Adrenomedullin Deficiency Increases the Susceptibility of Liver Fibrosis Induced by CCl₄

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Adrenomedullin (AM) is a peptide expressed in all body tissues, and its related receptors are increased in liver fibrosis. In this study, we evaluated the effect of AM deficiency on liver fibrogenesis induced by CCl₄ using AM heterozygous (HT) mice. The animals received a single injection of CCl₄ or olive oil for the acute experiment, and received CCl₄ or olive oil three times a week for 6 weeks for the chronic experiment. Fibrosis was assessed using histopathological analysis and the western blot. The AM HT mice showed mild pericentral lobular degeneration when compared to the AM wild type (WT) mice. In the acute experiment, there was no significant difference between the AM WT and AM HT mice. However, in the chronic experiment, the CCl₄-treated AM HT mice showed more severe liver fibrosis than that of the CCl₄-treated AM WT mice. The AST and ALT levels of the AM HT CCl₄ group were higher than those of the AM WT CCl₄ group. Additionally, the collagen deposition, α-SMA protein and TGF-β protein were increased in the AM HT CCl₄ group when compared to the AM WT CCl₄ group. The AM HT mice also exhibited severe lipid peroxidation through the GSH decrement. Taken together, our data suggest that AM deficiency increases the susceptibility to liver fibrosis induced by CCl₄ indicating a novel therapeutic target for patients with liver fibrosis.

Key words: Adrenomedullin, lipid peroxidation, liver fibrosis, TGF-β

Introduction

Liver fibrosis is a common manifestation of a variety of diseases such as alcohol-induced liver disease, viral hepatitis, drug toxicity, right heart failure, primary biliary cirrhosis and so on [9]. Liver is the principal site for CCl₄-induced effects such as severe oxidative stress in mitochondria resulting in apoptosis or cirrhosis and necrosis [6, 9]. There is no specific receptor for the action of CCl₄ in the liver, although CCl₄ inactivates cytochrome p450 2E1 (CYP2E1) which metabolizes various compounds such as xenobiotics. In addition, CCl₃OO⁻ radical results in lipid peroxidation and membrane damage [27]. When the liver is injured, quiescent hepatic stellate cells (HSCs) are stimulated by transforming growth factor-β (TGF-β) which is a key participant in the development of liver fibrosis [2], and activated with their subsequent transformation to myofibroblastic cells. The activated HSCs are characterized by overproduction of extracellular matrix proteins such as collagen [14, 17]. The relation between oxidative stress and collagen overproduction was first proposed in 1989 [4]. Hydroxyl radicals and peroxynitrite stimulate lipid peroxidation (LPO), which induce collagen expression and synthesis [21].

Adrenomedullin (AM) is a 54 amino acid peptide discovered in human pheochromocytoma extracts. It has been known to have several biological activities such as a circulating hormone and a vasodilator. AM is expressed in most tissues including peripheral blood monocytes and endothelial cells [7]. In fact, it has been thought to play an important role in the regulation of vascular tone and stability under physiological or pathological cellular conditions [8, 26], and to be closely involved in the formation of blood vessels, called angiogenesis [12, 22]. AM-induced angiogenesis is carried out through the activation of phosphatidylinositol-3 kinase (PI3K), mitogen-activated protein kinase (MAPK) and focal adhesion kinase (FAK) in endothelial cells [12]. In addition, AM reduces vascular permeability increased by reactive oxygen species, endotoxins or cytokines, thus limiting the formation of inflammatory exudates [18]. Moreover, AM...
is been known as a survival factor against hypoxia-induced cell death. It has been reported to protect epithelial cells from hypoxia/reoxygenation-induced cells by suppressing the generation of reactive oxygen species (ROS) via glutathione (GSH) production [11].

Here, we evaluated the effect of AM deficiency using AM heterozygous mice in a liver fibrosis animal model.

