# Effect of nicorandil on proteinuria in well controlled hypertensive patients

Tsung-Ming Lee[a] and Nen-Chung Chang[b]

***Objective*** **Proteinuria is an important risk factor for cardiovascular and renal morbidity and mortality. The effect of nicorandil on proteinuria in hypertensive patients well controlled by antihypertensive agents containing a low dose of valsartan has not been studied.**

***Patients and methods*** **A total of 136 proteinuric (300–3000 mg/day), valsartan-treated hypertensive patients with blood pressure less than 140/90 mmHg were randomized into three groups to receive placebo, isosorbide dinitrate (30 mg/day), or nicorandil (15 mg/day) for 6 months.**

***Results*** **The average dose of valsartan given to the patients was similar in the three groups. Creatinine clearance remained stable throughout the study in the three groups. Nicorandil, but not isosorbide dinitrate, significantly reduced proteinuria by 44% after 6 months ($P < 0.0001$). Urinary endothelin-1 levels significantly decreased after administration of nicorandil ($P = 0.002$), whereas placebo and isosorbide dinitrate had no effect. Urinary excretion of endothelin-1 was significantly correlated with improvement in urinary protein excretion in nicorandil-treated patients ($r = 0.69$, $P < 0.0001$). The urinary excretion of retinol-binding protein decreased after nicorandil administration, probably reflecting an improvement in tubular function. In contrast, the urinary excretion of immunoglobulin G did not change significantly throughout the study in the three groups. Multivariate analysis revealed that proteinuria was only significantly correlated with the use of nicorandil (model adjusted $r^2 = 0.35$, $P < 0.0001$).**

***Conclusion*** **The addition of nicorandil to treatment for patients with well controlled hypertension may have an additive effect on reducing proteinuria independent of hemodynamics and nitric oxide effects, possibly through inhibiting renal endothelin-1 synthesis and improving tubular function. *J Hypertens* 27:618–625 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

**Journal of Hypertension** 2009, **27**:618–625

**Keywords: ATP-sensitive potassium channels, endothelin-1, hypertension, proteinuria**

**Abbreviations: ACE, angiotensin-converting enzyme; ET-1, endothelin-1; ISDN, isosorbide dinitrate; $K_{ATP}$ channels, ATP-sensitive potassium channels**

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**Received** 15 July 2008 **Revised** 2 October 2008
**Accepted** 8 October 2008

## Introduction

Epidemiological studies have shown that urinary protein excretion is a strong independent predictor of renal and cardiovascular outcomes [1]. Hypertension has been shown to be an important cause of end-stage renal disease [2]. In a population of hypertensive patients, detection of those with increased risk of developing renal impairment is therefore a subject of great concern. Reducing proteinuria slows the rate of progression of renal failure in patients with nonnephrotic [3] or nephrotic [4] proteinuria. Thus, proteinuria regression emerged as a treatment goal in patients with well controlled hypertension. Ruilope *et al.* [5] have shown the occurrence of proteinuria in 17.5% of patients with well controlled hypertension. Although tight blood pressure (BP) control is known to be a crucial factor in preventing progression of renal disease, other factors are undoubtedly involved. Recent studies showed that angiotensin-converting enzyme (ACE) inhibitors [6], angiotensin II receptor blockers [7], and statins [8,9] slowed the progression rate of renal disease in patients with proteinuria. However, although progression is slowed, it is not arrested, indicating the need for adjunctive therapy. Thus, it is of interest to identify drugs other than ACE inhibitors, angiotensin II receptor blockers, and statins to reduce proteinuria.

The effects of ATP-sensitive potassium ($K_{ATP}$) channels on renal physiology were investigated in spontaneously hypertensive rats, a model of genetic hypertension. Renal $K_{ATP}$ channels are comprised of SUR2B in complex with Kir6.1 [10]. The SUR2B transcript was detected in proximal tubule, cortical thick ascending limb, distal collecting tubule, cortical collecting duct, and outer medullary collecting duct [11]. Nicorandil preferentially activates the $K_{ATP}$ channels containing SUR2B [12]. Thus, nicorandil may play a role in modulating the renal $K_{ATP}$ channels.

