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Effect of Immunosuppressive Drugs on the Metalloproteinase in the Glioma Cells and Osteoblasts

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Abstract

The matrix metalloproteinases (MMPs) play a key role in the normal physiology of connective tissue during development, morphogenesis, and wound healing. Dysregulation of their activity has been implicated in numerous diseases including encephalopathy and the process of bone loss. Thus, MMPs may play a role in the encephalopathy and post-transplantation bone disease by immunosuppressive drugs such as cyclosporine (CsA) and tacrolimus. Gelatin zymography of MMP-9 and MMP-2 was performed in the glioma cells and osteoblast after CsA or tacrolimus treatment. Glioma cells or rat osteoblast ROS17/2.8 cells were treated with CsA or tacrolimus to make final concentration from 2 to 250 μM. After incubation, gelatin zymography of MMP-9 and MMP-2 was performed. And the density for the MMP bands were measured using luminescent image analyzer system. Both MMP-9 and MMP-2 activities in the osteoblast cells were decreased depending on the concentration of CsA or tacrolimus. MMP-2 activity was increased after CsA or tacrolimus treatment in the glioma cells. However, MMP-9 activities were decreased after CsA or tacrolimus treatment in the glioma cells. These results indicate that dysregulation of MMPs in the osteoblast and in the glioma cells by immunosuppressive drugs may one of the contributing factors in post-transplantation bone disease and in the encephalopathy by tacrolimus or cyclosporine.

Key Words : Cyclosporine, MMP-2, MMP-9, Tacrolimus

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Introduction

Immunosuppressive drugs are widely used in the therapy of organ rejection in post-transplantation patients, and in the treatment of several autoimmune diseases [1-3]. Among the immunosuppressive drugs, cyclosporineA (CsA) and tacrolimus, are possess numerous side effects including post-transplantation bone disease, most notably, osteoporosis and osteopenia [4-7]. Despite the considerable attention to the CsA and tacrolimus, the mechanism by which CsA and tacrolimus causes bone loss remains unclear. And after the organ transplantation, some patients suffer from mild neurological symptoms such as tremor to severe complications including seizures and encephalopathy [8]. However, the mechanism by which CsA and tacrolimus causes encephalopathy remains unclear.

The matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases which belong to the metzincin family. They degrade extracellular matrix macromolecules in cell environment and therefore play a key role in the normal physiology of connective tissue during development, morphogenesis, and wound healing [9-11]. The dysregulation of their activity has been implicated in numerous diseases including arthritis, cancer development, tumor metastasis, and atherosclerosis [12,13]. Increased activity of MMP-9 is responsible for the decreased proliferation and the reduction of the number of cells synthesizing new osteoid matrix and thus contributing to the process of bone loss [5]. And MMPs are also known to play in the function of the blood-brain-barrier, in the acute encephalopathy following prolonged febrile seizures with neurological sequelae, and in the febrile seizure or encephalopathy in influenza virus infection [10,11].

To investigate the possible mechanism of the post-transplantation bone disease and encephalopathy by immunosuppressive drugs such as CsA and tacrolimus, the effects on the metalloproteinase in osteoblastic ROS 17/2.8 cells and in the glioma cells after CsA or tacrolimus treatment was studied.

Materials and Methods

1. Osteoblast culture

Rat osteoblast ROS17/2.8 cells were cultured in Dulbecco's modified Eagle's medium (GibcoBRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Hyclone; Logan, UT) and 100 μg/ml penicillin-streptomycin (100 U/ml) (GibcoBRL; Grand Island, NY, USA). Cells were grown in 10-cm diameter culture plates in a humidified atmosphere of CO_2/air (5%/95%) at 37℃. Cells were treated with CsA (Novartis Pharma Stein AG; Stein, Switzerland)at different concentrations for 24h after plating of cells.

2. Glioma cell culture

Rat glioma cells (Korean Cell Line Bank; Seoul, Korea) were cultured in Dulbecco's modified Eagle's medium (WelGENE; Daegu, Korea) supplemented with 10% fetal bovine serum (WelGENE; Daegu, Korea) and 1% penicillin-streptomycin (HyClone; Logan, UT, USA). Cells were grown in 10-cm diameter culture plates in a humidified atmosphere of CO_2/air (5%/95%) at 37℃.

3. Treatment of CsA or tacrolimus

Rat osteoblast ROS17/2.8 cells or glioma cells were seeded in 6-well plate at a density of 2×10^5

cells/well, and then were treated with CsA or tacrolimus (Astellas Pharma, Ireland, Co. Kerry, Ireland) to make final concentration from 2 to 250 uM, and incubated for 24 hours.