Materials and Methods

Animals and experimental design
AM heterozygous (AM+/−, AM HT) mice were used for this experiment because AM homozygous (AM−/−) is lethal within the uterus for unknown reason [11]. AM HT mice have approximately 50% of the serum and organ concentrations of AM compared to wild type (WT) mice [25]. To distinguish the genotypes of AM WT from AM HT type, the genomic DNA of mice was purified from the mouse-tail tissue and PCR was performed with the primers P1 (5′-GGCTCCTTAAGTTGCGCA-3′) and P2 (5′-ACGTAGAA-GAACTTATTAA-ACCGCA-3′) (Fig. 1E). The animals were kept in a room at 22±2°C and 50±10% relative humidity in a 12 hr light-dark cycle and fed standard laboratory chow and water, ad libitum. Chow was withheld from all mice 12 hr before being euthanized. Animal procedures were performed in accordance with the National Institutes of Health (NIH, Bethesda, USA) guidelines for the care and use of laboratory animals and approved by the Kyungpook National University Institutional Animal Care for the care and use of laboratory animals [Approval No.: KNU 2009-48].

Induction of acute and chronic liver fibrosis
For acute fibrosis, 15-week-old male AM WT mice (n=10) and AM HT mice (n=10) received a single injection of CCl4 (40% in olive oil, 1ml/kg). For chronic fibrosis, 7-week-old male AM WT mice (n=20) and AM HT mice (n=20) were injected with CCl4 (50% in olive oil) for 6 weeks, three times a week. The animals in the acute experiment were euthanized 24 hr after CCl4 injection.

Serum biochemical measurements
Serum ALT and AST concentrations were analyzed with the IFCC UV method (ADVIA, Bayer, USA), using commercial reagents, respectively.

Histopathology and immunohistochemistry
Hematoxylin and Eosin (H&E) staining and Masson’s Trichrome (MT) staining were done to detect collagen. The diameter of the portal veins varied depending on how it was cut; therefore, the minor axis was used to precisely measure the diameter. Image J software (National Institute of Health, Bethesda, MD, USA) was used to estimate the diameter and the coagulative centrilobular necrosis area. The area of the cross section of the portal veins was calculated mathematically: $\pi r^2$. The grade of portal fibrosis and perisinusoidal fibrosis was determined using blind assessment by four pathologists following the grading system of Mendier MH et al. [16]. The sections were immunostained

![Fig. 1. Histopathologic characteristics of AM transgenic mouse. (A) Histologic picture of control group mouse liver. (B) Histopathologic feature of the portal triad of AM WT control mouse and AM HT control. H&E stain. Original magnification: ×400. (C) The diameter of portal veins was measured in five fields (×200) per each five mice of AM HT and AM WT group. (D) The presumptive area of the portal vein is represented based on the result of (B) and the mathematical equation, $\pi r^2$. *p<0.05. (E) Genotyping of AM HT mouse.](image-url)
with anti-α-SMA antibody (Sigma, St Louis, MO, USA) and anti-CYP2E1 antibody (Chemicon International Inc., Temecula, CA, USA). The avidin biotin peroxidase complex method (Vector Laboratories, Burlingame, CA, USA) with 3, 3-diaminobenzidine (Zymed laboratories Inc., San Francisco, CA, USA) was used to visualize the antigen–antibody complex. For a negative control, the primary antibody was replaced by phosphate-buffered saline.

Terminal deoxynucleotidyl transferase-mediated nick-end labeling (TUNEL) assay

TUNEL assay was carried out to detect apoptotic cells using an in situ cell death detection kit (Roche, Indianapolis, IN). The cells were reacted with proteolytic enzyme (20 μg/ml of proteinase K) and rinsed with deionized distilled water (DDW). Then, the cells were reacted with a TdT mixture at 37°C for 1 hr, containing the following: 200 mmol/l potassium cacodylate; 25 mmol/l Tris-HCl (pH 6.5); 0.25 g/l bovine serum albumin; 1 mmol/l COCl₂; 5 mmol/l biotin-dUTP, and 100 U/ml TdT. After being rinsed with PBS, the cells were stained with an NBT/BCIP detection kit (Promega, WI, USA).

Immunoblotting

The concentration of liver protein was measured using the Bradford assay (Sigma). Equal amounts of proteins were separated by SDS-polyacrylamide gel electrophoresis. For immunoblotting, the proteins were electro-transferred to a PVDF membrane (Schleicher & Schuell, Dassel, Germany). Primary antibodies were applied to the membrane: anti-α-smooth muscle actin (α-SMA) antibody (Sigma), anti-CYP2E1 antibody (Chemicon International), anti-TGF-β1 antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA), anti-Glutathione peroxidase (GPx) antibody (Santa Cruz Biotechnology). Beta-tubulin was used as an internal standard protein. Blots were incubated with a horseradish peroxidase-conjugated anti-mouse and rabbit IgG (Santa Cruz Biotechnology). For detection, the Super Signal West Dura Extended Duration Substrate (Thermo Fisher Scientific Inc, Rockford, IL, USA) was used and exposed to Medical X-ray Film (Kodak, Rochester, NY, USA). The band intensities were quantified using the Image J software (NIH).