In animal models of renal disease, administration of a $K_{ATP}$ channel agonist (iptakalim) has reduced proteinuria

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[13]. Proteins filtered through the glomerular capillary might have intrinsic toxicity for the proximal tubular cells and a contributory role in the progression of renal damage. Protein overload on proximal tubular cells may induce a dose-dependent increase in synthesis and release of endothelin-1 (ET-1) in rat models of proteinuric renal disease [14]. ET-1 blockade has been reported to diminish proteinuria [15]. $K_{ATP}$ channel agonists have been shown to reduce the synthesis of ET-1 at the transcriptional level in rats [13]. However, no clinical studies have reported whether the effect of $K_{ATP}$ channel agonists as adjunctive therapy would further reduce proteinuria in hypertensive patients well controlled by drugs including angiotensin II receptor blockers. Thus, we investigated to assess whether nicorandil administration for 6 months was effective in reducing proteinuria when given to well controlled hypertensive patients already treated with angiotensin II receptor blockers, to test whether renoprotection is mediated by inhibiting renal ET-1 synthesis, to evaluate improvement of glomerular and tubular markers after administering nicorandil, and because nicorandil has a K channel opening effect that activates $K_{ATP}$ channels and induces nitric oxide in the same way as nitrates such as isosorbide dinitrate (ISDN), we added a group of patients treated with ISDN to exclude confounding effects of nitric oxide on proteinuria.

## Methods

### Patients

The present study was conducted in a prospective, parallel, double-blind, randomized, and placebo-controlled manner. Eligible patients were required to have stable well controlled hypertension, were treated with antihypertensive agents containing valsartan and had seated diastolic blood pressure (DBP) of 90 mmHg or less and systolic blood pressure (SBP) of 140 mmHg during a 3-month screening period. Cuff BP was selected for BP measurements to reflect standard office-based clinical practice. Proteinuria was defined as urinary protein excretion exceeding 300 mg/24 h in two consecutive 24-h urine samples without evidence of urinary tract infection or overt heart failure.

Patients were excluded from the study if they had diabetes mellitus, secondary hypertension, renal disease (plasma creatinine concentration $\geq$ 1.5 mg/dl or 133 μmol/l), proteinuria of 3 g/day or more, or treatment with corticosteroids or nonsteroidal anti-inflammatory drugs. Because body weight is a confounding factor for proteinuria [16], only patients with BMI less than 30 kg/m$^2$ were included. In order to evaluate the add-on effect of nicorandil, all antihypertensive agents were kept constant throughout the study. The patients were not given specific risk factor modification instructions such as diet control or smoking cessation. After a run-in period of 3 months during which the previously mentioned entry criteria were evaluated, proteinuric patients were randomized to 6-month treatment with placebo, ISDN [10 mg three times daily (t.i.d.)], or nicorandil (5 mg t.i.d.). Nicorandil was orally administered at a dose of 15 mg/day, which has been proven to activate $K_{ATP}$ channels [17]. We chose such a small dose to avoid substantial BP reduction with consecutive baroreceptor-mediated activation of the sympathetic nervous system. All patients were assessed at the outpatient clinic at regular intervals. Tolerability was assessed using spontaneously reported adverse events at each visit. For comparison of glomerular and tubular function, 10 healthy, normotensive control individuals without proteinuria were selected and matched according to age, sex, height, and body weight.

