·Basic Research·

Cirrhosis-induced morphological changes in the retina: possible role of endogenous opioid

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Abstract

• AIM: To investigate the impact of cirrhosis on retinal morphology and to evaluate the role of endogenous opioids as a mediator in cirrhosis induced retinal change.

• METHODS: Thirty-six male rats were divided into 3 main groups; the common bile duct ligated (BDL) group, the sham-operated (Sham) group and the unoperated (Unop) group. Then each of these three main groups was divided into two subgroups; the first subgroup received daily injection of naltrexone hydrochloride (NTX) and the second group was injected with normal saline (Saline) daily. After 28d, rats were anesthetized and their right eyes were enucleated and assessed for histological changes. The thickness of the rod and cons layer, outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer and ganglion cell layer for each eye were measured in micrometers by light microscope.

• RESULTS: Ganglion cell layer showed significant increase in thickness in the BDL group (P<0.05). This increase was eliminated in the group where BDL rats received daily intraperitoneal injection of naltrexone hydrochloride (20 mg/kg). No other histological changes were detected in the other 5 layers we measured

• CONCLUSION: The morphological change we detected in the retina of cirrhotic rats is probably due to opioids increased tone in cirrhosis since the increase in thickness in the ganglion cell layer was almost eliminated when naltrexone hydrochloride was injected. These results suggest a possible role for endogenous opioids in the morphological retinal changes detected in cirrhotic rats.

• **KEYWORDS:** cirrhosis; endogenous opioids; retina; ganglion cell layer
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INTRODUCTION

The pathologic features of cirrhosis consist of the development of fibrosis to the point that there is architectural distortion with the formation of regenerative nodules. This results in a decrease in hepatocellular mass, and thus function, and an alteration of blood flow[1]. Cirrhosis affects different organs through many mechanisms such as: impairment in nutrients absorption, increased endogenous opioids tone and alter in nitric oxide synthesis[2-4].

Opioids have been known as potent analgesics for a long time, and many studies have shown that opioids have a vast spectrum of non-analgesic effects on different tissues [5-7]. Opioid receptors were found to be distributed in the central nervous system, and later studies localized their expression in other organs[8,9]. The increase in endogenous opioids tone due to cirrhosis affects many organs through opioid receptors activation[5-7]. Recent studies have shown that opioid receptors activation in the retina causes some changes in the retinal morphology[10-14]. So we postulated that cirrhosis will probably lead to morphological changes in the retinal morphology through opioid receptors activation.

Despite the fact that opioid receptors are expressed in the retina, no pharmacological study has been conducted to investigate the impact of cirrhosis on the retina [15]. In this study we aimed to explore the cirrhosis-induced morphological changes in retina focusing on opioid receptors as the possible mediator.

MATERIALS AND METHODS

Animals and Procedures Thirty-six adult male Sprague-Dawley rats (230-250 g) were obtained from Pasteur

Table 1 The mean percent of retinal layers thicknesses in the six groups

Groups	Rod&Cons	Outer nuclear	Outer plexiform	Inner nuclear	Inner plexiform	Ganglion cell
BDL+NTX	16±1.56	30±0.88	4.3±0.37	15±0.57	29±0.83	4.3±0.51
Sham+NTX	18±0.77	31±1.41	4.0±0.45	16±0.39	27±0.66	4.1±0.42
Sham+Saline	19±0.36	29±0.76	4.4±0.67	15±0.65	28±1.77	4.5±0.35
BDL+Saline	18±0.45	28±0.75	4.2±1.48	15±0.55	27±0.90	7.8±0.50
Unop+Saline	17±1.36	31±0.80	4.1±0.59	16±0.45	28±0.77	4.2±0.53
Unop+NTX	18±0.38	30±0.86	4.3±0.87	15±0.78	28±1.82	4.3±0.32

Showing that retinal layers thicknesses were statistically similar in the six experimental groups except for the ganglion cell layer in the BDL+Saline group where a significant increase has been seen. BDL: Bile duct ligation; NTX: Naltrexone; Sham: Sham-operated; Unop: Unoperated.

Institute of Iran (Tehran, Iran). Rats were housed in temperature-controlled room on 12: 12h light-dark cycle and had free access to food and water. All animal procedures were performed in accordance with principles regarding the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

The rats were randomly divided into three main groups: 1) bile duct ligated (BDL) group in which the common bile duct had been double ligated and resected using Cameron and Oakley method[16]. 2) Sham-operated group (Sham) in which bile duct was manipulated but neither ligated nor resected. 3) Unoperated (Unop) control group in which the rats were intact. The bile duct ligation and sham procedure were performed under general anesthesia using a mixture of ketamine hydrochloride 50 mg/kg i.p (Sigma, Bristol, UK) and 10 mg/kg i.p xylazine (Sigma, Bristol, UK)[17].

