Fas Receptor Activation by Endogenous Opioids Is A New Mechanism for Cardiomyopathy in Cirrhotic Rats

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Background: **Cirrhosis, a common consequence of chronic liver inflammation is associated with various cardiovascular dysfunctions which are called cirrhotic cardiomyopathy (CC). Among the various possible causes of CC, apoptosis is considered to have a pivotal role.** ***Objectives:*** **To explore the contribution of endogenous opioids in the apoptosis process in a rat model of CC.** ***Material and methods:*** **Four genes were selected to cover both intrinsic and extrinsic pathways of apoptosis. Cardiac samples from 4 groups of rats were evaluated. Two groups were cirrhotic through bile duct ligation (BDL) receiving either naltrexone (BDL-naltrexone) or saline (BDL-saline), two others were normal rats as sham groups receiving either naltrexone (sham-naltrexone) or saline (sham-saline). Expression level of BCL2, Caspase3, Fas and FasL was explored in all groups using reverse transcriptase real-time PCR.** ***Results:*** **BDL-saline group showed significant over-expression of BCL2, caspase3 and Fas. BCL2 expression was 1.44 ($P < 0.001$) and caspasse3 was 1.35 ($P < 0.001$) times higher than sham–saline group, Fas was also overexpressed 1.3 ($P < 0.001$) times higher than BDL-naltrexone group and 1.91 ($P < 0.001$) compared to sham-naltrexone group. Caspase3 expression was 1.35 ($P < 0.001$) folds higher than sham-naltrexone group. The expression pattern of FasL revealed no statistically significant change among study groups.** ***Conclusion:*** **Fas molecule enrollment during CC is a novel finding. Fas molecule is activated during cirrhosis through elevated levels of endogenous opioids. This pathway is one of the leading causes of CC. Our findings also demonstrated the protective role of naltrexone as opioids antagonist on cardiomyocytes in a rat model of CC. (J Clin Exp Hepatol 2017;7:107–114)**

Apoptosis, or programmed cell death is a type of cell death which is not modulated by inflammation and is triggered either by the death receptor (extrinsic pathway through Fas ligand) or mitochondria (intrinsic pathway). It is known that changes in apoptosis pathway are responsible for some important medical diseases including some lymphoma subtypes, cardiomyopathies and cirrhosis.[1] Cirrhosis is a common consequence of chronic liver inflammation characterized by replacement of liver tissue by fibrous scar and regenerative tissue, leading to a progressive loss of liver function.[2] Cardiovascular dysfunction is a major complication of cirrhosis which includes increased cardiac output, decreased arterial pressure and total peripheral resistance, systolic incompetence, diastolic dysfunction, and electrophysiological abnormalities in the absence of any known cardiac disease. These abnormalities are named cirrhotic cardiomyopathy (CC).[3–5] Abnormalities in β-adrenergic signaling pathway, altered cardiomyocyte membrane fluidity and several mediators such as nitric oxide (NO) and carbon monoxide are involved in the pathogenesis of CC.[3]

Recently, the contribution of endogenous opioids system in impaired cardiac contractility in CC is demonstrated. Additionally, inhibiting opioid system has pharmacologically corrected bradycardia and reduced chronotropic and inotropic response to both α and β-adrenergic agonists in cholestatic rats.[6,7] Moreover, endogenous opioids can induce apoptosis in hepatocytes during liver cholestasis, and reducing liver antioxidant defense system is hypothesized as the main mechanism through which increased level of opioid system leads to hepatocytes damage and apoptosis.[8] Previously we tried to evaluate the effect of opioids on cardiomyocyte apoptosis in a rat model of CC.[9] We found increased amount of apoptotic cells in

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Abbreviations: BDL: bile duct ligation; CC: cirrhotic cardiomyopathy; H&E: Hematoxylin and Eosin; MAPKs: mitogen-activated protein kinases; NO: nitric oxide; qRT-PCR: Real-Time Reverse Transcription PCR
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bile duct ligated rats by immunohistochemical method which was decreased using naltrexone as an opioid antagonist.

In order to confirm our previous findings, and to find the main apoptotic pathway (intrinsic versus extrinsic) involved in the process, this time we tried to evaluate the effect of endogenous opioids in molecular level. So, here we evaluated the nitrogen-kept samples of the same formalin-kept specimens of our previous study to evaluate the molecular pathways of apoptosis. We tried to identify the molecular pathways through which apoptosis was mediated by opioids in CC.

