Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 489 (2004) 127–133
www.elsevier.com/locate/ejphar

Mycophenolate mofetil prevents autoimmune glomerulonephritis and alterations of intrarenal adrenomedullin in rats

Yi Shi[a], Fumiki Yoshihara[b], Hajime Nakahama[b,*], Rieko Goto[c], Masaharu Sada[c], Yuhei Kawano[b], Toshiki Moriyama[d], Koji Yazawa[a], Naotsugu Ichimaru[a], Shiro Takahara[a], Kenji Kangawa[c]

[a] *Department of Urology, Osaka University School of Medicine, Japan*
[b] *Division of Hypertension and Nephrology, National Cardiovascular Center, Fujishirodai 5-7-1, Suita 565-8565, Osaka, Japan*
[c] *Research Institute, National Cardiovascular Center, Japan*
[d] *School of Health and Sport Sciences, Osaka University, Japan*

Received 25 September 2003; received in revised form 20 January 2004; accepted 26 February 2004

Abstract

We studied the effects of mycophenolate mofetil, a specific inhibitor of inosine monophosphate dehydrogenase, on the mercuric chloride induced autoimmune glomerulonephritis in Brown Norway rats and also on the renal contents of adrenomedullin. In the rats with autoimmune glomerulonephritis, plasma and renal tissue adrenomedullin levels were increased significantly. Coadministration of mycophenolate mofetil resulted in prevention of autoimmune glomerulonephritis and also in maintaining of plasma and renal tissue adrenomedullin levels at control levels. Adrenomedullin mRNA expressions in the renal cortex were also higher in the rats with autoimmune glomerulonephritis. Significant positive correlations were found between renal cortical adrenomedullin levels and urinary Na^+ and *N*-acetyl-β-D-glucosaminidase excretion. A significant negative correlation between renal cortical adrenomedullin levels and creatinine clearance was also found. These results suggest that mycophenolate mofetil suppresses the renal damage in rats with autoimmune glomerulonephritis and renal adrenomedullin may participate in the pathophysiology of autoimmune glomerulonephritis.

Keywords: Adrenomedullin; Mycophenolate mofetil; Brown Norway, rat; Mercuric chloride; Autoimmune glomerulonephritis

1. Introduction

Adrenomedullin is a novel vasodilatory peptide originally discovered in human pheochromocytoma tissue (Kitamura et al., 1993). Subsequent studies demonstrated that adrenomedullin is widely distributed in the cardiovascular system, including the kidney, heart, lungs, and blood vessels (Kitamura et al., 1993). Adrenomedullin gene transcripts and specific binding sites are also present at high levels in the kidney, heart, lungs, and blood vessels (Ichiki et al., 1994). Immnohistochemistry of adrenomedullin in the canine kidney has revealed adrenomedullin immunoreactivity in glomeruli, cortical distal tubules, and medullary collecting duct cells. These results suggest that adrenomedullin may play a role in the regulation of kidney function (Jougasaki et al., 1995; Eto and Kitamura, 2001). Clinical studies have suggested the possible involvement of adrenomedullin in the pathophysiology of renal diseases (Eto and Kitamura, 2001). However, data are unavailable on the adrenomedullin in kidney tissues in glomerulonephritis. In the present study, using an animal model of autoimmune glomerulonephritis, we investigated the effect of glomerulonephritis on renal adrenomedullin content. We also studied the effects of mycophenolate mofetil, a specific inhibitor of inosine monophosphate dehydrogenase, which is involved in de novo purine synthesis, on the induced autoimmune glomerulonephritis and also on the renal contents of adrenomedullin.

2. Materials and methods

This study was performed in accordance with the guidelines of the Animal Care Committee of the National Cardiovascular Center Research Institute.

* Corresponding author. Tel.: +81-6-6833-5012; fax: +81-6-6872-7486.
E-mail address: hanakaham@hsp.ncvc.go.jp (H. Nakahama).

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.02.037

Table 1
Effects of $HgCl_2$ and mycophenolate mofetil administration on serum chemistry

