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Research Paper

Effects of emodin on the proliferation of the glomerular mesangial cell and correlative cytokines in rats

Xili Wu[a,*], Wanggang Zhang[b], Wansen Sun[a], Chenglin Qiao[a]

[a]*Department of Integrated Chinese Traditional and Western Medicine, the Second Affiliated Hospital of Xi' an Jiaotong University, Xi' an 710004, Shanxi Province, China*

[b]*Department of Hematology, the Second Affiliated Hospital of Xi' an Jiaotong University, Xi' an 710004, Shanxi Province, China*

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Abstract

Objective: To investigate the effects of emodin(EMD) on cell proliferation and correlative cytokines secretion of glomerular mesangial in rats. **Methods:**The effects of EMD on cell proliferation and IL-6 , TGF- β 1 secretion of glomerular mesangial in rats were observed. Cell proliferation was measured by MTT method. IL-6 and TGF- β 1 secretion was detected with ELISA. **Results:**EMD was able to inhibit the cell proliferation and down-regulate the IL-6 and TGF- β 1 secretion of glomerular mesangial, as compared to the model group in rats ($P < 0.05$). **Conclusion:**EMD could significantly inhibit the cell proliferation, and reduce the creation of extracellular matrix(ECM), this indicated that it could play an important role in alleviation and prevention of glomerular sclerosis. The mechanism may be that EMD can reduce the IL-6 and TGF- β 1 secretion of glomerular mesangial cell in rats.

Keywords: emodin; glomerular mesangial cell; mesangial cell proliferation; correlative cytokines

INTRODUCTION

Emodin(EMD), the most extensive uni-anthracene core group 1,8-ioxy-anthraquinone in anthracene ramification, is the main active constituent of traditional Chinese medicine, naturally occurring in plants, especially Polygonaceae, Giant Knotweed Rhizome, Rhubarb, Polygonum multiflorum, and so on[1]. Accordingly, as a quality control(QC) index of Chinese patent medicine, EMD may play an important role in application of quality criteria and production methods. For the past few years, it has been indicated that EMD possess many kinds of pharmacologic actions[2-4], for example, inhibiting tumor, anti-virus, anti-oxidation, immune suppression and so on. Some investigations consider that EMD has the effect of decreasing plasminogen activator inhibitor-1(PAI-1) activity which is excreted by human embryo kidney fibroblasts(KFB)[5], increasing degradation of extracellular matrix (ECM)[6-7] and inhibiting tumor necrosis factor in chronic renal failure(CRF)[8]. This study investigated the effects of EMD on the proliferation of the glomerular mesangial cell and correlative cytokines: interleukin-6(IL-6) and transforming growth factor-β1(TGF-β1) in rats, to further approach the mechanism of EMD on treating chronic re-nal glomerular disease.

MATERIALS AND METHODS

Reagents and experiment medication

Parenzyme, type Ⅳ collagenase, Hepes(production of Sigma), RPMI-1640(production of GIBCO, to contain Gln-glutamine 2 mmol/L), fetal calf serum (FCS, production of biological engineering institute of Hangzhou Sijiqing), ECM (preparation institute of Ruijin hospital).

Apparatus

Inverted phase contrast microscope (ECLIPSE TE type 300, Japanese Nikon company), CO_2 constant tem-

*Corresponding author.
E-mail address: wuxili1984@163.com

perature incubator(TC type 2323, American Shelolon Manufacture company), enzyme scale meter (Austria TECAN company Spectar Ⅲ).

Mesangial cell(MC) cultivation

12 healthy purebred male SD rats (180±20 g), were sacrificed. After bloodletting, the kidneys were dislodged under an asepsis conditions, the amicula were removed, the cortical were separated, and substance were punctuated. The cortical substance were minced, and then sieved through 80, 100, 150 stainless steel screen mesh. The constituents were put under a 200 screen mesh grit, and the glomerulum were collected from the grit. This was digested with 0.1% collagenase, then inoculated into the culture flask pretreated by collagen, and incubated at 37℃ in CO_2. The culture fluid consisted of RPMI-1640 containing 10% inactivated calf serum. The culture fluid was changed when glomcrulus adhered after 3-4days (at about 80%). 20 days later there were cells on the bottom of the culture flask. Those cells were then passaged with 0.125% trypsinization, and 5~6 generation cells were used in the experiment.

Grouping and administration

The fifth generation MC were cultured, with 20% FCS RPMI-1640, and subcultivated with 96 shadow masks, with the density of 1×10^5/ml, 200 μl per hole. After being cultivated for 24 hours, the culture fluid was exchanged with 0.5% FCS RPMI-1640, and went on cultivating for a further 24 hours, (achieving cells synchrony at the G0 stage). Grouping: ① control group; ② model group:10% FCS; ③ EMD group:10% FCS and EMD 80 μg; Involved the setting up of 6 ambiholes to every group, adjusting every hole cells concentration to 1×10^5/ml by means of FCS, proliferation of MC at 24 h and 48 h was detected.