Detecting hepatic hydroxyproline (HYP) content

The same volume of liver tissue homogenates in 6N HCl was hydrolyzed at 110°C for 16 hr. The hydrolysate was filtered, and aliquots were evaporated under a vacuum. The sediment was dissolved in isopropanol and incubated in 0.84% chloramines-T in an acetate-citrate buffer (pH 6.0) for 10 minutes at room temperature. Subsequently, Ehrlich's reagent was added and the mixture was incubated at 60°C for 25 minutes. The absorbance of the sample solution was measured at a wavelength of 560 nm (Tecan Instruments, Salzburg, Austria). The HYP content was calculated from a standard curve of 4-hydroxy-L-proline (Sigma).

Quantitation of ROS in the liver

Lever tissue homogenates with arachidonic acid were incubated for 5 min. 2′, 7′-dichlorodihydrofluorescein diacetate (DCFDA) was added to the liver homogenates in a 50 mM phosphate buffer. Changes in fluorescence intensity were evaluated every 5 min for 30 min with excitation and emission wavelengths of 485 and 530 nm, respectively (Genius, Tecan Instruments).

Glutathione (GSH) and oxidized glutathione (GSSG) assays

The method of Pandey and Katiyar [19] was used for the GSH and GSSG assays. Liver tissues with meta-phosphoric acid were centrifuged, and the supernatant was used for the assay. For the GSH assay, o- phthalaldehyde was added and then 1 mM EDTA/50 mM phosphate buffer was added. The fluorescence was measured at an excitation and emission wavelength of 360 nm and 460 nm, respectively. For the GSSG assay, the supernatant with N-ethylmaleimide was pre-incubated for 20 min and 0.1 M NaOH was added. The fluorescence was measured the same way as GSH.

TBARS assay for Lipid peroxidation (LPO) assessment

The content of hepatic thiobarbituric acid-reactive substances (TBARS) was determined to assess LPO. The liver homogenates with 8.1% SDS, 20% acetic acid and 1.2% 2-thiobarbituric acid (TBA) were heated for 30 min at 95°C. After cooling, n-butanol was added and the mixture was centrifuged. Then, the supernatant was evaluated at a wavelength of 550 nm and expressed as nanomoles of malondialdehyde (MDA).

Statistical analysis

The results are expressed as the means ± S.D. To compare values obtained from the two groups, Student t test was
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Results

Histopathologic characteristics of AM HT mice

First, we investigated the histopathologic characteristics of the AM HT mice. The AM HT mice showed mild pericentrallobular hepatocellular hypertrophy compared to AM WT mice (Fig. 1A). Pericentrallobular hepatocytes were mildly hypertrophic and had a hypereosinophilic cytoplasm and enlarged nucleus (Fig. 1B), which is the characteristics of pericentrallobular degeneration. The diameter of the portal veins in the AM HT mice was approximately two thirds of the AM WT mice (Fig. 1C). Furthermore, the presumptive area of the portal vein from the AM WT mice was approximately twice as much as the AM HT mice (Fig. 1D).

Acute CCl4-induced liver injury

Next, we examined the effect of AM deficiency on acute CCl4-induced liver injury. As shown in Fig. 2A, wide-spread centrilobular necrosis and coagulative necrosis of hepatocytes occurred in the CCl4 treated WT and HT groups. The inflammatory cells had not been recruited yet at 24 hr after acute treatment (Fig. 2A). The liver necrosis area of both groups was 38% of the total liver area (Fig. 2A and 2B). CCl4-induced inactivation of CYP2E1 was examined by immunohistochemistry and immunoblot data. CYP2E1 expression decreased in both AM WT and AM HT treated with CCl4 (Fig. 2C and 2D). Thus, there was no significant change in the AM WT and AM HT mice after acute CCl4 treatment.