	Group 1, Control (saline) group	Group 2, $HgCl_2$ only group	Group 3, Mycophenolate mofetil 50 mg/kg+$HgCl_2$	Group 4, Mycophenolate mofetil, 50 mg/kg only
Serum				
Albumin, g/dl	3.98±0.19	1.85±0.19[a]	3.24±0.53[a,b]	3.79±0.16[b,c]
BUN, mg/dl	24.39±4.11	34.01±13.16[a]	22.43±6.14[b]	28.81±5.09
Total cholesterol, mg/dl	60.38±4.75	242.25±30.72[a]	60.50±25.44[b]	47.38±5.55[b]
Creatinine, mg/dl	0.35±0.02	0.33±0.10	0.26±0.03[a,b]	0.31±0.03
Urine				
Protein, mg/day	11.81±3.01	672.56±124.65[a]	34.36±71.53[b]	3.07±1.18[b]
Ccr, ml/min	1.39±0.16	1.09±0.46	1.49±0.49[b]	1.25±0.13
N-acetyl-β-D-glucosaminidase, U/day	0.21±0.09	1.29±0.61[a]	0.16±0.04[b]	0.16±0.03[b]
Na^+, mEq/day	1.37±0.43	1.97±0.44[a]	1.50±0.32[b]	0.76±0.48[a,b,c]
Body weight	204.00±11.36	156.25±7.74[a]	156.50±10.89[a]	162.38±14.42[a]

Data are expressed as means±S.D. The One-way ANOVA test, followed by multiple comparisons test, was used for intergroup comparisons.
[a] $P<0.05$ vs. Group 1.
[b] $P<0.05$ vs. Group 2.
[c] $P<0.05$ vs. Group 3.

2.1. Animals

Six-week-old male Brown Norway rats were obtained from Oriental Yeast, Osaka, Japan. They weighed 150–160 g at the start of the experiments. They were fed standard food and tap water ad libitum.

2.2. $HgCl_2$ administration

$HgCl_2$ (1 mg/kg body weight) was dissolved in normal saline and inoculated subcutaneously 3 times/week for a total of 2 weeks.

2.3. Mycophenolate mofetil administration

Mycophenolate mofetil, donated by Chugai Pharmaceutical, Tokyo, Japan was given as a daily oral dose of 50 mg/kg sonicated in vehicle containing 0.9% benzyl alcohol, 5% L-dextrose, 0.9% NaCl, 0.5% carboxy-methylcellulose (7F), 0.4% polysorbate 80, and 88.7% water, pH 3.5.

2.4. Experimental design

Thirty-two animals were randomly divided into four groups of eight animals: group 1, rats received vehicle only; group 2, rats were treated with $HgCl_2$ alone; group 3, rats were treated with $HgCl_2$ and mycophenolate mofetil (50 mg/kg body weight), and group 4, rats were treated with mycophenolate mofetil (50 mg/kg body weight) alone. On day 13 of the experimental protocol, all rats were housed overnight in metabolic cages in order to collect urine. Serum and urine samples were obtained from all animals on day 14. Animals were sacrificed on day 14. Serum albumin, creatinine, total cholesterol, blood urea nitrogen, urinary protein, urinary *N*-acetyl-β-D-glucosaminidase, urinary creatinine and urinary Na^+ were determined using an automatic analyzer (Hitachi 7170, Hitachi, Japan).

Table 2
Effects of $HgCl_2$ and mycophenolate mofetil administration on renal tissue adrenomedullin

	Group 1, Saline only	Group 2, $HgCl_2$ only	Group 3, Mycophenolate mofetil, 50 mg/kg+$HgCl_2$	Group 4, Mycophenolate mofetil, 50 mg/kg only
Cortex mature adrenomedullin, fmol/mg	0.30±0.05	0.57±0.13[a]	0.38±0.03[a,b]	0.34±0.03[b]
Cortex total adrenomedullin, fmol/mg	0.61±0.13	0.95±0.17[a]	0.89±0.07[a]	0.68±0.03[b,c]
Medulla mature adrenomedullin, fmol/mg	0.33±0.04	0.59±0.20[a]	0.32±0.02[b]	0.24±0.03[b]
Medulla total adrenomedullin, fmol/mg	0.54±0.07	0.80±0.20[a]	0.68±0.07[a,b]	0.48±0.06[b,c]
Plasma total adrenomedullin, fmol/mg	26.58±10.67	62.48±21.34[a]	35.01±23.71[b]	30.68±5.24[b]

Data are expressed as means±S.D. The One-way ANOVA test, followed by multiple comparisons test, was used for intergroup comparisons.
[a] $P<0.05$ vs. Group 1.
[b] $P<0.05$ vs. Group 2.
[c] $P<0.05$ vs. Group 3.

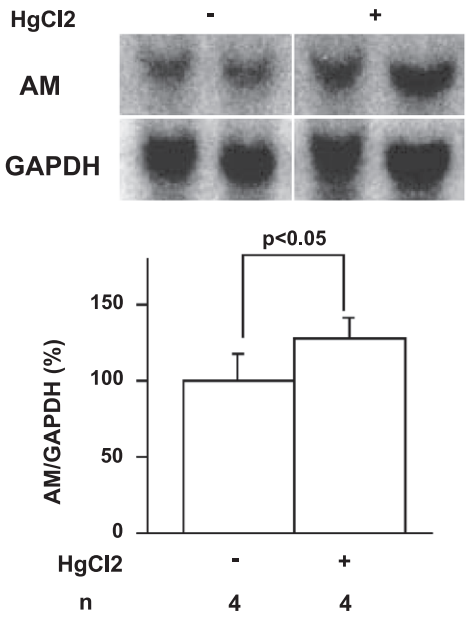

Fig. 1. Representative Northern blots showing adrenomedullin (AM) in the renal cortex in rats.