Test of MC proliferation by MTT

Grouping and handling were the same as above, 96 shadow masks which grown MC were added with MTT (5 mg/ml), 20 μl per hole, these continued to be cultivated at 37℃ in 5% CO_2 for 4 hours, and then the supernatant fluid was discarded carefully. Every hole was added 150 μl DMSO, agitating for 10 minutes, dissolving the crystals thoroughly. Then the value of OD was observed in the enzyme scale meter, at the wavelength of 490 nm.

Test of IL-6 and TGF- β 1

Grouping and handling were the same as above, the contents of IL-6 and TGF- β 1 were detected with ELISA method, specific operation followed the instruction of the kits(kits come from American Genzyme company).

Statistical analysis

All the data, formulated as mean ± standard deviation ($\bar{x} \pm s$), were processed with the statistics software package SPSS 10.0. *t*-test was used to compare data among groups before and after the treatment, $P < 0.05$ was considered as statistical significant difference.

RESULTS

Effects of EMD on the proliferation of the glomerular MC in rats

Compared with model group, treatment group inhibited the proliferation of the glomerular MC in rats significantly($P < 0.01$) (***Tab 1***).

Tab 1 **Effects of EMD on the proliferation of the glomerular MC in rats** ($\bar{x} \pm s$)

Groups	*n*	24h value of OD	48h value of OD
Control group	6	0.2701 ± 0.0305	0.2989 ± 0.0299**
Model group	6	0.3012 ± 0.0312	0.3572 ± 0.0184
Treatment group	6	0.2531 ± 0.0105**	0.2768 ± 0.0413**

compare with model group, **$P < 0.01$

Effects of EMD on the IL-6 secretion of glomerular MC in rats

Compared with model group, treatment group inhibited the IL-6 secretion of glomerular mesangial cell in rats significantly ($P < 0.01$) (***Tab 2***)

Tab 2 **Effects of EMD on the IL-6 secretion of glomerular mesangialcell in rats** (pg/ml, $\bar{x} \pm s$)

Groups	*n*	24 h	48 h
Control group	6	35.5 ± 9.52**	39.7 ± 11.43**
Model group	6	121.9 ± 33.23	130.1 ± 31.25
Treatment group	6	58.9 ± 10.98**	61.7 ± 11.33**

compare with model group, **$P < 0.01$

Effects of EMD on the TGF- β 1 secretion of glomerular MC in rats

Compared with model group, treatment group inhibited the TGF- β 1 secretion of glomerular mesangial cell in rats significantly($P < 0.05$, $P < 0.01$)(***Tab 3***).

Tab 3 **Effects of EMD on the TGF- β 1 secretion of glomerular MC in rats**

(pg/ml, $\bar{x} \pm s$)

Groups	*n*	24 h	48 h
Control group	6	844.72 ± 30.28	836.24 ± 30.35
Model group	6	855.74 ± 43.09	840.73 ± 32.23
Treatment group	6	763.38 ± 23.54*	720.82 ± 24.27**

compare with model group, *$P < 0.05$, **$P < 0.01$

DISCUSSION

In chronic renal glomerular disease, mesangial cell proliferation and ECM abnormal multiplication may play an important role in occurrence and development of glomerular sclerosis. Many studies[9] have already

confirmed that proinflammatory cytokines play a key role in the occurrence and development of glomerular sclerosis[10].Interleukin-1(IL-1)was the first cytokine confirmed autocrine by MC. It not only can make MC proliferate, induce MC and endothelial cells to excrete collagen and ECM, but also can strengthen the effect of MC on gene expression and secretion of IL-6, TGF-β and other polypeptide growth factors[11].TGF-β1 is an important cytokine which can induce glomerular sclerosis and interstitial fibrosis[12-14].It was confirmed[15]that TGF-β1 could regulate platelet-derived growth factor (PDGF), endothelin-1(ET-1), IL-1 and many other cytokines. Under the stimulatory function of PDGF and IL-1, the effect of MC on the synthesis and secretion of TGF-β1 can be strengthened[16-18]. Tumor necrosis factor(TNF) which can cause glomerular nephritis, and stimulate MC to contract and proliferate[19-20], generate IL-1, IL-6, PDGF and other polypeptide growth factors[21]. Thus, it is clear that there are complicated biological effects of linkage and coordination, magnified among proinflammatory cytokines, kidney cells, and cytokines. This causes MC proliferation endlessly, renal glomerular progressive sclerosis[22] and lastly renal failure. Therefore, it is important that to control MC proliferation and reduce ECM gathering for striving renal glomerular affection, to reverse and prevent it to develop glomerular sclerosis chronically.

This study confirmed that EMD could significantly inhibit the cell proliferation, and reduce the IL-6 and TGF-β1 secretion of glomerular mesangial cell in rats ($P<0.05$ or $P<0.01$). It is clear that MC is one of the main target cells on therapeutical effect of EMD. One of the main mechanisms that may be related is that EMD can lower the IL-6 and TGF-β1 secretion of glomerular mesangial, inhibit the cell proliferation and reduce ECM gathering in rats. It still remains to be studied whether there are other contribution channels. This experiment not only offered a theoretical basis for clinical application of the EMD, but also expanded therapic method of proliferative glomerulonephritis.

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