Original Article

Cholestasis progression effects on long-term memory in bile duct ligation rats

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Abstract

Background: There is evidence that cognitive functions are affected by some liver diseases such as cholestasis. Bile duct ligation induces cholestasis as a result of impaired liver function and cognition. This research investigates the effect of cholestasis progression on memory function in bile duct ligation rats.

Materials and Methods: Male Wistar rats were randomly divided into five groups, which include: control group for BDL-7, control group for BDL-21, sham group (underwent laparotomy without bile duct ligation), BDL-7 group (7 days after bile duct ligation), and BDL-21 group (21 days after bile duct ligation). Step-through passive avoidance test was employed to examine memory function. In all groups, short-term (7 days after foot shock) and long-term memories (21 days after foot shock) were assessed.

Results: Our results showed that liver function significantly decreased with cholestasis progression ($P < 0.01$). Also our findings indicated BDL-21 significantly impaired acquisition time ($P < 0.05$). Memory retrieval impaired 7 ($P < 0.05$) and 21 days ($P < 0.001$) after foot shock in BDL-7 and BDL-21 groups, respectively.

Conclusion: Based on these findings, liver function altered in cholestasis and memory (short-term and long-term memory) impaired with cholestasis progression in bile duct ligation rats. Further studies are needed to better insight the nature of progression of brain damage in cholestatic disease.

Key Words: Cholestasis, learning, memory, rat

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INTRODUCTION

Cholestasis is known as a severe manifestation of many liver diseases in humans and in rodents.[1,2] It has been shown that structural and functional impairment of the hepatobiliary systems[3] that cause a failure in bile secretion in hepatocytes or ductular cells or an impairment of bile flow and accumulation of bile salts in the body.[4-6]

Cholestasis may result in liver disease and other extra hepatic complications. It may affect many systems in the body such as cardiovascular,[7] renal,[8] and immune systems.[9] Also it is considered as one of the factors that can alter some of the brain functions.[10] Cognitive

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impairment is one of the complications in humans[11,12] and animal models of liver disease, too.[13] Children and adults with liver dysfunction such as biliary atresia showed deficits in their performance intelligence quotient, learning and memory, and visuospatial functions.[14-16] It has been reported that cholestatic rats were poorer in the Morris water maze task and passive avoidance test.[4,13,17] It has also been showed that the ability to discriminate between the novel object and the previously experienced sample object and memory were impaired in BDL rats[18] and in mice.[6]

Evidence suggests that the hippocampus is affected by cholestasis in rats. Besides, compelling evidence has confirmed the critical involvement of hippocampus upon memory processes in rats, monkeys, and humans.[6,16]

Nevertheless, there has not been enough information regarding the cholestasis progression effects on cognitive function such as learning, memory and about stages of memory (short-term and long-term memory) may affect in BDL rats. In the present study, we examined the cognitive function in rats with cholestasis using the passive avoidance test.[19] The study was designed to evaluate the effects of cholestasis progression and its effects on acquisition and retention times of memory 7 and 21 days after BDL in rats

MATERIALS AND METHODS

Animals

Male Wistar rats (220-250 g) were obtained from Joundishapoor University (Ahvaz, Iran), central animal house, Iran. The animals were kept in animal house and provided with food and water *ad libitum* and they experienced a 12:12-h light–dark cycle (07:00 to 19:00) in a temperature-controlled environment (22 ± 2°C) and humidity of 40-70%. The animals were allowed to adapt to the laboratory conditions for at least 1 week before surgery. Each rat was handled for about 3 min each day prior to behavioral testing. All experiments were performed between 9:00 and 12:00 AM and each rat was tested only once. All experiments were conducted in accordance with the international guidance principles for biomedical research involving animals, revised in1985. We used six animals in each group.

Bile duct ligation surgery and induced cholestasis

There were five experimental groups with six rats in each group:

1. Control-7 group (nonoperated, and kept in animal house for 7 days)
2. Control-21 group (nonoperated, and kept in animal house for 21 days)
3. Sham group (laparatomy surgery was performed without bile duct ligation)
4. BDL-7 group; experiments were performed 7 days after bile duct ligation, and they were killed 21 days after BDL
5. BDL-21 group; experiments were performed 28 days after BDL, and they were killed 42 days after BDL

Laparotomy was performed under general anesthesia, induced by injection of chloral hydrate (400 mg/kg, i.p). Sham group consisted of laparotomy and bile duct identification and manipulation without ligation or resection (with the aim of measuring possible stress induced by surgery). In the bile duct ligation groups, the main bile duct was first ligated using two ligatures approximately 0.5 cm apart and then transected at the midpoint between the two ligatures.[20] In the immediate postoperative period, each animal was placed in a cage by itself to prevent wound dehiscence and was moved to its original cage 4 h after the surgery.[21]