2.5. Radioimmunoassay for renal and plasma total and mature adrenomedullin

Each renal tissue to be used for radioimmunoassay was weighed, diced, and boiled in 10 volume of 1 mol/l acetic acid for 10 min to inactivate intrinsic proteases. After the boiled tissues were cooled, it was homogenized with a Polytron mixer for several min. The homogenate was centrifuged at 3000×*g* for 30 min, and the supernatant was centrifuged again at 15,000×*g* for 10 min. The supernatant was evaporated under vacuum until dry (Yoshihara et al., 2000). Rat total adrenomedullin and rat mature adrenomedullin were measured by immunoradiometric assay systems using two monoclonal antibodies against rat adrenomedullin, one specifically recognizing a ring structure of rat adrenomedullin in both assay kits and the other specifically recognizing the carboxy-terminal sequence in the rat mature adrenomedullin kit or adrenomedullin (25–36) in the rat total adrenomedullin kit. The assay measures rat total adrenomedullin or rat mature adrenomedullin by sandwiching it between the two antibodies (Nishikimi et al., 2002).

2.6. Northern blot analysis

An *Eco*RI/*Nae*I restriction fragment of rat adrenomedullin cDNA corresponding to nucleotides −153 to 436 was used as the rat adrenomedullin cDNA probe. Total RNA (20 μg/lane) for adrenomedullin mRNA evaluation was denatured, electrophoresed and transferred to nylon membrane. For hybridization with the cDNA probe, conditions for hybridization and washing have been previously described (Nishikimi et al., 1997).

2.7. Statistical analysis

Data from all the 32 animals (4 groups of 8 animals) were included in the analyses. Data are expressed as means±S.D. The One-way Analysis of variance (ANOVA) test, followed by multiple comparisons test, was used for intergroup comparisons. Differences were considered significant at a level of $P<0.05$. Univariate and stepwise multivariate regression analyses were used to detect independent predictors of renal tissue adrenomedullin levels among the eight variables, as listed in the following text. Correlation coefficients were calculated using linear regression analysis.

3. Results

3.1. Characteristics of $HgCl_2$-induced autoimmune glomerulonephritis

With subcutaneous inoculation of $HgCl_2$, a progressive proteinuria developed from 7 days onwards. The proteinuria stabilized at around 600 mg/24 h. Concomitant with the increase in proteinuria, there was a severe drop in serum albumin levels, reaching around 2 g/dl at day 14. A concomitant rise in serum total cholesterol was observed, reaching around 200 mg/dl at day 14.

Table 3
Univariate and stepwise multivariate regression analyses of factors predicting renal tissue mature adrenomedullin levels

Variable	Univariate		Multivariate	
	Correlation coefficient	*P*	Correlation coefficient	*P*
Cortical mature adrenomedullin				
Serum				
Albumin	−0.82	<0.0001	−0.363	<0.0001
Blood urea nitrogen	0.60	0.0002		NS
Creatinine	0.30	0.076		NS
Creatinine clearance	−0.49	0.003		NS
24-h urinary excretion				
Protein	0.86	<0.0001		NS
N-acetyl-β-D-glucosaminidase	0.87	<0.0001	0.578	<0.0001
Na^+	0.43	0.0101		NS
Medullary mature adrenomedullin				
Serum				
Albumin	−0.75	<0.0001		NS
Blood urea nitrogen	0.63	<0.0001		NS
Creatinine	0.50	<0.0001		NS
Creatinine clearance	−0.52	0.0012		NS
24-h urinary excretion				
Protein	0.85	<0.0001		NS
N-acetyl-β-D-glucosaminidase	0.90	<0.0001	0.899	<0.0001
Na^+	0.39	0.0206		NS

NS: Not significant.

3.2. Effects of mycophenolate mofetil (50 mg/kg body weight) on serum and urine chemistry

All animals were alive at the termination of the experiment. Effects of mycophenolate mofetil (50 mg/kg body weight) are shown in Table 1. Treatment with mycophenolate mofetil resulted in a 95% reduction in urinary protein excretion, a 75% reduction in total cholesterol levels and an 88% reduction in urinary excretion of *N*-acetyl-β-D-glucosaminidase. The reduction of proteinuria resulted in higher serum albumin levels in group 3. Urinary excretion of Na^+, which was significantly increased in group 2, was maintained at normal levels in group 3.

