Lactobacillus casei: influence on the innate immune response and haemostatic alterations in a liver-injury model

Cecilia Haro, Hortensia Zelaya, Sandra Lazarte, Susana Alvarez, and Graciela Agüero

Abstract: *Lactobacilllus casei* CRL 431 has the ability to modulate the local and systemic immune responses, which are significantly involved in liver injury caused by hepatotoxins. This work was conducted to determine whether *L. casei* has a preventive effect on the hepatic damage undergone during an acute liver injury (ALI). Methods: ALI was induced by an intraperitoneal injection of D-galactosamine (D-Gal). Different groups of mice received 1×10^9 *L. casei* cells/day/mouse for 2 days before D-Gal injection. Blood and liver samples were obtained 0, 6, 12, and 24 h after D-Gal administration. Results: D-Gal induced increases in serum aminotransferases, reduced the number of blood leukocytes, enhanced neutrophil myeloperoxidase activity, increased dead cells, and altered prothrombin time and plasma fibrinogen levels. The preventive treatment with *L. casei* for 2 days modulated the innate immune response. This effect was shown by the earlier normalization of white blood cell counts, myeloperoxidase activity and aminotransferases levels. However, the haemostatic parameters were only partially recovered. The favourable effects obtained could be due to the capacity of *L. casei* to moderate the inflammatory response at the site of the injury with less damage to liver tissue.

Key words: Lactobacillus casei, acute liver injury, blood coagulation.

Résumé : *Lactobacillus casei* CRL 431 a la capacité de moduler les réponses immunes locale et systémique, lesquelles sont significativement impliquées dans le dommage au foie causé par les hépatotoxines. Ce travail a été réalisé pour déterminer si *L. casei* pouvait prévenir des dommages hépatiques subits lors d'une atteinte hépatique aiguë (AHA). Méthode : l'AHA a été induite par une injection intrapéritonéale de D-galactosamine (D-Gal). Différents groupes d'animaux ont reçu 1×10^9 cellules/jour/souris (*L. casei*) pendant 2 jours avant l'injection de D-Gal. Des échantillons de sang et de foie ont été prélevés 0, 6, 12 et 24 h après l'administration de D-Gal. Résultats : La D-Gal a induit une augmentation de l'activité des aminotransférases sériques, une réduction du nombre de leucocytes sanguins et une augmentation de l'activité de la myéloperoxydase des neutrophiles. Un traitement préventif à *L. casei* pendant 2 jours a contribué à moduler la réponse immune innée. Cet effet a été mis en évidence par la normalisation précoce de globules blancs sanguins, de l'activité de la MPO ainsi que des niveaux d'aminotransférases. Cependant, les paramètres hémostatiques n'étaient que partiellement recouvrés. Les effets favorables obtenus pourraient être dus à la capacité de *L. casei* à moduler la réponse inflammatoire au site atteint, résultant en un dommage moindre du tissu hépatique.

Mots-clés : Lactobacillus casei, atteinte hépatique aiguë, coagulation sanguine.

[Traduit par la Rédaction]

Introduction

The liver, one of the main organs of synthesis and an important passageway of nutrients from the intestine to different systemic sites, can be damaged by various factors such as chemotherapeutic drugs, alcohol, toxics, viruses, aflatoxins, heavy metals, and chemical agents (Chandra 2004).

In acute liver injury (ALI), bacterial translocation from the gut may play an important role (Kasravi et al. 1997). Also, the innate immune response is significantly involved in the protection of the liver against injuries caused by hepatotoxins. It is characterized by an inflammatory response with infiltration of neutrophils and mononuclear cells, altered local and systemic expression of inflammatory mediators (Chandra 2004; Iwatsuki and Starzl 1988), and cell death (Williams and Gimson 1991). Unfortunately, in the liver, as in other organs, an excessive inflammatory response bears the risk of additional tissue damage (Chandra 2004). In addition, an inflammatory state leads invariably to the activation of the coagulation system and to the inhibition of anticoagulatory and fibrinolytic mechanisms (Opal and Esmon 2003). Alterations in the synthesis of coagulation fac-

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C. Haro, H. Zelaya, S. Lazarte, and G. Agüero.[1] Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Balcarce 747, CP 4000, San Miguel de Tucumán, Tucumán 4000, Argentina.
S. Alvarez. Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Balcarce 747, CP 4000, San Miguel de Tucumán, Tucumán 4000, Argentina; Centro de Referencia para Lactobacilos (CERELA-CONICET), Chacabuco 145, CP 4000, San Miguel de Tucumán, Tucumán 4000, Argentina.