### Laboratory tests

Proteinuria was measured in 24-h urine samples by using the trichloroacetic acid technique [18]. Twenty-four-hour urine samples for the determination of proteinuria were provided by patients, who were carefully instructed about proper collection methods. To verify the completeness of 24-h urine collections, the [measured creatinine content/(140-age) $\times$ weight (kg) $\times$ 0.2 ($\times$0.85, if women)] ratio was calculated as described previously [19]. The ratio from 0.90 to 1.1 was recognized as a complete 24-h collection. Protein intake was monitored by 24-h urinary urea nitrogen excretion using the following formula [16]: protein intake (g/day) = nitrogen content of urea and nonurea nitrogen (an estimated value of 31 mg/kg for nonurea nitrogen) $\times$ 6.25. Renal function was estimated through measurement of creatinine clearance using the Cockcroft–Gault equation [20]. All patients had creatinine clearance of more than 50 ml/min per 1.73 m$^2$. In patients with preserved renal function, creatinine clearance is a good index of glomerular filtration rate.

Plasma and urinary samples for ET-1 measurements were collected and extracted as previously described [21]. Samples were immediately centrifuged at 3000 $\times$ $g$ for 10 min, and the plasma samples were stored at −70°C until further analysis. ET-1 plasma concentrations were measured using an immunoassay (R&D System Inc., Minneapolis, Minnesota, USA). The detection limit for plasma ET-1 was 1 pg/ml. There was less than 1% cross-reactivity with big-endothelin 22–38. The intraassay and interassay coefficients of variation were 4.5 and 6.6%, respectively. Results were expressed as pg/ml for plasma and ng/g of urinary creatinine for urine.

Urinary protein excretion is determined not only by glomerular permeability, but also by tubular reabsorption. To assess the roles of glomerular and tubular function in reduction of urinary protein excretion, we measured the changes of urinary immunoglobulin G (IgG) as a glomerular marker and retinol-binding protein as a tubular marker. Because 24-h urine samples would entail samples remaining at 37°C for several hours, which

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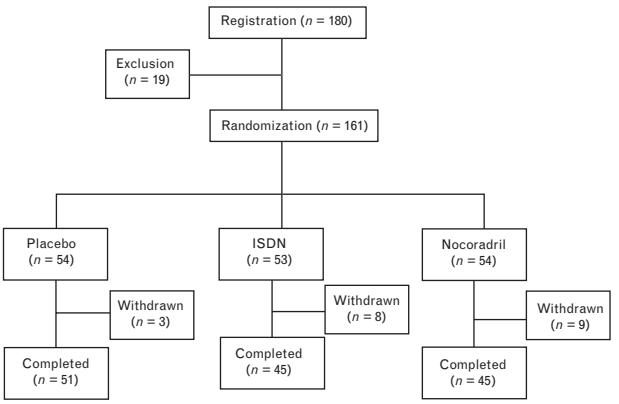
leads to the potential for significant losses of retinol-binding protein in the bladder [22], a random sample during daytime was collected instead. Polyclonal antibodies raised in rabbits against retinol-binding protein were obtained from Assaypro Ulc Company (Winfield, Missouri, USA). The detection limit was 8 μg/l. The intraassay and interassay coefficients of variation were 7 and 8%, respectively. Corrections for variations in urine flow rate can be made using the retinol-binding protein and creatinine ratio. Twenty-four-hour urine (protein excretion, creatinine, nitrogen, ET-1, and IgG), random urine (retinol-binding protein), plasma (creatinine and ET-1), and BP were measured at the beginning and at the end of the study. The protocol was approved by the ethics committee (no. IRB9507-003), and patients were required to sign an informed consent form before undergoing screening procedures.

**Fig. 1**




Randomization protocol. For exclusion criteria, see text. ISDN, isosorbide dinitrate.

### Statistics

All data were analyzed using Excel (Microsoft Inc., Seattle, Washington, USA) and SPSS (SPSS Inc., Chicago, Illinois, USA). Continuous variables were expressed as mean ± SD. For descriptive analysis, qualitative characteristics were summarized using median and interquartile range. Because many variables were not normally distributed, nonparametric statistical tests were used throughout the analysis. Proteinuria was analyzed after logarithmic transformation for its skewed distribution. For the categorical parameters, differences were compared by the chi-square test and by the Fisher exact test if the case number was less than 5. For multiple comparisons among three groups (three pairwise comparisons), the Mann–Whitney test was used for comparing each pair of groups, and the Bonferroni correction was applied to adjust the significance level. The relationships between the changes were analyzed by Spearman correlation and multiple linear regression. A $P$ value of less than 0.05 was considered statistically significant.

