Effect of Sepimostat Mesilate on the Development of Glomerulonephritis in NZB/W F1 Mice

Hiroshi Watanabe*, Shuzo Sato, Rie Saito, Haruyo Iwadate, Hiroko Kobayashi and Hiromasa Ohira

Department of Gastroenterology and Rheumatology, School of Medicine, Fukushima Medical University, Fukushima 960-1295, Japan

Abstract: *Objective*: To determine whether sepimostat mesilate inhibits activation of the complement pathways, and to evaluate the effectiveness of sepimostat mesilate on the development of glomerulonephritis in NZB/W F1 mice.

Methods: We used the Wielisa complement functional kit to assess the inhibitory effect of sepimostat mesilate on activation of the complement pathways. Groups of 10 NZB/NZW mice (age 18-22 weeks) were given sepimostat mesilate (200 μg/dose) or glucose (control) five times a week for 5 weeks after onset of proteinuria.

Results: Sepimostat mesilate dose-dependently inhibited the activity of all complement pathways. Administration of sepimostat mesilate after disease onset lowered the levels of blood urea nitrogen (243.2 ± 63.1 *versus* 120.9 ± 22.1 μg/dl; $p<0.0001$), C4d (0.244 ± 0.083 *versus* 0.153 ± 0.059 ng/dl; $p=0.011$), and delayed the development of proteinuria (0.822 ± 0.116 *versus* 0.470 ± 0.093 mg/mouse/day; $p=0.046$) at the end of treatment (22 weeks of age). After discontinuation of administration, blood urea nitrogen, C4d level, and proteinuria rapidly became elevated with no difference between the groups. Eventually, mortality was similar between treated and untreated mice.

Conclusions: Sepimostat mesilate could be a therapeutic option for lupus nephritis.

Keywords: Sepimostat mesilate, systemic lupus erythematosus, NZB/W F1 mouse.

INTRODUCTION

The complement system is intimately involved not only in host defenses against infectious diseases but also in the pathology of many autoimmune diseases such as systemic lupus erythematosus (SLE) [1]. Activation of the complement system is triggered by immune complexes (ICs) that are either deposited or formed in situ within tissues. The kidney is a major site of immune complex deposition and complement activation, and about half of all lupus patients will develop nephritis [2].

NZB/W F1 mice spontaneously develop antibodies against autoantigens such as DNA. These antibodies form ICs and are deposited in the glomeruli. The deposited ICs trigger activation of the complement system, which causes glomerulonephritis, vasculitis, and cellular infiltration in the interstitium of the kidney [3,4]. The clinical and immunopathological similarities to human SLE mean that the NZB/WF1 mouse model is commonly used for screening of drugs intended to treat human SLE [5-7].

Sepimostat mesilate (6-amidino-2-naphthyl[4-(4,5-dihydro-1H-imidazol-2-yl)amino] benzoate dimethane sulfonate) is a protease inhibitor developed by Torii Pharmaceutical Co., Ltd. (Tokyo, Japan) [8]. It has inhibitory potencies against proteases including C1r, C1s, Factor D, plasmin, kallikrein, thrombin, and trypsin. Furthermore, sepimostat mesilate (sepimostat) has inhibitory effects on various immunological reactions; it prevents complement-mediated hemolysis *in vitro* and *in vivo*, and protects against Forssman shock in guinea pigs [8,9]. As described above, sepimostat prevents activation of the classical and alternative complement pathways by inhibiting C1r, C1s, and factor D. However, the effect of sepimostat on activation of the lectin pathway has not yet been studied.

In this study: 1) we determined whether sepimostat inhibits the activation of the complement lectin pathway using the Wielisa complement function kit, an enzyme immunoassay for the qualitative determination of functional classical, lectin, and alternative pathways in serum; and 2) we administered sepimostat to NZB/W F1 mice to examine whether this agent can attenuate the severity of immunocomplex-mediated glomerulonephritis by inhibiting activation of the complement system.

MATERIALS AND METHODOLOGY

Mice

Six-week-old NZB/NZW F1 mice were purchased from Japan SLC (Hamamatsu, Japan) and maintained in the animal facility of Fukushima Medical University under specific pathogen-free conditions. This study was approved by the Fukushima Medical University Animal Protocols Review Board.

Inhibition Assay of Complement Activity by Sepimostat Using the Wielisa Kit

The Wielisa kit (Wieslab AB, Lund, Sweden) is an enzyme immunoassay for the qualitative determination of

*Address correspondence to this author at the Fukushima Medical University, Department of Gastroenterology and Rheumatology, School of Medicine, 1 Hikarigaoka, Fukushima 960-1295, Japan; Tel: +81-24-547-1111; Fax: +81-24-547-2055; E-mail: chiehiro@fmu.ac.jp