[1]Corresponding author (e-mail: gaguero@fbqf.unt.edu.ar).

tors during hepatic injury contributes to the modifications of haemostasis observed in some liver diseases.

Osman et al. (2007) and Adawi et al. (2001) demonstrated that some lactobacilli strains exert protective effects on ALI.

Probiotics are microorganisms that, when administered in adequate amounts, confer a health benefit to the host (WHO/FAO 2006). They stimulate the local and systemic immune response (Alvarez et al. 2001; Cross 2002; Villena et al. 2005), exert anti-inflammatory effects (Adawi et al. 2001; Osman et al. 2007), and can control bacterial translocation from the gut to the bloodstream (Nurmi et al. 2005). The probiotic strain *Lactobacillus casei* CRL 431 induces protective immunity in the gut (Alvarez et al. 1998), enhances bronchial immunity (Perdigón et al. 1999), and stimulates the gut immune cells to release inflammatory and regulatory cytokines (Perdigón et al. 2002).

The present study was conducted to examine in an ALI model whether oral administration of *L. casei* CRL 431 exerts protective effects through regulation of the inflammatory response and control of the coagulation alterations.

Materials and methods

Animals

Adult 8-week-old Balb/c mice weighing 22–28 g were obtained from the closed colony kept at Centro de Referencia para Lactobacilos (CERELA). The animals were housed in plastic cages, and the environmental conditions were kept constant following the standards for animal housing. Each experimental group consisted of 5–6 mice that were housed individually during the experiments. The experimental protocol was approved by the Ethical Committee for Animal Care of CERELA and the Universidad Nacional de Tucumán.

Microorganisms

Lactobacillus casei CRL 431 was obtained from the CERELA culture collection. It was cultured for 8 h at 37 °C (final log phase) in de Man – Rogosa – Sharpe broth (Oxoid, Wesel, Germany), and the bacteria were harvested through centrifugation at 3000*g* for 10 min and then washed 3 times with sterile 0.01 mol/L phosphate-buffered saline (PBS, pH 7.2). Subsequently, the lactic acid bacterium culture was suspended in 5 mL of sterile 10% nonfat milk.

Feeding procedures

The *L. casei* culture, suspended in 5 mL of sterile 10% nonfat milk, was added to the animals' drinking water (20% *v*/*v*). Different groups of mice received a dose of 1×10^9 *L. casei* cells/day/mouse for 2 days. The control group received sterile nonfat milk in the same conditions as the test group. All mice were fed ad libitum with a conventional balanced diet.

Acute liver injury induction

At the end of the *L. casei* administration, moderate ALI was induced by intraperitoneal injection of D-galactosamine (D-Gal) (catalogue G-0264; Sigma-Aldrich, St. Louis, Missouri) at a dose of at 800 mg per kg of body mass, in sterile saline solution.

D-Gal provokes depletion of hepatic uridine triphosphate, which is indispensable for the biosynthesis of macromolecules such as nucleic acids and proteins (Keppler et al. 1968).

The ALI control animals received the D-Gal injection without previous treatment with *L. casei* (D-Gal control group; DGC). The normal controls were animals injected with saline solution only. Blood samples were obtained through cardiac puncture from sodium pentobarbital anesthetized animals before acute liver injury induction (day 0) and 6, 12, and 24 h post D-Gal injection.

Alanine aminotransferase and aspartate aminotransferase as liver injury markers

To verify the induction of ALI by D-Gal, the serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined. Enzymatic activity was measured in mice serum by means of a spectrophotometric kinetics assay (Roche Diagnostics GmbH, Mannheim, Germany).

Total and differential blood leukocyte counts

Blood samples were obtained as described before and collected in tubes containing EDTA as an anticoagulant. The total number of white blood cells (WBC) was determined with a hemocytometer. Differential cell counts were performed by counting 200 cells in blood smears stained with May Grünwald – Giemsa stain using a light microscope (100×), and absolute cell numbers were calculated (Dacie and Lewis 1995).

Activation of blood neutrophils

Measurement of myeloperoxidase activity of blood neutrophils was carried out by use of the Washburn test, which is a cytochemical method that uses benzidine as a myeloperoxidase (MPO) chromogen (Kaplow 1968).

Cells were graded as negative or as weakly, moderately, or strongly positive and were used to calculate the score. The score was calculated by counting 200 neutrophils in blood smears. The score value was calculated by the addition of neutrophils with different positive grades.

Tumor necrosis factor-α concentrations in serum

Tumor necrosis factor-α (TNF-α) concentrations in serum were measured with commercially available enzyme-linked immunosorbent assay kits following the manufacturer's recommendations (Mouse TNF-α, CytoSet, BioSource Invitrogen Cytokines & Signaling, Belgium).