Each of the three main groups were also divided into two subgroups: naltrexone group (NTX) in which rats received daily injection of naltrexone hydrochloride 20 mg/kg i.p (Sigma, Bristol, UK) for 28d beginning one day after bile duct ligation operation, and Saline group in which daily injection of sterile normal saline (Saline) was given for 28d [18]. Each of the six subgroups contained six rats.

Histological Evaluation After twenty-eight days, the right eye of all the rats was enucleated, then we assessed the histological changes as described in previous publications[12]. Rats were anesthetized then euthanized with 100 mg/kg intravenous pentobarbital sodium. After fixation in 10% formaldehyde solution the eyes were processed and embedded in paraffin. The tissue sections were stained with hematoxylin and eosin and evaluated by light microscope. Values were calculated based on average of measurements in four adjacent areas within 1 to 2 mm of the optic nerve in the inferior peripapillary region. In order to reduce the possibility of regional anatomic variation, the measurements were performed in the same topographic region of the retina. The thickness of the rod and cons layer, outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer and ganglion cell layer were measured for each eye in micrometers. Previous publication has shown that the retina thickness may be influenced by weight variation, and since the rats in this study didn't have the same exact weight, the percent of every layer was calculated using this proportion: the layer thickness in percent= (layer thickness/total retina thickness) ×100% [19]. The histologist who did the measurement was masked to the distribution of rats in the groups.

Statistical Analysis The results are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using SPSS version16. Homogeneity of variance of data was evaluated with the Levene's test and statistical evaluation of data was performed using two way analysis of variance (ANOVA) followed by Tukey post hoc test.

RESULTS

One day after laparotomy, BDL rats started revealing manifestations of cholestasis (jaundice, dark urine and steatorrhea). After 28d the alkaline phosphatase activity was significantly higher in BDL rats (338 U/I±5) compared with Sham (104 U/I±5; P<0.05) and Unop rats (98U/I±3; P<0.05).

We compared the five layers of retina (rod and cons layer, outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer and ganglion cell layer) in the different six groups with each other. The percent of the mean six layers thicknesses are shown regarding their groups in Table 1. We found that the percent of the mean ganglion cell layer thickness in the cirrhotic rats treated with saline (BDL+Saline group; mean=7.8±0.50; Figure 1) was significantly increased compared with the other groups(Figure 2; P<0.05). No other significant difference was detected between the six groups (P>0.05).

Administration of naltrexone did omit the cirrhosis effect on retina. Ganglion cell layer thickness in cirrhotic rats treated with naltrexone (BDL+NTX group; mean =4.3 ±0.51) was significantly less than BDL+Saline group (mean=7.8±0.50; P<0.05) , and as shown in (Figure 1) thickness of ganglion cell layer BDL+NTX group didn't have significant difference with sham and control groups (P >0.05). Naltrexone administration didn't induce changes in the ganglion cell layer in the Sham and Unop groups; the ganglion cell layer thickness in Sham+Saline group (mean =4.5 ±0.35) didn't change significantly after treatment with naltrexone (Sham+NTX group; mean=4.1±0.42; P>0.05) and the same

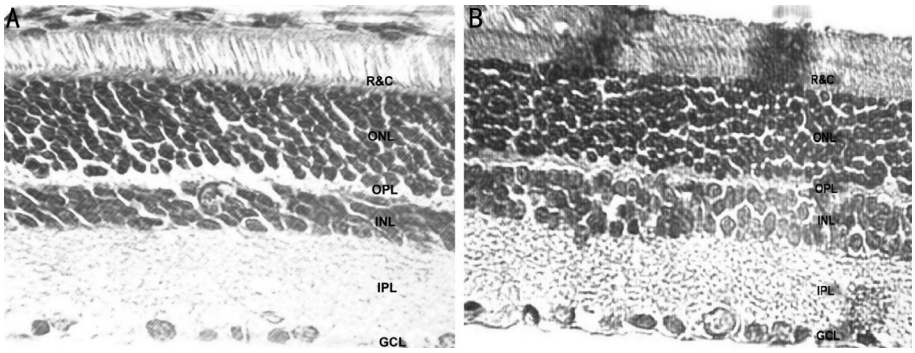

Figure 1 The histologic apperance of the different layers of retina A: Sham-operated rats; B: BDL rats after 28d of surgery. The thickness of ganglion cell layer in BDL (B) rats was significantly increased in comparison with Sham group (A) (P<0.05). While, the thickness of the rod and cons layer, outer nuclear layer, outer plexiform layer, inner nuclear layer, and inner plexiform layer, significantly did not change in BDL group (B) in comparison with sham operated group (A) after 28d of surgery (P>0.05). R&C: Rods and cones; ONL: Outer nuclear layer; OPL: Outer plexiform layer; INL: Inter nuclear layer; IPL: Interplexiform layer; GCL: Ganglion cell layer; Hematoxylin and eosin; original magnification, ×400.