3.3. Effects of mycophenolate mofetil (50 mg/kg body weight) on plasma and renal tissue adrenomedullin levels

$HgCl_2$ administration increased plasma and renal tissue adrenomedullin levels significantly. Coadministration of $HgCl_2$ and mycophenolate mofetil resulted in maintaining of plasma and renal tissue adrenomedullin levels at control levels (Table 2). Adrenomedullin mRNA expressions in the renal cortex were also higher in the rats of group 2 (Fig. 1). To determine the independent predictors of renal tissue mature adrenomedullin levels, stepwise multivariate regression analysis was used with eight variables such as serum albumin, blood urea nitrogen, serum creatinine, creatinine clearance, 24-h urinary protein excretion and 24-h urinary *N*-acetyl-β-D-glucosaminidase excretion, 24-h urinary Na^+ excretion. Among these eight variables, serum albumin and 24-h urinary *N*-acetyl-β-D-glucosaminidase excretion were independent and significant predictors of renal cortical mature adrenomedullin and 24-h urinary *N*-acetyl-β-D-glucosaminidase excretion was an independent and significant predictor of renal medullary mature adrenomedullin (Table 3). Significant positive correlations were found between renal mature adrenomedullin levels and urinary Na^+ and *N*-acetyl-β-D-glucosaminidase excretion. A significant negative correlation was found between renal mature adrenomedullin levels and creatinine clearance (Fig. 2). A significant negative correlation was

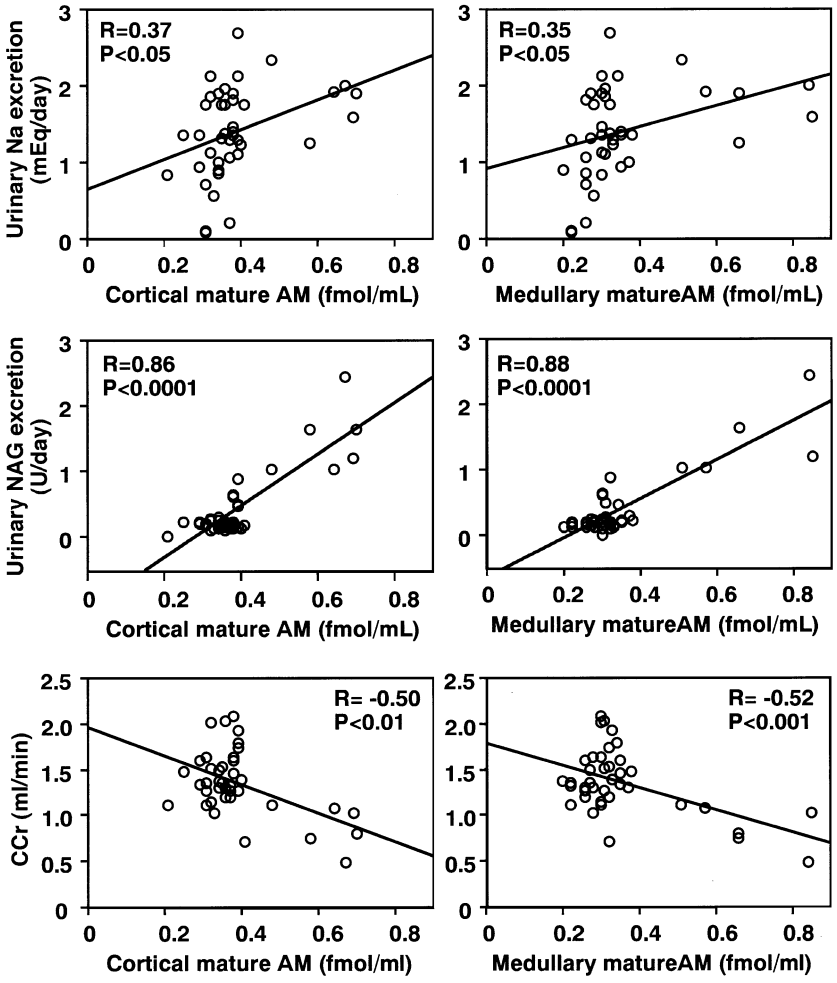

Fig. 2. Correlations between renal tissue adrenomedullin (AM) levels in the renal cortex and medulla and urinary Na^+, *N*-acetyl-β-D-glucosaminidase (NAG) and creatinine clearance (CCr).