Cell death quantification: propidium iodide staining

The animals' livers were removed, washed with a sterile saline solution, and homogenized in sterile 0.01 mol/L PBS. The homogenates were centrifuged at 3000*g* for 10 min and then washed 3 times with PBS. Subsequently, 2.5×10^5 pelleted cells were resuspended in 200 μL of PBS. Two microlitres of staining solution (50 μg/mL propidium iodide; catalogue No. P-4170; Sigma-Aldrich) were added, and the cell suspension was incubated for 5 min at room temperature. The suspension was then diluted with 200 μL of PBS and analyzed using a Partec Pas flow cytometer.

For each sample, data from 100 000 events were recorded in list mode and registered on logarithmic scales. Analyses were performed using FloMax software (Partec CyFlow space, Partec Pas).

Protein in blood serum

The concentration of proteins in blood serum was determined by means of a colorimetric assay by the Biuret method (Roche Diagnostics). The results were expressed as g/L.

Platelet count

Blood samples were obtained as described for the leukocyte counts. Manual platelet counting was performed by visual examination of diluted whole blood with 1% aqueous ammonium oxalate. The total number of platelets was determined with a hemocytometer (Dacie and Lewis 1995).

Coagulation tests

Blood samples were collected in a 3.2% solution of trisodium citrate at a 9:1 ratio of blood to trisodium citrate, according to Agüero et al. (2006). Routine coagulation tests, such as prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen concentration, were performed manually on fresh plasma samples. PT was determined by a one-step method (Thromboplastin-S, Biopool International, Buenos Aires, Argentina). APTT was determined by a one-step technique (STA APTT, Diagnostica Stago, Asnière, France). Fibrinogen was measured by the Clauss method using a commercial kit and following the manufacturer's instructions (FIBRI PREST 2, Diagnostica Stago) (Agüero et al. 2006). The activity of factors V (FV) and VIII (FVIII) in blood plasma was determined by coagulation tests in a single-step coagulation assay (Grupo CAHT 2003).

Statistical analysis

Experiments were performed in triplicate (5–6 animals per experiment), and the results were expressed as means ± SD. After verification of a normal distribution of the data, two-way ANOVA was used. Tukey's test (for pairwise comparison of the means) was used to test for differences between the groups. Differences were considered significant at $p < 0.05$.

Results

Alanine aminotransferase and aspartate aminotransferase

D-Gal induced a significant increase in ALT activity 6 h post injection. Then a slight decrease in enzyme levels was observed, although normal values were not reached by the end of the experiment. However, animals whose water was supplemented with *L. casei* showed normal levels of both enzymes 12 h after D-Gal administration (Table 1).

AST activity significantly increased 6 h post D-Gal injection. Then the enzyme activity decreased gradually to reach normal values at the end of the experiment. No differences were found between animals in the *L. casei* group and those in the DGC group (Table 1).

Total and differential blood leukocyte counts

Twelve hours after injection with D-Gal, we observed a significant decrease in total leukocyte and lymphocyte counts in both groups of mice (Table 2). These values did not become normal until the end of the experiment in the DGC group, whereas the animals whose water was supplemented with *L. casei* showed leukocyte and lymphocyte numbers similar to the normal control at 24 h post injury. The neutrophils demonstrated a kinetic similar to other blood cells. However, at 6 h post liver damage, mice in the *L. casei* group showed significantly higher values than those in the DGC group. At 24 h post D-Gal injection, normal values were observed in both groups.

Activation of blood neutrophils

After D-Gal inoculation, an increase in peroxidase scores was observed, and the maximum values were reached at 12 h in both experimental groups. For the mice treated with *L. casei*, the score returned to basal values 24 h post injury, whereas peroxidase activity remained significantly increased in the DGC group (Fig. 1).

TNF-α in serum

In the experimental model of liver injury, the serum levels of TNF-α reached a maximum at 6 h post injury in both groups (Fig. 2). At the end of the experiment, the animals treated with *L. casei* had normal levels, whereas the level of TNF-α remained high in the DGC group.

Percentage of dead cells

Twelve hours after administration of D-Gal, there was a significant increase in the percentage of dead cells in both experimental groups, but in mice treated with *L. casei*, the increase was significantly lower than in the DGC group. Afterward, the percentage of dead cells decreased gradually in both groups of mice, with normal values being found in those previously treated with *L. casei* (Fig. 3).