Memory testing and apparatus

The training apparatus had two compartments comprising a dark chamber (25 × 25 × 20 cm) and a light compartment (25 × 25 × 20 cm). They were separated by a guillotine door. Electric shocks were delivered to the grid floor by an isolated stimulator. At the beginning, each rat was placed in the apparatus for 5 min to habituate. On the second day, an acquisition trial was performed; rats were placed individually in the illuminated chamber. After a habituation period (1 min), the guillotine door was lifted. The latency to enter dark chamber was recorded as initial latency (IL). When the rat entered the dark chamber, the door was lowered and an inescapable scrambled single electric shock (0.2 mA, 50 HZ) was delivered for 3 s. In probe trial, the interval between placement in the illuminated chamber and entry into the dark chamber was measured and compared with own IL.

Biochemical analysis

Twenty-one days after surgery, a sample of blood (3–4 ml) was collected then plasma bilirubin (total bilirubin, direct and indirect bilirubin)[22,23] levels were determined by using a commercially available kit (Zist Shimi, Tehran, Iran).

Statistical analysis

Repeated measure analysis of variance (ANOVA) was used for data analysis. *Post-hoc* analysis (Tukey-test) was performed for assessing specific inter-group variations. Differences with $P < 0.05$ between experimental groups at each point were considered

statistically significant. The results are presented as the mean ± S.E.M.

RESULTS

Induction of cholestasis

One day after bile duct ligation, the animals showed signs of cholestasis (jaundice, dark urine, and steatorrhea), which were tested qualitatively and quantitatively [Table 1]. BDL rats showed biochemical evidence of cholestasis with significant elevations in serum bilirubin and alanine amino transaminase levels.

Table 1: Liver biochemistries from BDL and sham-operated rats 21 days

	Sham-operated	BDL
Alanin Trans Aminase (IU/L)	177.15±13.42	5533.07±85.19**
Alkalin Phosphatase (IU/L)	435±79.38	732±70.3**
Total Bilirubin (mg/dl)	0.52±0.036	6.53 ± 1.22**
Direct Bilirubin (mg/dl)	0.29±0.03	4.69±1.18**
In direct Bilirubin (mg/dl)	0.42±0.01	3.06±0.42**

Data represent the mean±SEM of data from 6 rats per group, ** P<0.01 vs. respective sham operated

Passive avoidance learning test

The latencies were measured in prefoot shock (IL), as well as 7 and 21 days postfoot shock (retention time or STL). Decrement of the latency (shorter time to enter the dark chamber after receiving foot shock) indicates that memory functions were impaired.

Repeated measure ANOVA and *post-hoc* Turkey's analysis revealed that there were not significant differences between controls and sham groups, thus surgery did not affect preshock latencies (IL) in experimental groups [Figure 1a].

Also, there were no significant differences in IL between control-7 and BDL-7 groups. In contrast, There were significant differences between control-21 and BDL-21 groups in IL ($P < 0.05$) and preshock

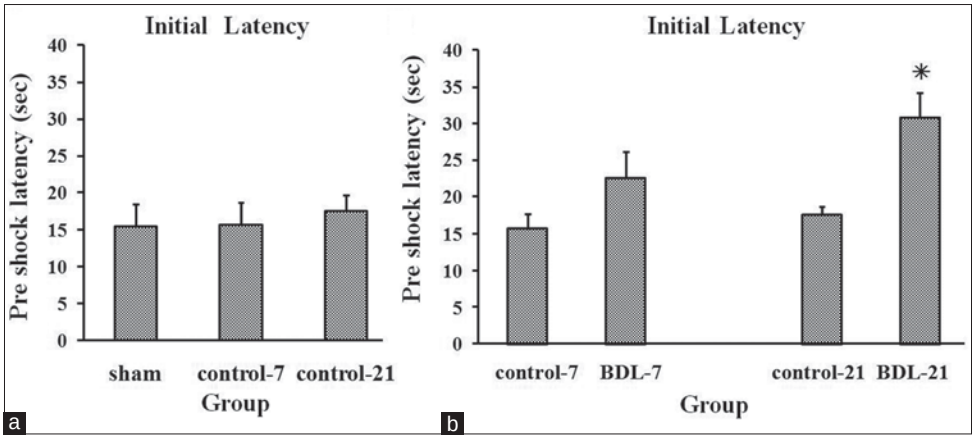

Figure 1: Comparison of latency to enter the dark chamber before receiving foot shock (Initial latency). Each bar represents the mean ± S.E.M. Panel A- There were no significant differences between the control-7, control-21, and sham groups; Panel B- Differences between the Control-7 and BDL-7 groups were not significant, but there were significant differences between the control-21 and BDL-21 groups (* P<0.05). Each group consisted of six rats