Chronic CCl4-induced liver injury

H&E stained liver showed abnormal hepatic architecture with bridging fibrosis between the central veins and periportal region, necrotic hepatocytes, and multifocally in-
Liver fibrosis was promoted in the AM HT mouse

Liver fibrosis was promoted in the AM HT mouse. Increased inflammatory cells in the groups (Fig. 3A). The WT group showed milder fibrosis than HT group with CCl4 treatment. Notably, the CCl4 treated AM HT mice show hepatic spongiosis from HSCs degeneration. The degenerative HSCs are characterized by anisocytosis and cyst-like structures that contain foamy granular eosinophilic materials in the cytoplasm (Fig. 3A, black arrows). The serum AST and ALT level were increased by chronic exposure to CCl4. The average level of AST and ALT of AM HT group was higher than that of AM WT group in CCl4 treated mice (Fig. 3B). TUNEL staining is negative on liver parenchyma in both groups, yet is positive on few HSCs in AM HT groups (Fig. 3C). Immunoblot data of CYP2E1 displayed a declined level in the CCl4 treated groups (Fig. 3D). Reduction of CYP2E1 expression in the CCl4 treated group was detected in both AM WT mice and AM HT mice. The relative protein level of CYP2E1 in the AM HT control mice was approximately twice as much as the AM WT control mice (Fig. 3D). Also, CYP2E1 protein level of AM HT CCl4 group was about 20% of that of AM HT control whereas AM WT CCl4 group showed about 50% of AM WT control.

Liver fibrosis was promoted in the AM HT mouse

MT staining shows collagen fibers as blue and smooth muscles as red. The accumulation of collagen in the AM HT CCl4 treated group occurred to a greater extent compared to the AM WT CCl4 treated group (Fig. 4A). The fibrosis grade was evaluated based on the MT stained histological data. For an objective estimate of fibrosis scoring, four pathologists graded liver fibrosis through a blind assessment. The control groups revealed no fibrotic changes. However, the CCl4 - treated AM WT group (grade 2.43 by portal fibrosis grading) showed fibrous expansion into most portal areas, with or without short fibrous septa, and (grade 0.8 by perisinusoidal fibrosis grading) represented perivenular and/or periportal involvement of some lobules. The CCl4 treated AM HT group exhibited severe fibrosis (grade 4.55 for portal fibrosis grading and grade 1.6 for perisinusoidal fibrosis grading) (Fig. 4B and 4C).

The number of α-SMA-immunopositive cells were increased in the CCl4 treated group around the central vein branches and portal area (Fig. 4D). Compared to the AM WT CCl4 group, the AM HT CCl4 group exhibited intensely stained α-SMA positive, and active HSCs were observed in the liver parenchyma. Additionally, α-SMA positive cells in the AM WT control and AM HT control mice were hardly detected in the liver by immunohistochemistry (Fig. 4D). In agreement with the immunohistochemistry data, the immunoblot data showed a significant increase in α-SMA protein expression in the CCl4 treated groups, especially in the AM HT CCl4 group. The protein level of α-SMA in the AM WT CCl4 mice increased approximately 5-fold compared to the AM WT control mice (Fig. 4E). And the AM HT CCl4 group showed about a 7-fold increase compared to the AM HT control (Fig 4E). The hepatic hydroxyproline (HYP) content in the CCl4 treated groups was significantly higher than
Fig. 4. Hepatic fibrosis was promoted in the AM HT mice. (A) Histopathologic pictures of Masson’s Trichrome staining. Collagen fibers shown as blue areas was more intensive in the CCl4 treated AM HT group compared to the CCl4 treated AM WT group. Original magnification: ×200. (B, C) Fibrosis grade. (D) Immunohistochemical analysis of α-SMA. CCl4 treated groups showed an increased level of α-SMA, which represents activated HSCs. Original magnification: ×200. (E) Immunoblot analysis for α-SMA. (F) The relative expression level of α-SMA in the immunoblots. The graph represents the relative band densities normalized with α-tubulin. (G) Relative HYP level was standardized in the AM WT control group. The HYP level of the CCl4 treated groups was similar in the AM WT and AM HT groups. Data is represented as the mean ± SD. *p<0.05, **p<0.01