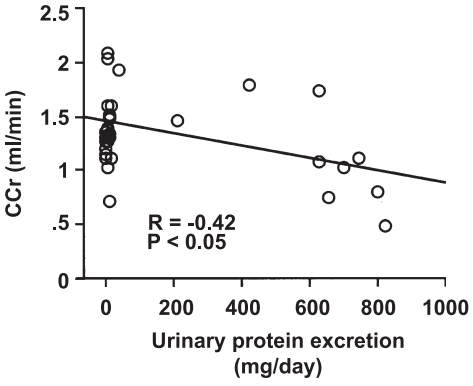

Fig. 3. Correlation between 24-h urinary protein excretion and creatinine clearance (CCr).

found between 24-h urinary protein excretion and creatinine clearance (Fig. 3).

4. Discussion

Mercuric chloride ($HgCl_2$) induces a lymphproliferative disorder and autoimmune glomerulonephritis in Brown Norway rats (Sapin et al., 1997). In the kidney, early deposition of antiglomerular basement membrane antibodies occurs and is associated with heavy proteinuria. Subsequently, a superimposed immune complex type glomerulonephritis occurs. It has often been proposed as having many of the features of membranous nephropathy (Fukatsu et al., 1987).

Mycophenolate mofetil is a suppressor of both T- and B-cell lymphocyte proliferation and has been used successfully for the prevention of acute and chronic rejection of renal allograft (The Mycophenolate Mofetil Acute Renal Rejection Study Group, 2001). Mycophenolate mofetil is considered to be effective in the treatment of glomerulonephritis as a result of a combination of its immunosuppressive properties and its other mechanisms of action. Mycophenolic acid, the pharmacologically active metabolite of mycophenolate mofetil, inhibits both T- and B-lymphocyte proliferation, B-lymphocyte antibody production and expression of adhesion molecules (Allison and Eugui, 1996). In addition, mycophenolic acid has been shown to inhibit vascular smooth muscle cell (Allison et al., 1993) and mesangial cell proliferation (Hauser et al., 1999), to be a selective inhibitor of inducible nitric oxide synthase (Senda et al., 1995), and to induce apoptosis in activated T cells (Cohn et al., 1999). One or another, or combination of these actions could account for the observed amelioration of various experimental models of glomerular disease, including active Heymann nephritis (Penny et al., 1998), hyperfiltration injury in remnant kidney (Lui et al., 2001), mesangial proliferative nephritis (Ziswiler et al., 1998) and murine nephritis (Zoja et al., 2001). A few trials of mycophenolate mofetil in human glomerulopathy have reported partial amelioration of the disease, albeit non-randomized and anecdotal in nature (Briggs et al., 1998; Chan et al., 2000; Choi et al., 2002; Day et al., 2002; Mogyorosi et al., 2002). Given the lack of nephrotoxicity and adverse hemodynamic and metabolic effects (Suthanthiran et al., 1996), mycophenolate mofetil represents a suitable alternative to the calcineurin-inhibitors as an adjuvant treatment for many patients.

We have reported that tacrolimus hydrate (FK 506), a potent immunosuppressive agent with a similar mode of action to cyclosporine A, is effective both in the prevention (Nakahama et al., 1999a) and the treatment (Nakahama et al., 1999b) of mercuric chloride ($HgCl_2$) induced autoimmune glomerulonephritis in Brown Norway rats. In the present study, treatment of the induction phase of autoimmune glomerulonephritis with mycophenolate mofetil prevented disease, with no significant proteinuria at day 14. The degree of suppression of proteinuria was comparable to that exerted by tacrolimus hydrate, which virtually provided complete prevention of autoimmune glomerulonephritis and consequent proteinuria despite the different mechanisms of immunosuppression between these two drugs. These results offer a strong case for exploring the possibility that in humans, mycophenolate mofetil has a role in the treatment options for autoimmune glomerulonephritis.

A novel finding of our present study was the increase in the plasma and renal tissue levels of adrenomedullin. Namely, $HgCl_2$ increased plasma and renal tissue adrenomedullin levels significantly. Coadministration of $HgCl_2$ and mycophenolate mofetil resulted in recovery of plasma and renal tissue adrenomedullin levels to the control levels (Table 2).