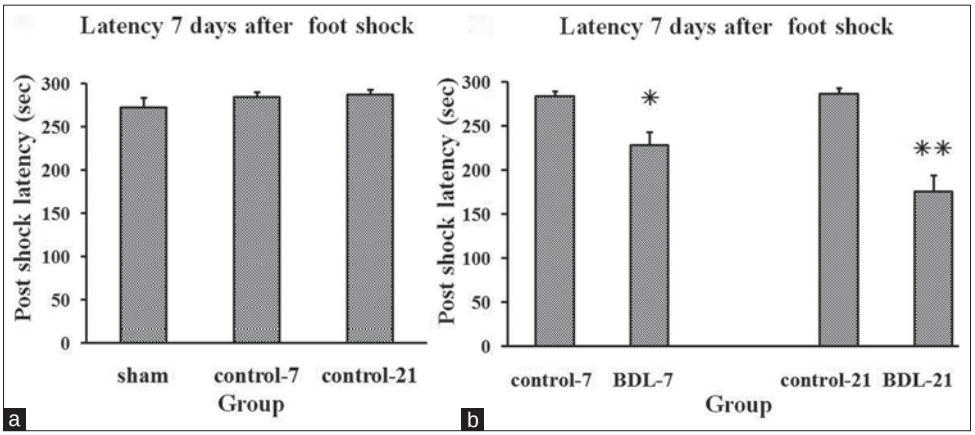

Figure 2: Comparison of latency to enter the dark chamber 7 days after receiving foot shock (short-term memory). Each bar represents the mean ± SEM. Panel A- There were no significant differences between the control-7, control-21, and sham groups. Panel B- The retention time was significantly decreased in the BDL-7 and BDL-21 groups when compared with the corresponding controls groups (* P<0.05, ** P<0.01). Each group consisted of six rats

latency to enter dark room was longer in BDL-21 group compared with control-21 group [Figure 1b].

Our results showed that there were no significant differences between control-7, control-21, and sham groups in latency to enter dark chamber 7 days after receiving foot shock. So surgery has no effect on the results [Figure 2a]. A memory deficit was observed in BDL-7 (14 days after BDL) and BDL-21 (28 days after BDL) groups in latency to enter dark chamber 7 days after receiving foot shock. There were significant differences between BDL-7 and BDL-21 groups in 7 days after receiving foot shock compared with the corresponding controls groups ($P < 0.05$ and $P < 0.01$, respectively; Figure 2b).

Also, there were no differences among control-7, control-21, and sham groups in 21 days after receiving foot shock to enter the dark chamber [Figure 3a].

Our results showed that there were significant differences between control-7 and BDL-7 groups in the latency to enter the dark chamber 21 days after receiving foot shock (28 days after BDL) compared with the corresponding controls groups ($P < 0.001$, Figure 3b).

There were no significant differences within control-7, control-21, and sham groups in 7 and 21 days after foot shock to enter dark chamber, although latencies to enter dark chamber after 7 and 21 days were significant in BDL-7 and BDL-21 groups [Figure 4].

DISCUSSIONS

The main findings of this study were that memory retrieval (short-term memory) impaired after 7 days of BDL, and it has worsened with cholestasis progression after 21 (BDL-7 group) and 42 days (BDL-21 group, long-term memory) after bile duct ligation rats [Figures 2b, 3b and 4]. Also IL did not alter in early stages of disease, but it impaired 21 days post-BDL [Figure 2b]. In the passive avoidance test after training, animals learn to avoid entering the dark chamber after receiving electrical foot shock.[18] Also our previous data (not presented here) showed that short-term memory (24 h after receiving foot shock) did not alter in early stage of cholestasis in BDL rats. Some articles reported that BDL causes biliary cirrhosis, fibrosis, portal hypertension, portal-systemic shunting, and immune system dysfunction. It has been shown that bile duct ligation after 3–4 weeks cause cirrhosis.[5,24-26] Mild cognitive impairment reported in patients with the liver cirrhosis.[27] Furthermore, in some patients with liver disease and signs of hyperamonia it has been showed that impaired attention, memory, cognitive function, and motor function.[6,28]