Lipid peroxidation increased in the AM HT mouse

MDA and HAE, ROS, GPx, GSH, GSSG, and GSH/GSSG were examined to assess lipid peroxidation (LPO) in AM WT and AM HT mice. The MDA and HAE levels derived from the LPO process were increased in the livers of the CCl4 administered groups compared to the control groups. However, the AM HT CCl4 group displayed a dramatically and significantly higher level of MDA and HAE (Fig. 5A). In the AM WT group, a significant increase in ROS was triggered by CCl4 treatment. However, the AM HT group generated substantial amounts of ROS in both the control and CCl4 groups (Fig. 5B). The GPx protein level showed no dramatic changes induced by CCl4 treatment in the AM WT mice (Fig. 5C). However, the GPx protein level decreased because of the CCl4 treatment in the AM HT mice. In addition, the AM HT control mice showed a significant increase in the GPx protein level compared to the AM WT control mice. The GSSG level was augmented in the CCl4 treated groups, which showed a higher concentration in the AM HT CCl4 than in the AM WT CCl4 groups (Fig. 5D). The GSH
FIG. 5. Lipid peroxidation was increased in the AM HT mouse. (A) Lipid peroxidation level was investigated via malondialdehyde (MDA) and 4-hydroxyalkenals (HAE). (B) The ROS level was increased because of the CCl₄ treatment and AM deficiency. (C) Immunoblotting assay for GPx. GPx was decreased in the treated groups. The relative GPx expression normalized with α-tubulin (D) GSSG level increased after CCl₄ treatment. (E) GSH level increased from the CCl₄ treatment. (F) GSH/GSSG ratio was increased in the CCl₄ treated group; the ratio of the AM HT groups was almost half of that of the AM WT groups. Data are represented as the mean ± SD. *p<0.05, **p<0.01.

Discussion

This study investigated the effect of AM deficiency in liver fibrosis using AM heterozygous type (HT) mice. We demonstrated that (1) AM heterozygous mice have smaller vascular size and reduced angiogenesis in the liver compared to control mice; (2) AM HT mice show little differences from AM WT mice for acute CCl₄ treatment; (3) AM deficiency increases the accumulation of proteins related to fibrosis such as α-SMA, TGF-β and collagen and enhances lipid peroxidation by suppressing GSH activity, resulting in increased fibrosis in the liver.

As shown in Fig. 1A and 1B, small vascular formations suppressed angiogenesis in the liver were present. The reduced lumen diameter of vessels generally causes an increase in peripheral resistance, which is one of the key characteristics of hypertension [24]. Indeed, the AM HT CCl₄ treated mice showed less fibrosis when losartan, a drug to treat hypertension by antagonizing angiotensin II receptor, was applied (unpublished data). Additionally, AM is considered a hypoxia-regulated gene because AM is increased in hypoxic hepatocytes and protects organs from hypoxia and hypertensive injury from such as liver transplantation [5]. Therefore, the liver of the AM HT mouse could be assumed to be hypoxic with hypertension condition, susceptible to injury.

To elucidate the effect of AM on liver injury, we designed and performed two types of experiments: acute and chronic. AM WT and AM HT groups exhibited little differences in liver injury induced by a single injection of CCl₄ in the acute experiment (Fig. 2). AM would not have a critical effect on acute liver injury. However, the AM HT group showed vulnerability to liver injury in the chronic experiment (Fig. 3). Repeated CCl₄ injections triggered more severe biochemical changes (ALT and AST) in the AM HT mice. A substantial decrease in CYP2E1 expression was detected in the AM HT CCl₄ mice, which represents reduced capacity to metabolize chemical compounds and vulnerability to liver injury induced by CCl₄ in AM HT mice. Additional, TUNEL staining is negative on liver parenchyma and positive on a few HSCs. The HSCs located in perisinusoids is considered as non-parenchymal cells in liver. This confirms that the liver undergoes not apoptosis but necrosis by CCl₄ treatment.

As shown in Fig 4, liver fibrosis was exacerbated in the AM HT mouse. Histopathologic data as well as Western blot analysis confirmed the promoted liver fibrosis in the AM
However, the AM HT group did not show any fibrotic changes compared to the AM WT group. There was little collagen staining and almost a 0' fibrosis grade in the AM HT control group. In addition, the hydroxyproline level of this group was same as that of the AM WT control group. Thus, this TGF-β increase did not seem to be directly related to liver fibrosis. Recently, Masuoka et al. reported that TGF-β production was significantly increased under hypoxia condition in vitro, stimulating epithelial-mesenchymal transition (EMT) of gastric cancer. TGF-β induced EMT transformed gastric cancer cells into spindle-shaped cells which might be responsible for the initial invasion and metastasis of cancer [15]. Based on a previous report and our data, we assumed that mild hypoxia condition due to AM deficiency could trigger TGF-β production in the AM HT control mice. As we mentioned above, AM function is closely associated to hypoxia [5]. The AM HT mice had narrow vessels and a small number of vessels (Fig. 1). Thus, we hypothesized that the down-regulated AM protein forces the liver to a mild hypoxia condition, resulting in an increase of TGF-β production and fibroblast deposition. An elevated basal level of TGF-β could give the AM HT mice susceptibility to fibrosis. However, to evaluate our hypothesis, further study should be needed.