Adrenomedullin, a potent hypotensive peptide, is a 52-amino acid peptide originally isolated from human pheochromocytoma (Kitamura et al., 1993). Recently, a RIA method was developed for measurement of "mature adrenomedullin" and "total adrenomedullin", which is a sum of mature and immature adrenomedullin (Ohta et al., 1999). Therefore, it is worthwhile to measure the level of mature adrenomedullin to investigate the pathophysiological role of adrenomedullin. A novel finding of our present study was the increase in renal tissue levels of mature adrenomedullin and that its levels correlated with urinary Na^+ excretion, urinary *N*-acetyl-β-D-glucosaminidase excretion and creatinine clearance (Fig. 2), suggesting that renal adrenomedullin might be involved in part in the pathophysiology of glomerulonephritis. It has been well documented that plasma concentrations of a large number of compounds, which under normal conditions are excreted by the healthy kidney, are elevated in renal dysfunction. Adrenomedullin is no exception (Vanholder et al., 2003). A part of the observed increase in plasma and renal tissue adrenomedullin levels may be attributable to retention. However, it seems that the reduction of creatinine clearance (−22%) is too small to account for the 39.4% increase in plasma adrenomedullin concentration. Furthermore, the enhanced renal tissue adrenomedullin mRNA expression in nephritic rats supports our

hypothesis that adrenomedullin production per se is enhanced in nephritic state.

Several clinical observations suggest possible role of adrenomedullin in human glomerulonephritis. In a study with patients with Immunoglobulin A (IgA) nephropathy (Kubo et al., 1998a), the plasma adrenomedullin level was higher in patients with IgA nephropathy than in healthy volunteers. The plasma adrenomedullin concentration was positively correlated with serum creatinine and blood urea nitrogen and with fractional excretions of Na^+ and potassium. When the disease activity was scored based on the histological findings of renal biopsy specimen, the urinary adrenomedullin level was lower in the high-activity group as compared with the low-activity one, which suggest that urinary adrenomedullin may reflect the activity in IgA nephropathy. It has also been shown that plasma adrenomedullin was positively correlated and urinary adrenomedullin was negatively correlated with the degree of proteinuria in patients with a variety of types of glomerulonephritis (Kubo et al., 1998b). Kinoshita et al. (1999) measured plasma and urine levels of adrenomedullin in patients with chronic glomerulonephritis including IgA nephropathy, minor glomerulopathy, focal glomerular sclerosis, membranous nephropathy and lupus nephritis, one third of whom showed nephrotic syndrome. Contrary to the previous reports, the plasma adrenomedullin level in the patients with chronic glomerulonephritis did not differ from that in control subjects, whereas the urinary adrenomedullin concentration was significantly less for the patients with chronic glomerulonephritis. The plasma adrenomedullin concentration was negatively correlated with plasma renin activity and aldosterone concentration. The urinary concentrations of adrenomedullin showed a significant correlation with the urinary excretion of Na^+. The findings suggested that adrenomedullin might have a role in the regulation of urinary Na^+ excretion as paracrine or autocrine factors, in addition to the endocrine actions of plasma adrenomedullin in patients with chronic glomerulonephritis.

Recent studies of dogs (Majid et al., 1996) and rats (Nagaya et al., 1999) have shown that an intravenous injection of adrenomedullin increases glomerular filtration rate and fractional Na^+ excretion indicating that adrenomedullin acts as a natriuretic factor. Moreover, adrenomedullin is suggested to be an autocrine/paracrine regulator of mesangial function and is secreted by renal tubular cell line (Sato et al., 1998). We have reported that in aortocaval shunt rats, a rat model of heart failure, renal adrenomedullin levels were increased in decompensated heart failure and there was a significant positive correlation between renal adrenomedullin levels and urinary Na^+ excretion, indicating that adrenomedullin affects ion and water transport in renal diseases by acting as a natriuretic hormone or local regulator (Yoshihara et al., 2001). In addition, chronic adrenomedullin infusion has been reported to reduce proteinuria, glomerular injury score and renal tumor growth factor-β (TGF-β) mRNA expression in Dahl salt-sensitive rats, suggesting that its infusion has renoprotective effects in this type of hypertension model (Nishikimi et al., 2002). Taken together, our present findings suggest that renal adrenomedullin is increased in compensation for Na^+ retention tendency in nephritic syndrome and for renal damage in glomerulonephritis.

References

Allison, A.C., Eugui, E.M., 1996. Purine metabolism and immunosuppressive effects of mycophenolate mofetil (MYCOPHENOLATE MOFETIL). Clin. Transplant. 10, 77–84.

Allison, A.C., Eugui, E.M., Sollinger, H.W., 1993. Mycophenolate mofetil (RS-61443): mechanism of action and effects in transplantation. Transplant. Rev. 7, 129–139.

Briggs, W.A., Choi, M.J., Scheel, P.J., 1998. Successful mycophenolate mofetil treatment of glomerular disease. Am. J. Kidney Dis. 31, 213–217.