Total proteins

In the experimental model of liver injury, no significant modifications in the serum concentrations of total proteins was observed. The preventive administration of *L. casei* for 2 days before D-Gal injection did not induce modifications in this parameter (data not shown).

Haemostatic parameters

During liver injury, a marked decrease in prothrombin activity was observed in both groups, with values <40% between 6 and 12 h post D-Gal injection. From then until the end of the experiment, prothrombin activity increased to 80% in the DGC group and to 100% in the *L. casei* group. The percentage obtained by in vitro testing in the DGC group (80%) is sufficient for normal clotting of blood in vivo. Therefore, mice treated with *L. casei* showed the same behaviour as mice in the DGC group (Fig. 4A).

No modifications in APTT were observed. Mice treated with *L. casei* for 2 days showed the same behaviour as those in the DGC group (Fig. 4B).

In our model, we saw a decrease in FV activity. This parameter was normalized in both experimental groups 24 h post D-Gal injection (Fig. 4C).

In the DGC group, no modifications were observed in plasmatic fibrinogen during the first 12 h. From then on, there was a gradual increase until 24 h, with values significantly higher than the basal level. In the mice that received *L. casei*, an increase in fibrinogen was observed at 6 h post injury, with values significantly higher than those in the DGC group. The fibrinogen levels had returned to normal at 24 h

Table 1. Aminotransferase levels in serum.

	Serum levels (mean ± SD)			
	ALT (U/L)		AST (U/L)	
Hours post D-Gal injection	DGC	*L. casei*	DGC	*L. casei*
0	48.3±3.1	47.0±3.0	167.0±9.9	169.0±9.0
6	72.5±2.1*	77.5±7.7*	371.5±6.4*	330.5±15.0**
12	59.2±3.7*	43.6±6.0**	193.5±13.5	168.5±14.5
24	58.7±5.0*	41.3±3.0**	140.7±14.0	140.5±14.1

Note: The serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined at different times post D-galactosamine (D-Gal) injection. *Lactobacillus casei* mice were treated with acid lactic bacteria for 2 days before D-Gal injection; the D-Gal control (DGC) group received the D-Gal injection without previous treatment. *, $p < 0.05$ with respect to the basal values; **, $p < 0.05$ with respect to the DGC group.

Table 2. Total and differential blood leukocyte counts.

	Blood leukocyte counts (mean ± SD; $\times 10^9$ cells/L)					
	WBC		PMN		Lymphocytes	
Hours post D-Gal injection	DGC	*L. casei*	DGC	*L. casei*	DGC	*L. casei*
0	5.9±0.4	5.8±0.4	1.0±0.3	1.0±0.3	4.9±0.3	4.8±0.3
6	6.8±0.5	6.6±0.5	1.9±0.3	2.5±0.2**	4.9±0.4	4.1±0.4
12	3.8±0.6	3.2±0.5	0.7±0.3	0.6±0.1	3.1±0.4	2.6±0.3
24	4.8±0.4	6.1±0.4*	1.0±0.3	1.1±0.3	3.8±0.4	5.0±0.4*

Note: Total and differential blood leukocyte counts were determined at different times post D-galactosamine (D-Gal) injection in the D-Gal control (DGC) and *Lactobacillus casei* groups. Mice whose water was supplemented with *L. casei* were treated with acid lactic bacteria for 2 days before D-Gal injection; those in the DGC group received the D-Gal injection without previous treatment. PMN, polymorphonuclear leukocytes; WBC, white blood cells. *, $p < 0.05$ with respect to the DGC group; **, < 0.05 with respect to the DGC group and basal values.

Fig. 1. Myeloperoxidase (MPO) activity scores in polymorphonuclear leukocytes (PMN) from peripheral blood. *Lactobacillus casei* was orally administrated to mice at a dose of 1×10^9 cells for 2 days before D-galactosamine (D-Gal) injection; mice in the D-Gal control (DGC) group received the D-Gal injection without previous treatment. Results are expressed as means ± SD ($n = 5$ or 6). *, Significantly different from the DGC group and basal values ($p < 0.05$).