Because lipid peroxidation is considered as one of the hypoxia characteristics of AM deficient mice, we examined lipid peroxidation (LPO). MDA and HAE derived from the LPO process were significantly increased in the AM HT CCl₄ group. In addition, there was a slight increase in MDA and HAE in the AM WT CCl₄ group and the AM HT control group (Fig. 5A). Because the generation of free radicals induces the LPO process, we examined ROS production. The ROS level was greatly increased in the AM HT mice and the AM WT CCl₄ group. Previous reports have shown that AM plays a role as an antioxidant in two ways: suppressing ROS production [1, 3, 8] and promoting the ROS scavenging system [10]. Consequently, the increased ROS might be due to the down-regulated AM.

Next, the ROS scavenging system such as GPx, GSH, GSSG and GSH/GSSG was investigated. GSH is a key regulator of cellular redox and is involved in the maintenance of interior cellular redox balance in mammals [10, 23]. Surprisingly, the GSH/GSSG level of the AM HT mice was almost half the level of the AM WT mice due to a decrease in the GSH level (Fig. 5D, 5E, 5F). The expression of GPx in the HT control group was enhanced compared to the WT control group and was greatly decreased after CCl₄ treatment (Fig. 5I, 5J). An increased GPx expression in the AM HT control group may be a protective reaction from the enhanced ROS. The diminished GSH/GSSG as well as GPx increased hydrogen peroxide and thus induced hydroxyl radicals, which is a source of LPO. Moreover, hydrogen peroxide may also act as a vasodilator in many vascular beds under certain conditions [13, 20]. In summary, our findings suggest that AM can provide a novel therapeutic strategy in the development of medication for patients with liver fibrosis or chronic liver disease.

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초록: 아드레노메둘린 결핍은 사염화탄소로 유도된 간경화 감수성을 상승시킴

지애리1・황미열1・이은미1,2・이은주1,2・김아영1,2・서은영1,2・김상협1,2・박진규1・정규식1,2*
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아드레노메둘린은 모든 조직에서 발견되는 콜라이는 아드레노메둘린 수용체가 간경화 유발 시 증가한다. 이번 연구에서는 사염화탄소(CCH) 투여로 유도되는 간경화에서 아드레노메둘린의 효과를 아드레노메둘린 유전자 한 쪽이 결핍된 생쥐에서 관찰하였다. 사염화탄소를 일회투여 받은 급성 군과 6주 동안 지속적으로 투여 받은 만성군으로 나누어 효과를 관찰하였다. 간경화 정도는 병리조직적 검사와 웨스턴 블롯 방법을 사용하여 측정하였다. 급성에서는 결핍된 생쥐와 야생형 생쥐에서 별다른 차이점이 없었다. 만성의 경우 아드레노메둘린이 결핍된 생쥐에서 심각한 간경화가 관찰되었다. 아스파르테이트 아미노전이요소(AST)와 알라닌 아미노전이요소(ALT) 수치가 아드레노메둘린이 결핍된 생쥐 군에서 높게 관찰되었다. 간경화 마커 단백질인 콜라젠 알파-SMA와 TGF-beta가 아드레노메둘린이 결핍된 생쥐 군에서 높게 관찰되었다. 또한 아드레노메둘린 결핍 생쥐 군은 심각한 지질 과산화 반응이 확인되었고 이는 글루타시오 폐로시마이아게(GSH)의 감소가 원인이었다. 따라서 이 연구는 아드레노메둘린의 결핍은 사염화탄소로 유도된 간경화 감수성을 증가시키는 것으로 결론할 수 있다. 이로써 아드레노메둘린은 간경화 새로운 치료 타겟으로 사용될 수 있음을 나타낸다.