4. Gelatin zymography

Gelatin zymography of MMP-9 and MMP-2 was performed as follows; samples were subjected to electrophoresis on 7.5% SDS-PAGE co-polymerized with gelatin (0.2%) as the substrate. After electrophoresis was complete, the gel was incubated at 37℃ for 24 hour in 1 M Tris-HCl buffer, pH 7.6, containing 1M $CaCl_2$. The gels were stained with 0.5% Coomassie Brilliant BlueG-250, and destained with 10% acetic acid. Gelatinolyitc activities were detected as unstained bands against the background of Coomassie blue stained gelatin. The density for the MMP bands was measured using luminescent image analyzer system (LAS-3000, Fuji Film, Tokyo, Japan).

Results

The density for MMP-9 and MMP-2 bands after gelatin zymography in osteoblast was expressed in table 1. Both MMP-9 and MMP-2 activities were decreased depending on the concentration of CsA or tacrolimus.

The density for MMP-9 and MMP-2 bands after gelatin zymography in glioma cells was expressed in table 2. MMP-2 activity was increased after CsA or tacrolimus treatment. However, MMP-9 activities were decreased after CsA or tacrolimus treatment.

Discussion

Type IV collagenases (gelatinases) are members of the family of MMPs and are thought to play an important role in degradation of extracellular components. The gelatinase subclass can be divided into gelatinase-A (MMP-2) and gelatinase-B (MMP-9) and they are capable of degrading types IV and V collagens, elastin, and gelatin [14-16].

The reduction in bone mineral density after organ transplantation results in post-transplantation bone disease and remains an unsolved problem [4]. Calcineurin inhibitors such as cyclosporine and tacrolimus, have serious effects causing rapid and severe bone loss in animal models and humans

Table 1. Effect of immunosuppressant on the metalloproteinase in osteoblasts

MMP	MMP-9		MMP-2	
Reagent / Conc.	CsA	Tacrolimus	CsA	Tacrolimus
2 μM	75	99	93	88
10 μM	96	99	59	91
50 μM	87	87	35	36
250 μM	80	76	43	34

The density for the MMP bands were measured using luminescent image analyzer system (LAS-3000, Fuji Film, Tokyo, Japan). The density of the control was calculated as 100%. Conc; concentration of the immunosuppressants, CsA; cyclosporine A, MMP; matrix metalloproteinase.

Table 2. Effect of immunosuppressant on the metalloproteinase in glioma cells.

MMP	MMP-9		MMP-2	
Reagent / Conc.	CsA	Tacrolimus	CsA	Tacrolimus
2 μM	80	102	108	125
10 μM	90	90	119	112
50 μM	79	86	123	101
250 μM	90	82	101	102

The density for the MMP bands were measured using luminescent image analyzer system (LAS-3000, Fuji Film, Tokyo, Japan). The density of the control was calculated as 100%. Conc; concentration of the immunosuppressants, CsA; cyclosporine A, MMP; matrix metalloproteinase.

[5,6]. A connection with the long-term application of nonglucocorticoidal immunosuppressants is the subject of controversial discussion [4]. The author hypothesized that CsA and tacrolimus have an influence on the skeletal system on the cellular level by dysregulation of the MMPs. In this study, both MMP-9 and MMP-2 activities were decreased depending on the concentration of CsA or tacrolimus.

And among the several immunosuppressants used after the organ transplantation, tacrolimus and cyclosporine which are known calcineurin inhibitors can cause neurological side effects [8]. According to Rosendal *et al*. [17], twenty percent of the patients immunosuppressed with CsA develop neurological side effects such as tremor, paresthesias, headache, seizures, visual disorders, paresis, and coma. And Kiemeneij *et al*. [18] reported a tacrolimus encephalopathy. However, the mechanisms of encephalopathy by CsA and tacrolimus are not fully understood. In this study, MMP-2 activity was increased after CsA or tacrolimus treatment. However, MMP-9 activities were decreased after CsA or tacrolimus treatment.

These results suggest that CsA and tacrolimus may play a role in post-transplantation bone disease by inhibiting MMP activity in osteoblasts. And the dysregulation of the MMP-2 and MMP-9 by tacrolimus or cyclosporine may one of the contributing factors in the encephalopathy by tacrolimus or cyclosporine.

Summary

The dysregulation of MMP activity has been implicated in numerous diseases including encephalopathy and the process of bone loss. Thus, MMPs may play a role in the encephalopathy and post-transplantation bone disease by immunosuppressive drugs such as CsA and tacrolimus. Glioma cells or rat osteoblast ROS17/2.8 cells were treated with CsA or tacrolimus to make final concentration from 2 to 250 μM. After incubation, gelatin zymography of MMP-9 and MMP-2 was performed. And the density for the MMP bands were measured using luminescent image analyzer system. Both MMP-9 and MMP-2 activities in the osteoblast cells were decreased depend on the concentration of CsA or tacrolimus. MMP-2 activity was increased after CsA or tacrolimus treatment in the glioma cells. However, MMP-9 activities were

decreased after CsA or tacrolimus treatment in the glioma cells. These results indicate that dysregulation of MMPs in the osteoblast and in the glioma cells by immunosuppressive drugs may one of the contributing factors in post-transplantation bone disease and in the encephalopathy by tacrolimus or cyclosporine.

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