were streaked onto CaCO3-agar plates gram-positive staining, rod shape, and catalase-negative phenotype. Biochemical analyses, including sugar fermentation profile and gas production in MRS broth (pH 5.5, Himedia, India), growth at 6.5% NaCl, ammonia gas production from arginine, growth at 10, 15, 45 °C, growth at pH4 and pH9 were conducted to differentiate the species of *Lactobacillus* as described in (Holt *et al.*, 1994; Hammes, and Hertel, 2006).

Quantification of lactobacilli suspensions were adjusted to approximately 1.5 X 10^8 CFU/ml by comparison to McFarland turbidity standards confirmed by enumeration using the spread plate technique (Forbes, *et al.*, 2007).

Lipase activity assay

Lipase activity was detected according to Lopes *et al.* (1999) using olive oil as substrate.

Inoculation protocol

All animals were anaesthetized with sodium pentobarbital (25 mg / kg) (Satoh *et al.*, 1984). Abdomen and surrounding area were sterilized with 75 % ethanol then 50 µl of the bacterial suspension was injected intraperitoneally by aid of 1 ml syringe.

Group A was injected with *L. bulgaricus* (isolated from yoghurt), group B was inoculated with *L. plantarum* (isolated from vinegar), group C was inoculated with *L. acidophilus* which has been isolated from the vagina, while group D was inoculated with normal saline to be considered as a control group.

Three days later, animals were sacrificed; livers and kidneys were taken for histopathological study (Humason, 1972).

Results and Discussion

Isolation and identification

Results revealed that *L. bulgaricus*, *L. plantarum* and *L. acidophilus* were isolated from yoghurt, vinegar and vagina, respectively; all of them are lipase positive.

Histopathological study

All lactobacilli isolates were able to cause histopathological damage to rat liver tissues represented by infiltration of inflammatory cells and necrosis (figure 1B and C). While kidney suffered from congestion of blood inside the vessels (figure 2)
Figure 1: Cross section in rat liver. A) Normal control. B&C) Post injection with 1.5 X 10^8 CFU/ml *Lactobacillus acidophilus*, signs of necrosis (N) and infiltration of inflammatory cells (I) can be seen. X400. H&E.
Figure 2: Cross section in rat kidney: A) Normal control. B) Post injection with $1.5 \times 10^8$ CFU/ml *Lactobacillus acidophilus* haemorrhage and congestion (arrow) can be seen. X400. H&E.

Regarding previous results will emphasize the pathogenicity of lactobacilli. However, such pathogenicity could be attributed to the effect of cellular component such peptidoglycan, DNA, RNA, cytoplasmic membrane (Van Amersfoort, et al., 2003).

Cannon and his coworkers (2005) reported that *Lactobacillus* was found to be frequently associated with endocarditis and bacteremia. *Lactobacillus* was also associated with a variety of other infections including, but not limited to, peritonitis, abscesses, and meningitis.

Veckman et al. (2003) suggested that direct as well as indirect cytokine-mediated mechanisms are involved in the regulation of chemokine gene expression by Gram-positive bacteria. They demonstrated that *L. rhamnosus* stimulate Th1 cell chemotaxis.
Also the ability of this bacteria to induce IL-12, IL-18, and IFN α/β production suggest that nonpathogenic *L. rhamnosus* are able to stimulate Th1 immune responses.

Although *Lactobacillus* spp. had traditionally been considered to be relatively avirulent, they could cause lifethreatening infections with serious outcomes. Several cases of liver abscess due to *Lactobacillus* spp. (*L. rhamnosus, Lactobacillus acidophilus, and Lactobacillus paracasei*) were reported. Moreover, bacteremia due to anaerobic, nonsporulating, Gram-positive bacilli, 25% of the isolates were identified as *Lactobacillus* spp. by 16S rRNA gene sequencing, and the isolation of *Lactobacillus* spp. was associated with clinically significant bacteremia (Chan *et al.* 2010).

As a conclusion, non pathogenic lactobacilli showed serious histopathological changes in kidney and liver of rats when injected intraperitoneally.

**References**


