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PPARγ provides anti-inflammatory and protective effects in intrahepatic cholestasis of pregnancy through NF-κB pathway

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ABSTRACT

Aims: Intrahepatic cholestasis of pregnancy (ICP) is a pregnancy-specific hepatic disorder with potentially deleterious consequences of fetuses. Although the intimate relationship between ICP and peroxisome proliferator-activated receptor γ (PPARγ) has been previously reported in physiological and pathological conditions, the detailed mechanisms in the process of intrahepatic cholestasis of pregnancy has been unclear. The aims of this study are to assess the role of PPARγ regulating the reactive oxygen species (ROS) and inflammation in the process of the ICP.

Methods: Clinical data of the pregnant women were collected. And the serum of cytokines, hepatic function, the expression of PPARγ and NF-κB were measured. The rat and fetal rat ICP model were constructed and detection of the expression of PPARγ and NF-κB, evaluation the level of ROS and inflammation.

Results: The clinical data showed that the new-born information in severe ICP group were significantly different as compared to that in control group ($P < 0.05$), and part of information in mild ICP group were also difference to that in control group ($P < 0.05$). The expression of PPARγ and NF-κB were significantly higher in clinical pregnant women, rat, fetal rat ICP model groups and taurocholate acid (TCA) treated HTR-8/SVneo cell ($P < 0.01$). PPARγ inhibited the production of ROS and decreased the level of inflammation. PPARγ down-regulated the NF-κB pathway.

Conclusions: PPARγ provides the anti-inflammatory and protective effects in intrahepatic cholestasis of pregnancy through NF-κB pathway, which might be a probably one of the mechanisms of ICP.

1. Introduction

Intrahepatic cholestasis of pregnancy (ICP) is a pregnancy-specific hepatic disorder, characterized by severe pruritus and disturbed bile acid levels [1–3]. It can affect about 2% pregnant women [4]. ICP is benign to the mother, but is undesirable to fetus because it may increases the fetal mortality rate [5]. So the third trimester is crucial for the treatment of the women with ICP and their fetus. So the pharmacological treatment of ICP is very important, but unfortunately, researchers and clinicians have not discovered the etiology of ICP due to its complexity, and they have not identified an optimal therapeutic strategy. Many factors, such as environmental factors, hormonal changes, immune factors, and so on, have been found and demonstrated as the key factors in the pathophysiological processes of ICP [6]. So finding new and key molecular mechanisms of ICP is very important for development of new drugs to treat the ICP.

Peroxisome proliferator-activated receptors (PPARs) are transcription factors which are primary expressed in adipose [7]. PPARs participate in many biological processes, for example, regulating the adipogenesis [8]. There are three isoforms in the PPARs family, including PPARα, PPARβ, and PPARγ. PPARγ is expressed in some target tissues for insulin, such as skeletal muscle tissues [9], human peripheral blood mononuclear cells (PBMC) [10], and so on. And also, PPARγ plays an important role in regulating inflammatory processes. It can block the inflammatory effect through inhibiting the nuclear necrosis factor α pathway (NF-κB) [11].

The nuclear factor kappa B (NF-κB) pathway is very important and has been revealed be associated with many diseases, including immune and inflammatory processes [12]. For example, NF-κB can

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regulate the Th1 and Th2 immune responses [13]. It can be activated by many factors, such as proinflammatory cytokines. The inflammation has been demonstrated as an important regulators in the process of the cholestatic liver disease [14]. Bile acids (BA) could trigger inflammation in liver and induce various classes of inflammatory cytokines, including neutrophils, macrophages and lymphocytes [15].

In our previous studies, we collected and evaluated the expression of peroxisome proliferator-activated receptor γ (PPARγ) and nuclear factor kappa B (NF-κB) in placenta of patients and HTR-8/SVneo cell. And we found that PPARγ and NF-κB plays an important role in the process of ICP. So in order to in-depth evaluate the molecular mechanisms of the PPARγ and NF-κB in the process of ICP [16]. Firstly, we collected some ICP women and confirmed that PPARγ, NF-κB pathway, and inflammation are all activated. So the aim of this paper is to evaluate the expression of PPARγ and NF-κB in the placenta tissues of ICP women, rat, fetal rat ICP model, and cultured HTR-8/SVneo cell; to measure the maternal serum levels of proinflammatory cytokines and ROS; to further investigate the roles of PPARγ in regulating the NF-κB signaling pathway, which might provide new therapy targets for the treatment of ICP.