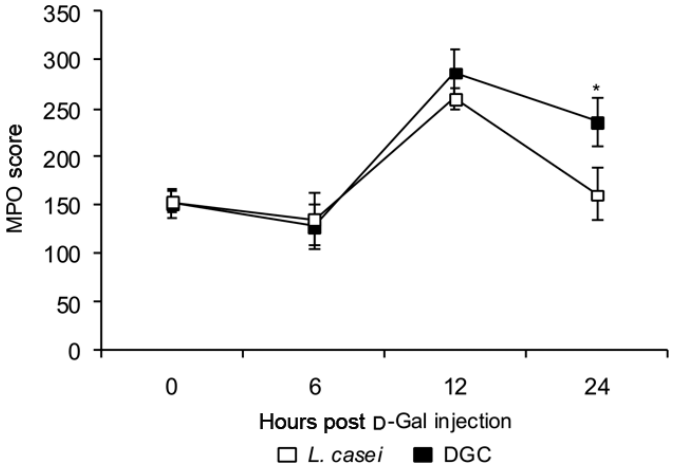
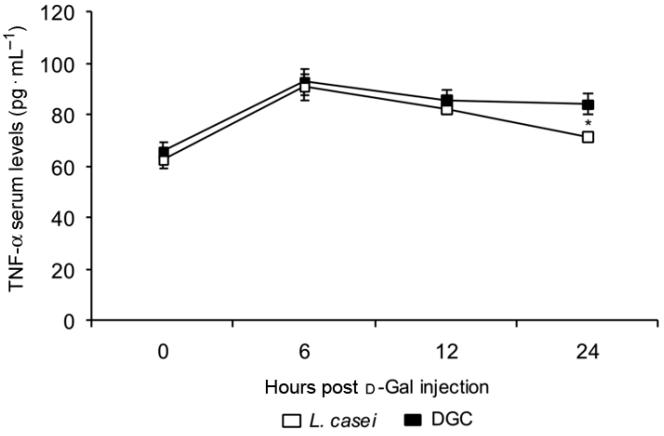


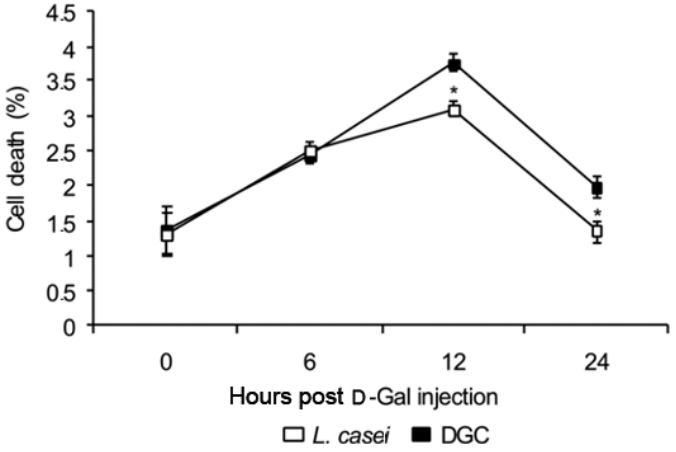
Fig. 2. The serum levels of tumor necrosis factor-α (TNF-α). *Lactobacillus casei* was orally administrated to mice at a dose of 1×10^9 cells for 2 days before D-galactosamine (D-Gal) injection; mice in the D-Gal control (DGC) group received the D-Gal injection without previous treatment. Results are expressed as means ± SD ($n = 5$ or 6). *, Significantly different from the DGC group at the same time point ($p < 0.05$).



post D-Gal injection (Fig. 5A). A significant increase in FVIII was observed at 6 h post D-Gal injection. However, in the group previously treated with *L. casei*, FVIII values were significatively lower than those in the DGC group ($p < 0.05$). After that, both groups showed similar behaviour (Fig. 5B).

The injection of D-Gal induced a significant decrease in the number of platelets in peripheral blood, with the lowest values found 6 h post injury. Platelet counts returned to basal values 24 h post D-Gal injection. Both experimental groups showed similar behaviour (data not shown).

Fig. 3. Quantification of cell death in liver homogenate using the cytofluorimetric method. *Lactobacillus casei* was orally administrated to mice at a dose of 1×10^9 cells for 2 days before D-galactosamine (D-Gal) injection; mice in the D-Gal control (DGC) group received the D-Gal injection without previous treatment. Results are expressed as means ± SD ($n = 5$ or 6). *, Significantly different from the DGC group at the same time point ($p < 0.05$).