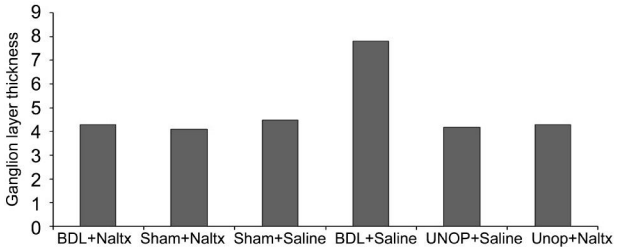

Figure 2 Effects of chronic administration of naltrexone on ganglion cell layers thickness of retina in sham-and BDL rats These groups were treated with saline, or naltrexone for 28d after surgery. The ganglion cell layer thickness in the BDL+saline group was significantly increased compared with Sham+saline group. While, treatment with naltrexone significantly decreased the ganglion cell layers thickness in comparison with BDL+saline group. Data are presented as mean±S.E.M. Each group consisted of 6 rats and differences in ganglion cell layers thickness were analyzed by repeated measures analysis of variance (ANOVA). BDL: Bile duct ligated; Naltx: Naltrexone; Sham: Sham-operated; UNOP: Unoperated.

result was seen in the Unop group (Figure 1) after naltrexone treatment; Unop group (mean=4.2±0.53) and Unop+NTX group (mean=4.3±0.32; P>0.05).

There was no significant change in layers of retina in naltrexone treated sham and Unop groups compared to non-treated Unop (Figure 1) and Sham groups (P>0.05).

Statistically the total thickness of the six retinal layers we measured was the same in the six experimental groups (P>0.05).

DISCUSSION

In the current study we detected that cirrhosis induced morphological changes in the retina of rats. We demonstrated that the thickness of ganglion cell layer significantly increased 28d after bile duct ligation, but there was no other change in thickness of the other layers which we assessed. We also identified that naltrexone (a non-selective opioid receptors antagonist) decreased the thickness of ganglion cell layer in BDL rats. While, daily injection of naltrexone did not change the thickness of ganglion cell layer in sham operated group. In this regard, we supposed that endogenous opioids as the possible candidate substance mediating the excess of the thickness of ganglion cell layer after bile duct ligation.

Many diseases and factors induce changes in retinal layer thicknesses [20,21]. Diabetes has been found to induce changes in retinal layer thicknesses mainly in ganglion cell layer, and in early stages in rod and cones layer [22]. Hypoxia and ischemia also were found to induce changes in the ganglion cell layer through different mechanisms[23].

Since the liver function is deeply compromised in cirrhosis, the metabolism of some substances is altered, causing changes in their serum levels such as: carbohydrate, estradiol, androgen and opioids which in turn lead to modification in their function [3,24-26]. Between these substances the increased tone of opioids in cirrhosis was widely studied and the cirrhosis effect on kidney, gonads and seizures, mediated through opioid receptors was shown in previous publications [5-7]. Early studies demonstrated that opioid receptors are expressed in the retina and specific types of opioid receptors were also detected in retina[15].

Opioids have been known to affect the ganglion cell layer activity [10]. Isayama *et al* [11] concluded that endogenous opioids serve as natural inhibitory factors that tonically regulate cell proliferation in developing rats. Other studies showed the protective role of exogenous morphine in the retina; by studying the changes in the retinal layer thickness, Riazi-Esfahani *et al* [12] found that opioid receptors activation by exogenous morphine protects the ganglion cell layer when ischemia is induced. Husain *et al* [13] found that exogenous morphine could protect the ganglion cell layer when intraocular pressure is increased and this protective role was not seen when opioid antagonist naltrexone was used. Peng

et al [14] found that δ-Opioid receptor-mediated activation of extracellular signal-regulated kinase triggers cellular events that correct the redox imbalance in the post-ischemic retina.

Exogenous opioids could preserve the retinal ganglion cells during stresses like ischemia and increased intraocular pressure through mechanisms that involved opioid receptors activation so it is possible to postulate that the morphological change in retinal ganglion cells in cirrhotic rats was due to the increase in the endogenous opioids tone during cirrhosis. In addition we found that the morphological changes was absent when cirrhotic rats were treated with naltrexone daily for 28d. These observations suggest that the retinal morphological change detected in cirrhotic rats is mediated at least partially through opioid receptors activation in retinal ganglion cells.

More studies should be carried out to detect the specific opioid receptors mediating changes in retina in cirrhosis and the impact of these changes on the eye function should be assessed using electroretingraphy.

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