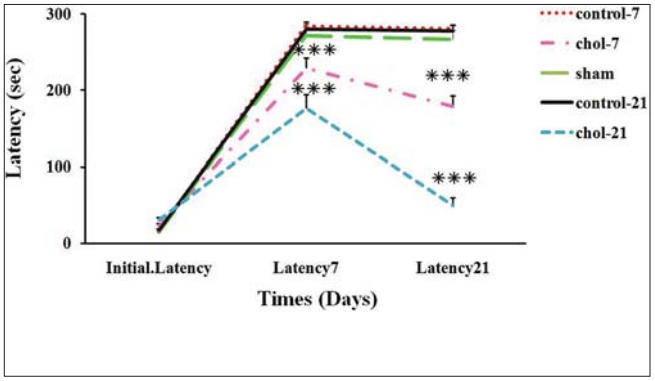

Figure 4: Time delay for entering into the dark room before and after 7 and 21 days receiving electrical foot shock; the reduction of the latency in the control-7, control-21, and sham groups was not significant during 7 and 21 days. Comparison of the latency 7 with 21 days in the BDL-7 and BDL-21 groups showed a significant decrease

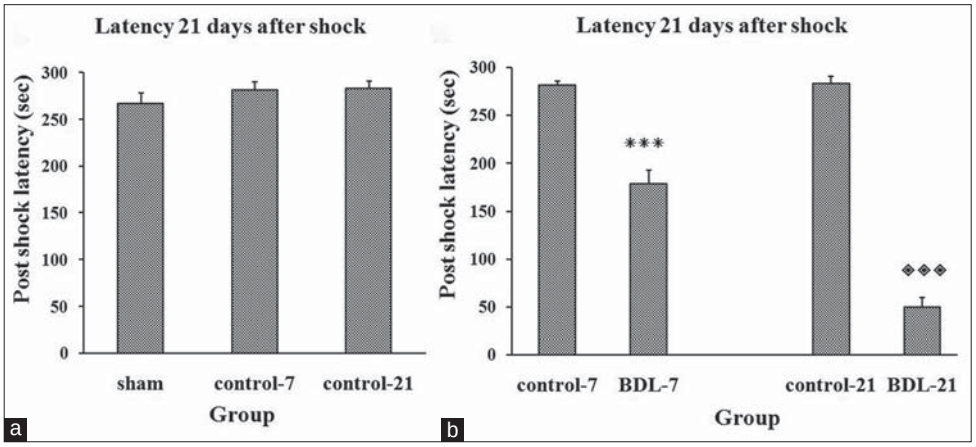

Figure 3: Comparison of latency to enter the dark chamber 21 days after receiving foot shock (the long-term memory). Each bar represents the mean ± SEM. Panel-A; There were not significant differences between the control-7, control-21 and sham groups. Panel-B; The retention time was significantly decreased in the BDL-7 and BDL-21 groups when compared with the corresponding controls groups (***P<0.001, ◆◆◆P<0.001). Each group consisted of six rats

Our findings are consistent with some studies that reported an impaired spatial memory and ability to discriminate the novel object after BDL in rodents.[12,29,30] Of course the beginning time of disturbances has not been studied. Moreover, in the patients with liver disease have been reported deficits in attention, visual perception, and working memory.[31,32]

The BDL is a model of chronic liver injury. It has been showed that both acute and chronic liver failure induces cholestasis that affects brain functions.[13,33,34] The molecular mechanisms by which liver failure impairs cognitive function remain unknown. Some studies suggested that in the liver disease hyperammonia is one of the main factors responsible for the neurological alterations.[35] Also it has been suggested that some mechanisms for glutamatergic system involvement in amnesia induced such as change of brain NO, oxidative stress, disruption of calcium homeostasis, membrane damage, and cell death.[4,36-39] All of the above-mentioned biologic effects can result in cognitive deficits in amnesia induced in BDL rats. Although the mechanisms of amnesia that were induced by cholestasis in BDL rats, have not been fully elaborated.

Previous studies indicated low locomotor performances in swimming[40] and treadmill running tests in BDL rats after 5 days.[41] Our previous data showed despite a decline in locomotor activity, this change was not significant 12 days post-BDL[17] and locomotor activity significantly decreased after 21 days in cholestatic rats (data not shown here). Probably parts of our results could be due to decreased motor function in cholestatic rats. Although, some of the studies proposed that fatigue is responsible for locomotor disturbances in BDL. The mechanisms involved in fatigue that accompanies with cholestasis may occur as a result of changes in the central nervous system as shown previously.[42,43] Among the neurotransmitter systems, serotonergic and adrenaline pathways are both implicated in fatigue states.[44] In the literature, it was shown that these systems are intimately involved in the control of central corticotropin-releasing hormone (CRH) release.[44] Not only the serotonergic system but also the opioidergic system was demonstrated to be affected in cholestasis.[45-47] Studies indicated that in BDL animals without obvious signs of infection, fever, or signs of sepsis showed activation of pro-inflammatory cytokines,[10] similar to those found in human with liver disease[48] that associated with activation of inflammatory mediators, provokes a greater behavioral impairment.

In summary, the results showed that chronic liver failure leads to developed cognitive impairments with progression of cholestasis in BDL rats.

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