Discussion

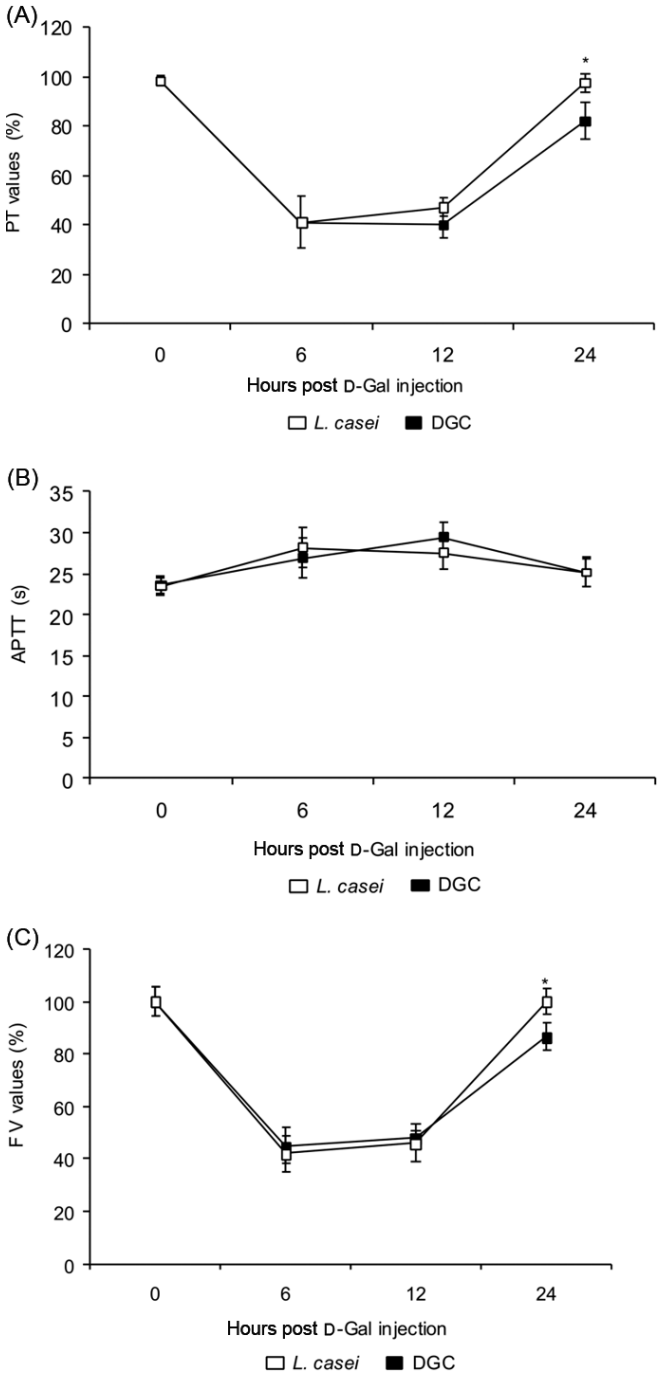
In liver injury, probably the most important consequence of drug-induced stress is the amplification of the inflammation via the activation of the transcription of cytokines, chemokines, and adhesion molecules (Lacour et al. 2005). In tissue damage induced by different drugs, the innate immune response is strongly involved. Probiotic microorganisms can stimulate this response in a magnitude that does not enhance the inflammatory mechanisms (Maldonado Galdeano et al. 2007; Osman et al. 2007). In the present work, after D-Gal administration, a marked decrease in leukocytes was observed in both experimental groups (DGC and *L. casei*). The decrease in leukocytes could be attributed to the well-known process of leukocyte migration towards the site of the injury (Lavnikova et al. 1993; Franková and Zidek 1998).

The recruitment of WBC induced by the D-Gal injection would probably be promoted by, among other factors, an increase in TNF-α, whose main function is to stimulate the attraction of neutrophils and monocytes to the site of the injury and amplify the inflammatory response (Chosay et al. 1997; Gujral et al. 2003; Abbas and Lichtman 2004). In liver injury, TNF-α is released by Kupffer cells together with other inflammatory mediators with cytotoxic potential (Bautista et al. 1991).

In the present study, we observed that the plasma levels of TNF-α increased 6 h post injury in both experimental groups. *Lactobacillus casei* administration significantly decreased the TNF-α values at the end of the period analyzed. In previous works, we observed a similar kinetic of TNF-α serum levels in a respiratory-infection model (Racedo et al. 2006).