Chan, T.M., Li, F.K., Tang, C.S.O., Wong, R.W.S., Fang, G.X., Ji, Y.L., Lau, C.S., Wong, A.K.M., Tong, M.K.L., Chan, K.W., Lai, K.N., 2000. Efficacy of mycophenolate mofetil in patients with diffuse proliferative lupus nephritis. N. Engl. J. Med. 343, 1156–1162.

Choi, M.J., Eustage, J.A., Gimenez, L.F., Atta, M.G., Scheel, P.J., Sothinathan, R., Briggs, W.A., 2002. Mycophenolate mofetil treatment for primary glomerular diseases. Kidney Int. 61, 1098–1114.

Cohn, R.G., Mirkovich, A., Dunlap, B., Burton, P., Chiu, S.H., Eugui, E., Caufield, J.P., 1999. Mycophenolic acid increases apoptosis in lysosomes and lipid droplets in human lymphoid and monocytic cell lines. Transplantation 68, 411–418.

Day, C.J., Cockwell, P., Lipkin, G.W., Savage, C.O.S., Howie, A.J., Adu, D., 2002. Mycophenolate mofetil in the treatment of resistant idiopathic nephritic syndrome. Nephrol. Dial. Transplant. 17, 2011–2013.

Eto, T., Kitamura, K., 2001. Adrenomedullin and its role in renal diseases. Nephron 89, 121–134.

Fukatsu, A., Brentjens, J.R., Killen, P.D., Kleinman, H.K., Martin, G.R., Andres, G.A., 1987. Studies on the formation of glomerular immune deposits in Brown Norway rats injected with mercuric chloride. Clin. Immunol. Immunopathol. 45, 35–47.

Hauser, I.A., Renders, L., Radeke, H.H., Stertel, R.B., Goppelt-Struebe, M., 1999. Mycophenolate mofetil inhibits rat and human mesangial cell proliferation by guanosine depletion. Nephrol. Dial. Transplant. 14, 58–63.

Ichiki, Y., Kitamura, K., Kangawa, K., Kawamoto, M., Matsuo, H., Eto, T., 1994. Distribution and characterization of immunoreactive adrenomedullin in human tissue and plasma. FEBS Lett. 338, 6–10.

Jougasaki, M., Wei, C.-M., Aarhus, L.L., Heublein, D.M., Sandberg, S.M., Burnett, J.C., 1995. Renal localization and actions of adrenomedullin: a natriuretic peptide. Am. J. Physiol. 268, F657–F663.

Kinoshita, A., Fujimoto, S., Kitamura, K., Yokota, N., Kawamoto, M., Tokura, T., Hisanaga, S., Eto, T., 1999. Plasma and urine levels of adrenomedullin and proadrenomedullin N-terminal 20 peptide in chronic glomerulonephritis. Am. J. Kidney Dis. 34, 114–119.

Kitamura, K., Kangawa, K., Kawamoto, M., Ichiki, Y., Nakamura, S., Matsuo, H., Eto, T., 1993. Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. Biochem. Biophys. Res. Commun. 192, 553–560.

Kubo, A., Iwano, M., Minamino, N., Sato, H., Nishino, T., Hirata, E., Akai, Y., Shiiki, H., Kitamura, K., Kangawa, K., Matsuo, H., Dohi, K., 1998a. Measurement of plasma and urinary adrenomedullin in patients with IgA nephropathy. Nephron 78, 389–394.

Kubo, A., Kurioka, H., Minamino, N., Nishitani, Y., Sato, H., Nishino, T., Iwano, M., Shiiki, H., Kangawa, K., Matsuo, H., Dohi, K., 1998b. Plasma and urinary levels of adrenomedullin in chronic glomerulonephritis patients with proteinuria. Nephron 80, 227–230.

Lui, S.L., Chan, L.Y.Y., Zhang, X.H., Zhu, W., Chan, T.M., Fung, P.C.W.F., Lai, K.N., 2001. Effect of mycophenolate mofetil on nitric oxide production and inducible nitric oxide synthase gene expression during renal ischaemia–reperfusion injury. Nephrol. Dial. Transplant. 16, 1577–1582.

Majid, D.S.A., Kandowitz, P.J., Coy, D.H., Navar, L.G., 1996. Renal responses to intra-arterial administration of adrenomedullin in dogs. Am. J. Physiol. 270, F200–F205.

Mogyorosi, A., Lippman, H.R., Feldman, G.M., 2002. Successful treatment of steroid-resistant minimal change disease with mycophenolate mofetil. Am. J. Nephrol. 22, 569–572.

Nagaya, N., Nishikimi, T., Horio, T., Yoshihara, F., Kanazawa, A., Matsuo, H., Kangawa, K., 1999. Cardiovascular and renal effects of adrenomedullin in rats with heart failure. Am. J. Physiol. 276, R213–R218.