## Results

Of a total of 180 patients who were screened, 161 underwent randomization. Fifty-four patients were assigned to placebo, 53 were assigned to ISDN, and 54 were assigned to nicorandil (Fig. 1). The intention-to-treat population consisted of 160 patients (53 in the placebo, 53 in the ISDN, and 54 in the nicorandil). Of these patients, two in the placebo group, eight in the ISDN group, and nine in the nicorandil group did not complete the trial. There was a significantly higher withdrawal rate in the nicorandil group as compared with placebo ($P = 0.047$) because of headache. In addition, five patients had incomplete 24-hr urine collection assessed by the [measured creatinine content/(140-age) × weight (kg) × 0.2 (×0.85, if women)] ratio. Thus, a total of 136 evaluable patients completed the study (48 in the placebo group, 45 in the ISDN group, and 43 in the nicorandil group).

Table 1 presents the baseline demographic characteristics of patients in each group. The number of antihypertensive agents was similar in three groups, with a mean number of 3.0 in the placebo group, 2.9 in the ISDN group, and 2.6 in the nicorandil group. The average dose of valsartan was 92 ± 38 mg/day in the placebo group, 91 ± 35 mg/day in the ISDN group, and 97 ± 40 mg/day in the nicorandil group. There were no

**Table 1 Baseline clinical characteristics of patients**

| Parameters | Placebo ($n = 48$) | ISDN ($n = 45$) | Nicorandil ($n = 43$) |
|---|---|---|---|
| Age | 60 ± 9 | 62 ± 10 | 59 ± 11 |
| Sex (male/female) | 25/23 | 25/20 | 23/20 |
| Blood pressure (mmHg) | | | |
| Systolic | 124 ± 8 | 122 ± 10 | 120 ± 7 |
| Diastolic | 77 ± 5 | 78 ± 5 | 76 ± 6 |
| Duration of hypertension (years) | 14 ± 5 | 13 ± 6 | 15 ± 5 |
| BMI (kg/m$^2$) | 25.7 ± 2.3 | 26.4 ± 1.8 | 26.7 ± 1.4 |
| Cigarette smoking, *n* (%) | 28 (58) | 30 (67) | 25 (58) |
| Antihypertensive agents | | | |
| β-blocking agents, *n* (%) | 22 (46) | 23 (51) | 20 (47) |
| Calcium blocker, *n* (%) | 30 (63) | 28 (62) | 27 (63) |
| Diuretics, *n* (%) | 19 (40) | 21 (47) | 15 (35) |
| Statin treatment, *n* (%) | 26 (54) | 27 (60) | 25 (58) |

Data values are mean ± SD. ISDN, isosorbide dinitrate.

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**Table 2 Renal function and endothelin-1 at baseline and 6-month intervention therapy**

| | Placebo ($n=48$) | | ISDN ($n=45$) | | Nicorandil ($n=43$) | |
|---|---|---|---|---|---|---|
| Parameters | Baseline | Follow-up | Baseline | Follow-up | Baseline | Follow-up |
| Dietary protein intake (g/day) | 74 ± 8 | 72 ± 9 | 75 ± 10 | 74 ± 9 | 76 ± 9 | 75 ± 7 |
| Creatinine clearance (ml/min per 1.73 $m^2$) | 90 ± 13 | 92 ± 12 | 88 ± 16 | 92 ± 13 | 86 ± 14 | 90 ± 17 |
| Plasma samples | | | | | | |
| Creatinine (mg/dl) | 1.13 ± 0.28 | 1.05 ± 0.28 | 1.10 ± 0.23 | 1.15 ± 0.14 | 1.07 ± 0.25 | 1.03 ± 0.21 |
| ET-1 (pg/ml) | 1.67 ± 0.43 | 1.70 ± 0.50 | 1.72 ± 0.45 | 1.69 ± 0.48 | 1.66 ± 0.42 | 1.57 ± 0.44 |
| Urine samples | | | | | | |
| Protein excretion (mg/24 h) | 825 ± 483 | 809 ± 471 | 818 ± 557 | 823 ± 573 | 781 ± 536 | 438 ± 345*,† |
| ET-1 (ng/g urinary creatinine) | 32.3 ± 4.1 | 31.6 ± 5.9 | 32.4 ± 4.5 | 32.0 ± 4.5 | 30.8 ± 5.3 | 27.7 ± 5.1*,† |
| IgG (mg/24 h) | 24.7 ± 7.8 | 29.3 ± 7.5 | 25.3 ± 6.8 | 29.9 ± 8.3 | 26.9 ± 9.3 | 29.9 ± 8.5 |
| Retinol-binding protein (μg/mmol) | 305 ± 84 | 285 ± 53 | 288 ± 79 | 295 ± 75 | 293 ± 65 | 174 ± 59*,† |