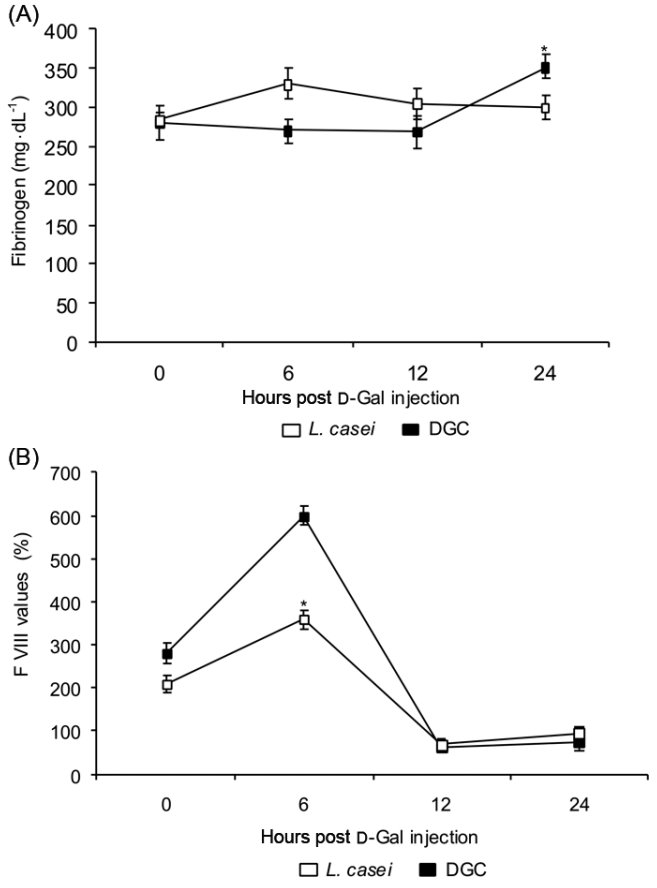
We observed that the decrease in TNF-α correlated with the normalization in the number of leukocytes, probably because of lower recruitment to the liver. Regulation of the process of leukocyte migration could probably moderate the inflammatory response at the site of the injury. The ability of certain probiotic bacteria to inhibit neutrophil migration has been also demonstrated by Roselli et al. (2006) via in vitro assays.

Fig. 4. Haemostatic parameters in liver injury. *Lactobacillus casei* was orally administrated to mice at a dose of 1×10^9 cells for 2 days before D-galactosamine (D-Gal) injection; mice in the D-Gal control (DGC) group received the D-Gal injection without previous treatment. (A) Prothrombin time (PT), (B) activated partial thromboplastin time (APTT), and (C) factor V activity (FV) were studied. Results are expressed as means ± SD ($n = 5$ or 6). *, Significantly different from the DGC group ($p < 0.05$).



We also observed that D-Gal injection induced, in both groups, activation of phagocytic cells, which was shown by the strong activity of neutrophil MPO, presumably because of the increase in TNF-α (Ding et al. 2008). The DGC group had the highest values of this enzyme, whereas the mice that

Fig. 5. Fibrinogen levels and factor VIII activity. *Lactobacillus casei* was orally administrated to mice at a dose of 1×10^9 cells for 2 days before D-galactosamine (D-Gal) injection; mice in the D-Gal control (DGC) group received the D-Gal injection without previous treatment. (A) Fibrinogen levels and (B) factor VIII activity were studied. Results are expressed as means ± SD (n = 5 or 6). *, Significantly different from the DGC group ($p < 0.05$).



received *L. casei* showed normal levels at the end of the experiment. This result could be due to the decrease of TNF-α induced by *L. casei*. Similarly, Tok et al. (2007) demonstrated in a lung-injury model in rats that lactobacilli induced a reduction in MPO activity. This effect, added to the normalization in the number of leukocytes, would lead to a smaller release of oxidative agents and, as a consequence, to a decrease in tissue damage.

Liver injury caused by hepatotoxins is also characterized by hepatocyte degeneration and an increase in the percentage of dead cells, which could be due to apoptosis or necrosis (Wu et al. 1999). At the moment, apoptosis and necrosis are beginning to be considered as often representing alternate outcomes of the same cellular pathways to cell death (Malhi et al. 2006). This phenomenon can be caused by different toxic mechanisms such as mitochondrial damage, reactive oxygen species, and activation of Kuppfer cells and sinusoidal endothelial cells, which, after activation, secrete cytokines (Holst et al. 1996; Lacour et al. 2005). Twelve hours post liver injury in our experimental model, we observed a substantial increase in the percentage of dead cells, whereas the increase was smaller in the animals that received previous treatment with *L. casei*. This effect could be due to a control of the inflammatory response caused by *L. casei*. Kano et al. (2002) also demonstrated that skimmed milk fermented with *Lactobacillus delbrueckii* inhibited secretion of proinflammatory cytokines in mice. The most prominent effect was inhibition of TNF-α.

Also, by use of an in vitro assay, Llopis et al. (2009) confirmed that live *L. casei* can counteract the proinflammatory effects of *Escherichia coli* on Crohn's disease-inflamed mucosa by specific downregulation of key proinflammatory mediators.