MATERIALS AND METHODS

Specimens

We previously[9] showed that opioid blockade using naltrexone can reduce apoptosis in CC. Briefly, we created a rat model of cirrhosis by ligating rats' bile ducts (BDL) for 4 weeks—during which cirrhosis took place according to various studies in literature.[10–12] We examined four groups of rats each containing six rats treated as following: BDL-naltrexone (group A): six rats which underwent bile duct ligation (BDL) and then treated by subcutaneous naltrexone injection for 29 ± 1 days. Sham-naltrexone (group B): six rats which treated by naltrexone as like as the first group without BDL. BDL-saline (group C): six rats which underwent BDL and treated by normal saline for the same time-period as group A. Sham-saline (group D): six rats treated by normal saline for 29 ± 1 days. After treatment, the rats were sacrificed and samples from various organs were obtained both in formalin solution and liquid nitrogen for further histologic and molecular studies, respectively. Formalin-kept ventricular samples were evaluated in our previous study. Presence of cirrhosis was documented using pathologic evaluation which showed marked fibrosis and nodule formation within liver samples of BDL rats (10), Figure 1. Cardiomyopathy was confirmed by evaluating left ventricular wall thickness which significantly decreased LV wall thickness was observed in BDL rats, Table 1. Pathologic evaluation to confirm cardiomyopathy was also performed using Hematoxylin and Eosin (H&E) and Masson's trichrome staining methods which showed attenuated, stretched and irregular cardiomyocytes in H&E staining and presence of focal fibrosis in Masson's trichrome staining which are evidences of cardiomyopathy,[13] Figure 2a and b. Finally, we found increased amount of apoptotic cells in bile duct ligated rats by immunohistochemical method. We also found that endogenous opioids blockade using naltrexone had decreased apoptosis density in BDL rats (Figures 3a and b and 4). In the present study the liquid nitrogen-kept samples from our previous study were examined for molecular analysis. We analyzed the expression levels of FASL, FAS (extrinsic apoptotic pathway genes), BCL2 (mitochondrial apoptotic pathway gene) and caspase3 (active executioner) by qRT-PCR in four above mentioned experimental groups, A, B, C and D. Expression level of these 4 genes was compared in the studied groups.

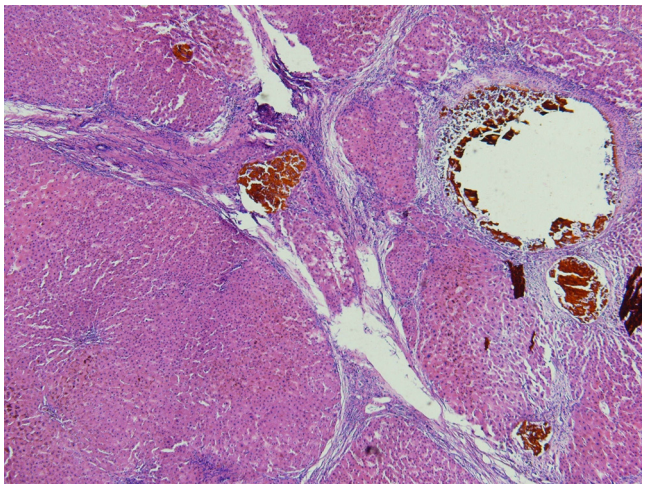

Figure 1 Showing fibrotic bundles and nodule formation with cholestasis in a cirrhotic liver of bile duct ligated rat.

RNA Extraction and cDNA Synthesis

Rat ventricular myocardium total RNA was extracted using TRIzol reagent (Invitrogen, Inc.) following the manufacturer's instructions. Quantity and quality assessments were determined by NanoDrop_ND-1000 UV–Vis

Table 1 Left Ventricular Thickness in Studied Groups.

Groups	Subgroups	Left ventricular thickness (mm)	*P* value
BDL	Naltrexone (group A)	2.75 ± 0.14	0.1
	Saline (group C)	2.26 ± 0.26	
Sham	Naltrexone (group B)	3.8 ± 0.2	0.3
	Saline (group D)	3.6 ± 0.1	
BDL	A + C	2.5 ± 0.16	<0.001
	B + D	3.75 ± 0.1	

BDL: bile duct ligation.
P value under 5% is considered as significant. The differences between groups A and B, A and D, C and B and C and D are also significant.