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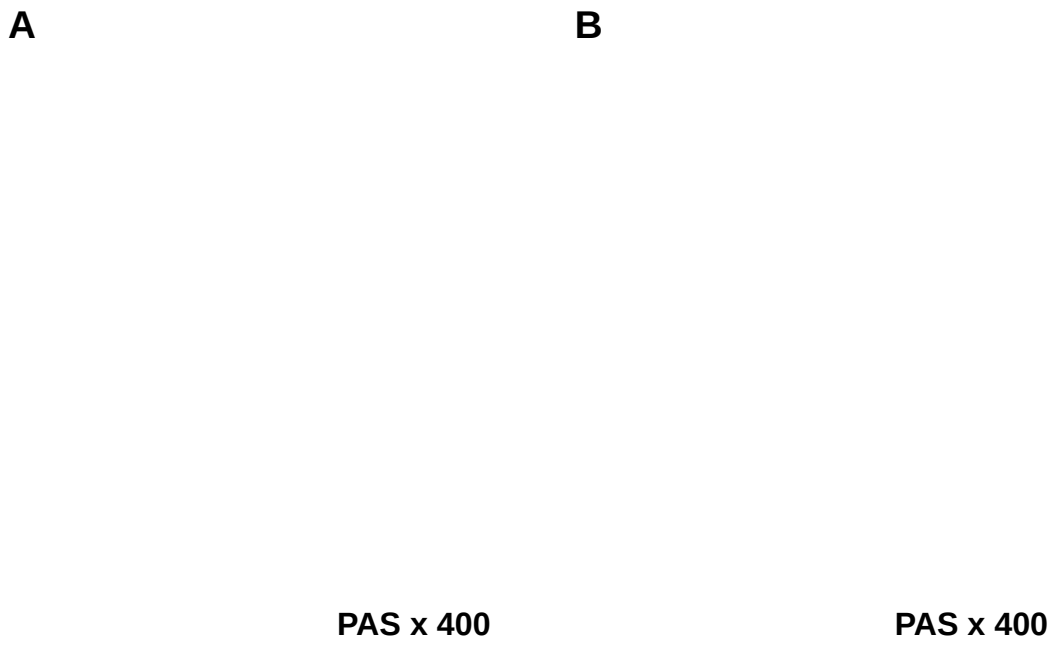

Fig. (6). Renal findings in sepimostat-treated mice (**A**) and control mice (**B**). In both groups, kidneys show diffuse glomerular hypercellularity, wire loop lesions, mesangial expansion, and glomerular hyalinization.

of the lectin pathway by binding mannose-binding lectin (MBL). The lectin complement pathway is closely related to the classical complement pathway in adaptive immunity, in terms of the structures as well as functions of their components. Both pathways are triggered by complexes consisting of collagenous proteins and serine proteases of the MASP/C1r/C1s family [12-14]. Considering that MASP is a member of the same family as C1r and C1s, it is possible that sepimostat may inhibit the lectin pathway by inhibiting the potency of MASP.

We also observed that the administration of sepimostat after disease onset lowered the levels of BUN, C4d, and delayed the development of proteinuria. However, once the treatment was stopped, serum levels of BUN, C4d, and proteinuria became elevated with no difference between the groups. Eventually, mortality as well as histological damage were similar between treated and untreated mice.

Although it could not be proven by immunopathological methods, the decreased serum level of C4d in the sepimostat-treated group suggests that sepimostat may alleviate glomerulonephritis in the NZB/w F1 mouse by inhibiting activation of the classical pathway, the lectin pathway, or both. Clearly, sepimostat does not have the function of inhibiting the formation of ICs as well as their deposition in the kidney. Therefore, the presumed mechanism is that, by inhibiting C1r and C1s, sepimostat inhibits the activation of the classical pathway triggered by deposition of ICs in the kidney, thereby alleviating inflammation and tissue damage. It seems likely that, because of the short half-life, the effects of sepimosat cease rapidly after it is stopped, and disease activity returns.

Sepimostat inhibits proteases other than those in the complement system (C1r, C1s, Factor D), such as plasmin, kallikrein, thrombin, and trypsin. Therefore, it is possible that the reduction in proteinurea and serum BUN levels were caused not only by the inhibition of complement pathway activation, but also by the inhibition of these other serine proteases that may contribute to tissue damage in autoimmunity.

Inhibition of complement activation has been considered a potential therapeutic approach for SLE. However, the complement system appears to play a dual role in the progression of SLE; it has not only a pathogenic role in inducing local inflammation but also a beneficial role in the clearance of apoptotic cells and ICs [1]. Given that the complement system also has important physiological roles in host defense and immune homeostasis, potential risks are associated with systemic complement inhibition, particularly when prolonged treatment is required. Therefore, long-term administration of sepimostat could cause diseases (e.g., lupus-like disease, Neisseria infection) in patients with complement deficiency, due to the inhibition of the beneficial effects of complement; i.e., clearance of apoptotic cells and ICs, and host defense.

Several serine protease inhibitors (i.e., nafamostat mesilate, camostat mesilate, gabexate mesilate) with similar chemical structures to sepimostat are commonly used in pancreatitis and disseminated intravascular coagulation in Japan. Although these protease inhibitors strongly inhibit activation of the complement system, there have been no reports of adverse effects of opportunistic infections resulting from immune suppression. This may relate to the short half-life of these drugs (no more than 10 minutes). Furthermore, nafamostat mesilate has been reported to ameliorate glomerulonephritis in immunocomplex disease as well as in the NZB/W F1 mouse [15-17]. Therefore, it is possible that sepimostat could be a therapeutic option for lupus nephritis patients with steroid resistance or severe immune compromise.

In conclusion, our study suggests that sepimostat may represent a novel treatment for immune-complex nephritis. Further long-term studies are needed to confirm the utility of this drug as a treatment for lupus nephritis.

CONFLICT OF INTEREST

The authors declare that we have no financial and personal relationships with other people or organizations that could inappropriately influence this work.

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