In addition, hepatic tissue damage was demonstrated by an increase in serum levels of aminotransferases. This increase could be attributed to alterations in the permeability of the cell membranes as a consequence of the inflammatory process triggered by D-Gal (Drotman and Lawhorn 1978). In this study, the serum levels of ALT and AST increased 6 h after D-Gal injection, but these increases were attenuated by pretreatment with *L. casei*. These results could indicate that the lower levels of TNF-α caused by *L. casei* modulate the inflammatory response and preserve the structural integrity of the hepatocellular membrane. Osman et al. (2007) reported a decrease in the levels of aminotransferases, TNF-α values, and MPO activity in animals fed *Lactobacillus plantarum* or *Bifidobacterium infantis*.

To study the effect of *L. casei* on liver function after damage induced by D-Gal, the concentration of total serum proteins was determined. No significant modifications were observed, which could be due to the fact that various factors have an influence on the levels of total serum proteins, such as different mean half-lives, alterations in their synthesis, and increases or decreases in the proteins considered to be acute-phase reactants (Aoi et al. 2007).

Drug- or toxics-induced liver damage is known to be associated with coagulopathies (Ganey et al. 2007) as a consequence of the altered synthesis of coagulation factors. In this study, we observed that the PT and the percentage of activity of FV were strongly decreased by the effect of D-Gal. These results could be due to the activation of the extrinsic coagulation pathway owing to a greater expression of FT induced by TNF-α, a cytokine that was significantly increased by D-Gal. It is known that the activation of the coagulation mechanism leads to the consumption of factors with a resulting decrease in their concentration (Holst et al. 1996). Similar results were observed in a paracetamol-induced ALI model (Kerr 2003; Kerr et al. 2003; Dabos et al. 2005).

In this work, we observed that previous administration of *L. casei* induced the recovery of prothrombinic activity and FV levels at 24 h post D-Gal injection. However, the lower value obtained in the DGC group is sufficient for the normal clotting of blood in vivo (Grupo CAHT 2003). Thus, it can be concluded that mice treated with *L. casei* showed similar behaviour as those in the DGC group.

When the intrinsic coagulation mechanism was evaluated using the APTT, no significant modifications were observed. This result could be attributed to the fact that this mechanism is not too strongly involved in the inflammatory process induced by the toxin (Kerr 2003; Kerr et al. 2003).

In the DGC group, we found modifications in the concentrations of fibrinogen and FVIII, which are also involved in the intrinsic pathway. Fibrinogen levels responded with a slow increase at 24 h post injury and did not return to nor-

mal during the observation period. However, the mice that received *L. casei* showed maximum fibrinogen concentration at a previous stage (6 h). The fibrinogen increase could be attributed to the action of TNF-α on the hepatocytes, which may have caused an increase in the synthesis of this protein (Abbas and Lichtman 2004), which, in this case, would have acted as an acute-phase protein. Fibrinogen can reduce and eliminate the undesirable consequences of the inflammatory reaction and have very diverse effects, including immunomodulatory action, which also could be involved in the regulation of liver regeneration after injury (Jensen 2001).

FVIII may be used for coagulation at the site of bleeding. In addition, FVIII levels depend on the immune status and the inflammatory microenvironment (Lacroix-Desmazes et al. 2008). We observed that FVIII reacted immediately and strongly to D-Gal administration, possibly because it is a more sensitive acute-phase protein than fibrinogen (Noe et al. 1989). When the lactic acid bacterium was administered, a lower increase in FVIII was observed, perhaps because of the inflammatory microenvironment modulation by *L. casei*.

The decrease in platelet counts observed in both groups after D-Gal injection could be attributed to the contribution of these cells to the pathogenesis of liver injury (Pearson et al. 1995). We did not see any effect of *L. casei* on platelet number.

With these results, we conclude that the preventive administration of *L. casei* for 2 days could modulate the innate immune response. This effect was shown by the rapid normalization of WBC counts and MPO activity, as well as aminotransferase levels. However, the haemostatic parameters only were partially recovered. The favourable effects obtained could be due to the capacity of *L. casei* to prevent the massive secretion of proinflammatory cytokines, particularly TNF-α (Peran et al. 2005). Similar results were observed by Peran et al. (2007) in the trinitrobenzenesulphonic acid model of rat colitis.

On the basis of the previous findings and our results, we propose that the decrease in TNF-α serum levels induced by *L. casei* regulates leukocyte migration to the liver and the degree of activation of leukocytes. These effects probably moderate the inflammatory response at the site of the injury with less damage to liver tissue. The results obtained in this study open up important perspectives for the use of *L. casei* as an oral hepatoprotective agent.

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