Nakahama, H., Obata, K., Matsuyama, T., Sugita, M., Horio, M., Oka, K., Moriyama, T., 1999a. Effect of a novel immunosuppressant, FK 506, on autoimmune glomerulonephritis in Brown Norway rats. Nephron 81, 215–220.

Nakahama, H., Obata, K., Sugita, M., Horio, M., Oka, K., Moriyama, T., 1999b. Effect of FK 506 in the treatment of autoimmune glomerulonephritis in Brown Norway rats. Nephron 81, 421–427.

Nishikimi, T., Horio, T., Sasaki, T., Yoshihara, F., Takishita, S., Miyata, A., Matsuo, H., Kangawa, K., 1997. Cardiac production and secretion of adrenomedullin are increased in heart failure. Hypertension 30, 1369–1375.

Nishikimi, T., Mori, Y., Kobayashi, N., Tadokoro, K., Wang, X., Akimoto, K., Yoshihara, F., Kangawa, K., Matuoka, H., 2002. Renoprotective effect of chronic adrenomedullin infusion in Dahl salt-sensitive rats. Hypertension 39, 1077–1082.

Ohta, H., Tsuji, T., Asai, S., Sasakura, K., Teraoka, H., Kitamura, K., 1999. One-step direct assay for mature-type adrenomedullin with monoclonal antibodies. Clin. Chem. 45, 244–251.

Penny, M.J., Boyd, R.A., Hall, B.M., 1998. Mycophenolate mofetil prevents the induction of active Heymann Nephritis: association with Th2 cytokine inhibition. J. Am. Soc. Nephrol. 9, 2272–2282.

Sapin, C., Druet, E., Druet, P., 1997. Induction of antiglomerular basement membrane antibodies in the Brown Norway rat by mercuric chloride. Clin. Exp. Immunol. 28, 173–179.

Sato, K., Imai, T., Iwashina, M., Marumo, F., Hirata, Y., 1998. Secretion of adrenomedullin by renal tubular cell lines. Nephron 78, 9–14.

Senda, M., DeLustro, B., Eugui, E., Natumeda, Y., 1995. Mycophenolic acid, an inhibitor of IMP dehydrogenase that is also an immunosuppressive agent, suppresses the cytokine-induced nitric oxide production in mouse and rat vascular endothelial cells. Transplantation 60, 1143–1148.

Suthanthiran, M., Morris, R.E., Strom, T.B., 1996. Immunosuppressants: cellular and molecular mechanisms of action. Am. J. Kidney Dis. 28, 159–172.

The Mycophenolate Mofetil Acute Renal Rejection Study Group, 2001. Mycophenolate mofetil for the treatment of a first acute renal allograft rejection: three-year follow-up. Transplantation 71, 1091–1097.

Vanholder, R., De Smet, R., Glorieux, G., Argilés, A., Baurmeister, U., Brunet, P., Clark, W., Cohen, G., De Deyn, P.P., Deppisch, R., Descamps-Latscha, B., Henle, T., Jörres, A., Lemke, H.D., Massy, Z.A., Passlick-Deetjen, J., Rodriguez, M., Stegmayr, B., Stenvinkel, P., Tetta, C., Wanner, C., Zidek, W., For the European Uremic Toxin Work Group (EUTox), 2003. Review on uremic toxins: Classification, concentration, and interindividual variability. Kidney Int. 63, 1934–1943.

Yoshihara, F., Nishikimi, T., Horio, T., Yutani, C., Nagaya, N., Matuso, H., Ohe, T., Kangawa, K., 2000. Ventricular adrenomedullin concentration is a sensitive biochemical marker for volume and pressure overload in rats. Am. J. Physiol., Heart Circ. Physiol. 278, H633–H642.

Yoshihara, F., Nishikimi, T., Okano, I., Horio, T., Yutani, C., Matsuo, H., Takishita, S., Ohe, T., Kangawa, K., 2001. Alterations of intrarenal adrenomedullin and its receptor system in heart failure rats. Hypertension 37, 216–222.

Ziswiler, R., Steinmann-Niggli, K., Kappeler, A., Daniel, C., Marti, H.-P., 1998. Mycophenolic acid: a new approach to the therapy of experimental mesangial proliferative glomerulonephritis. J. Am. Soc. Nephrol. 9, 2055–2066.

Zoja, C., Benigni, A., Noris, M., Corna, D., Casiraghi, F., Pagnoncelli, M., Rottoli, D., Abbate, M., Remuzzi, G., 2001. Mycophenolate mofetil combined with cyclooxygenase-2 inhibitor ameliorates murine lupus nephritis. Kidney Int. 60, 653–663.