2. Materials and methods

2.1. HTR-8/SVneo cell culture treatments

The human placenta trophoblast cell line HTR-8/SVneo, an immortalized human trophoblast cell line established from first-trimester human cytotrophoblast cell and proved to be an important tool for the study of placental function, was a kind gift from Dr Charles H. Graham (Queen's University, Ontario, Canada). The HEK293T cell was obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China).

The HTR-8/SVneo was cultured in RPMI 1640 media and the HEK293T was grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS (fetal bovine serum), 100 U/ml penicillin and 100 μg/ml streptomycin under a humidified 5% CO_2/95% air atmosphere at 37 °C.

2.2. Patient participation and tissue collection

Placental tissues were obtained from women who were hospitalized in department of gynecology and obstetrics of Wuxi maternal and child health hospital, the affiliated hospital of Nanjing medical university. Written consent was received from women after full explanation of the purpose, nature and risk of all procedures used before surgery. The hospital ethic committee approved the consent forms and the protocols to utilize the tissue. One hundred consecutive pregnant women with ICP were enrolled in the study. The severity of ICP was based on the occurrence of fetal complications and the results of laboratory tests. A control group comprised 61 consecutive healthy women with physiological pregnancies. All subjects were nulliparous Chinese women with singleton pregnancy. The women with ICP attending the department of obstetrics and gynecology between 1 January 2014 and 31 December 2015, the diagnosis of ICP was based on the following criteria: (1) the presence of pruritus, predominantly located on hands and feet, that resolved within hours or days after delivery; (2) the abnormalities in liver-function tests suggestive of ICP, such as serum levels of ALT and AST greater than 40 IU/L, respectively; (3) elevated levels of fasting total serum bile acid (SBA) 0>10 μmol/L; and (4) no skin lesions caused by systemic diseases that could lead to pruritus. All the patients were referred during pregnancy and were confirmed for the absence of infection by hepatitis viruses (HAV, HBV, and HCV). The exclusion criteria included autoimmune diseases, moderate to severe alcohol intake, biliary obstruction, and the use of drugs or alternative medicine therapy known to precipitate cholestasis. Information was provided by the same doctor to all patients on enrolment. Data on occurrence of cholelithiasis or other liver diseases were recorded according to information provided by the patients [16].

Tissues was dissected and repeatedly rinsed until free from blood in chilled (4 °C) PBS, dried with clean gauze, placed in dry vials, treated with diethylpyrocarbonate (DEPC) and snap-frozen in liquid nitrogen and stored at −280 °C for RNA or protein extraction.

2.3. Blood samples collection

The peripheral blood was collected and then was centrifuged within 2 h (4 °C, 35,000 r/min, 10 min), and the supernatant was transferred to a sterile 1.5 ml centrifuge tube. All plasma samples were aliquoted and stored at −80 °C until further use for IL-4, IL-6, IL-12, TNF-α, TBA, TBIL, DBIL, CG, ALT, AST, ALP, TCH, TG, HDL-C and LDL-C measurement by routine automated techniques [16].

2.4. Inflammatory cytokine assay

The maternal serum levels of cytokines IL-4, IL-6, IL-12 and TNF-α were measured by ELISA according to manufacturer's instructions as described previously [14,16].

2.5. RNA extraction and RT-PCR

According to the manufacturer's instruction, total RNA was extracted from HTR-8/SVneo cells and placentas from patients with Trizol. Then total RNA (5 μg) was used for the RT reaction and then performed using specific PCR primers. The PCR products were separated on 1.5% agarose gel and quantitated by densitometry using an Image Master VDS system and the associated software (Applied Biosystems, USA).

2.6. Protein extraction and western blotting

Protein was extracted from HTR-8/SVneo cells and placentas from patients using the commercial kit in accordance with the manufacture's instruction, and the protein concentration was determined by a bicinchoninic acid (BCA) kit. Western blotting assay was performed against specific antibodies as described previously [16].

2.7. Immunohistochemistry

Placental tissue samples were fixed in buffered formalin for 24 h at 4 °C, dehydrated in 70% ethanol, paraffin embedded, and sectioned. Serial sections were then incubated and analyzed against specific antibodies. The sections were examined by conventional light microscopy as described previously [16].

2.8. Luciferase assay

The p−NF−κB luciferase was transiently transfected into HEK293T cells as described previously [17]. The luciferase activity evaluation method was performed using a luciferase assay system (Promega) as described previously [17].

2.9. Statistical analysis

Unpaired *t*-test for comparisons between groups (Data are expressed as mean ± SD). X^2-test was used to evaluate the comparisons of the rates. The statistical analysis was conducted using SPSS 13.0, $p < 0.05$ was considered statistically significant.

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