EXPERIMENTAL RESEARCH

Effect of Emodin in Suppressing Acute Rejection Following Liver Allograft Transplantation in Rats

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ABSTRACT Objective: To investigate the mechanism of action of emodin for suppressing acute allograft rejection in a rat model of liver transplantation. Methods: Brown Norway (BW) recipient rats of orthotopic liver transplantation (OLT) were divided into three groups, Group A receiving isografting (with BW rats as donor), Group B receiving allografting (with Lewis rats as donor), Group C receiving allografting and emodin treatment (50 mg/kg daily). They were sacrificed on day 7 of post-transplantation, and their hepatic histology, plasma cytokine levels, and T-cell subset expression were detected. Results: Compared with those in Group A, rats in Group B exhibited severe allograft rejection with a rejection activity index (RAI) of 7.67 ± 0.98, extensive hepatocellular apoptosis with an apoptosis index (AI) of 35.83 ± 2.32, and elevated plasma levels of interleukin-2 (IL-2), interleukin-10 (IL-10), tumor necrosis factor-α (TNF-α), CD4+ and CD4+CD8- ratio. However, recipients in Group C showed a decrease in histological grade of rejection and hepatocellular apoptosis, as well as a decrease in plasma levels of IL-2, TNF-α, CD4+ and CD4+CD8+ ratio, but elevated levels of IL-10 as compared with the allograft group. Conclusion: Post-OLT acute rejection could be attenuated by emodin, its mechanism of action may be associated with protecting hepatocytes from apoptosis, polarizing the Th1 paradigm to Th2, and inhibiting the proliferation of CD4+ T cell in plasma.

KEYWORDS emodin, liver transplantation, acute allograft rejection, apoptosis, immuno-suppression

Liver transplantation is currently the choice for treatment of acute or chronic liver diseases(1). Advances in immunosuppressive therapy have played a major role in the success of liver transplantation(2). However, liver allograft rejection still occurs in 30%-60% of transplanted patients, 5%-10% of whom eventually lead to liver dysfunction, representing 10%-20% of patients who die of liver transplantation(3). The liver is a vital organ for drug metabolism and its acute graft rejection after transplantation is distinct from other organs, which necessitates the administration of immunosuppressive agents with high efficiency and low toxicity.

A variety of studies have revealed that several kinds of Chinese herbal extracts exhibit immunosuppressive activities(4-6). Our previous study also demonstrated that emodin, as an immunosuppressive agent, has a potential inhibitory effect on acute allograft rejection following liver transplantation in rats(7). The aim of this study was to investigate the mechanism of the emodin-induced immunosuppressive effect, providing a theoretical basis for developing a novel immunosuppressive agent in Chinese medicine.

METHODS

Animals

Inbred male Lewis (LEW) and Brown Norway (BN) rats weighing 230 to 260 g served as donors and recipients for an acute allograft rejection model, respectively. All animals were purchased from the Beijing Experimental Animal Center (Beijing, China) and housed in the Experimental Animal Center, Wenzhou Medical College. They were maintained in a temperature- and humidity-controlled environment with a 12-hour light-dark cycle and had free access to water and standard rat chow. All the operations were performed under sterile conditions. All animal experiments were carried out according to the regulations of the Institutional Animal Care Committee.
out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, with the approval of the Scientific Investigation Board of Wenzhou Medical College.

Reagents
Emodin powder (purity, 98%) was purchased from Xi’an Huachui Co. (Xi’an, China). The TUNEL apoptosis detection kit was obtained from Maixin Biological Company (Fuzhou, China). The other reagents were purchased from Sigma (St. Louis, MO) except those stated otherwise.

Establishment of Transplantation Model and Grouping
Both donors and recipients were anesthetized with an intraperitoneal injection of sodium pentobarbital (30-50 mg/kg). Orthotopic liver transplantation (OLT) in rats was performed according to Kamada’s two-cuff technique, as modified by Tsuchimoto, et al. The recipients whose survival time was more than 3 days were used for the next experiments.

Forty-eight recipients were divided into three groups equally: Group A received isografts with donor livers from BN rats, Group B received allografts with donor livers from LEW rats, the two groups were treated with saline, Group C received allografts but were treated with emodin in a dose of 50 mg/kg per day. Emodin or normal saline was injected intraperitoneally into the recipients once a day from day 1 to day 5 after grafting.

Items and Methods of Observation
Histopathologic Changes
On the seventh day after transplantation, six rats in each group selected randomly were sacrificed. Their liver tissues were harvested and fixed in 10% formalin at 4 °C overnight, then embedded in paraffin and cut into 4-μm sections. Hematoxylin-eosin (HE) staining, rejection activity index (RAI) measurement based on Banff schema, and hepatocellular apoptosis with the use of TUNEL to determine the apoptosis index (AI) were used for morphological assessment. The severity of acute rejection (AR) was defined as nondeterministic AR when RAI scores were< 3; as mild AR when 3<RAI ≤5; as moderate AR with 5<RAI≤7 and as severe AR when RAI score were within 7-9.

All specimens were reviewed by a pathologist who was blinded to the treatment groups.

Enzyme-Linked Immunosorbent Assay
A total of 200 μL serums for each rat was collected from the tail-vein on the postoperative days 1, 3, 5, and 7. Serum levels of tumor necrosis factor-α (TNF-α), interleukin (IL)-2 and IL-10 were assayed by two-site sandwich enzyme-linked immunosorbent assay (ELISA). All reagents, samples, and standards were prepared according to the manufacturer's instructions. Diluted standards and samples (100 μL) were added into the appropriate wells for incubation (covered) at 37 °C for 120 min. Serum levels were determined by incubation with biotinylated anti-TNF-α/IL-2/IL-10 (0.1 ml/well) at 37 °C for 60 min. The coating solution was removed and the plates were washed three times with PBS-T (0.01 mol/L) before incubation with avidin-biotin complex (0.1 ml/well) at 37 °C for 60 min. Plates were washed again before adding tetramethylbenzidine microwell peroxidase substrate. All the measurements were evaluated on a plate reader at 450 nm.

Analysis of CD4+ and CD8+ T-lymphocyte Subsets in Plasma
Blood samples (250 μL) were collected via the portal vein 7 days after transplantation. Red blood cells were depleted using lysis buffer, and leukocytes (2 × 10^7/50 μL) were incubated with FITC-conjugated anti-CD3 mAb, PE-conjugated anti-CD4 mAb, and PE-cyanine (Cy)5-conjugated anti-CD8 mAb for 30 min on ice (all mAbs were from BD Pharmingen, San Diego, Calif., USA). Incubation with primary mAbs was followed by Cy-Chrome-streptavidin (BD Pharmingen).

Cells were fixed in 2% paraformaldehyde and analyzed using a BD FACScan (BD Pharmingen). Fluorochrome-conjugated species- and isotype-matched irrelevant mAbs were used as negative controls.

Statistical Analysis
The data were analyzed statistically using the software SPSS, version 11.0 (SPSS Inc, Chicago, IL). The parametric data are presented as mean ± standard deviation, and statistical significance was determined using ANOVA with subsequent Student-Newman-Keuls test. P value less than 0.05 was considered statistically significant.