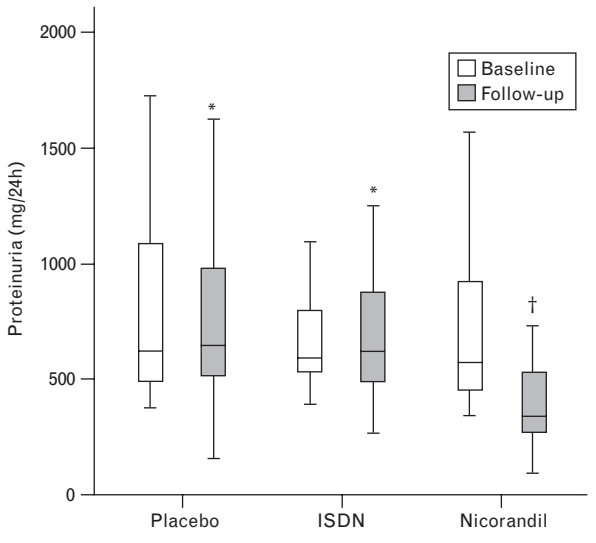
Data values are mean ± SD. ET-1, endothelin-1; IgG, immunoglobulin G; ISDN, isosorbide dinitrate. *$P<0.05$ compared with respective baseline data. †$P<0.05$ compared with placebo and ISDN-treated patients at follow-up.

significant changes in hemodynamic parameters after administering either ISDN or nicorandil. There was no difference at baseline or at 6 months in protein intake calculated from urea excretion among the three groups (Table 2). We observed no clinically meaningful changes in the parameters indicative of renal function such as plasma creatinine and creatinine clearance in the three groups during the course of the study.

### Effect on renal glomerular and tubular markers

The baseline values and the effect of nicorandil on renal glomerular and tubular markers are shown in Table 2. The baseline urinary excretion of markers for glomerular size selectivity, IgG, proximal tubular function, and retinol-binding protein were pathologically elevated in the three hypertensive groups compared with normal controls (11.7 ± 4.2 mg/24 h for IgG and 78 ± 25 μg/mmol creatinine for retinol-binding protein), which were similar to those in previous studies in healthy individuals [23]. Retinol-binding protein, a marker for proximal tubular function, was significantly reduced during nicorandil treatment compared with baseline, whereas IgG levels remained stable throughout the study.

**Fig. 2**




Box plot of the median and interquartile range of 24-h urine protein excretion at baseline and at follow-up. The ends of the boxes are the 25th and 75th percentiles; whiskers indicate the minimum and maximum values, excluding outliers. *$P<0.05$ compared with nicorandil-treated patients at follow-up; †$P<0.05$ compared with baseline data in the nicorandil group. ISDN, isosorbide dinitrate.

### Proteinuria and endothelin-1

Changes in urinary protein excretion are shown in Table 2 and Fig. 2. The urinary protein excretion did not differ among the three groups at baseline. In nicorandil-treated patients, proteinuria was significantly reduced by 44% (from 781 ± 536 to 438 ± 345 mg/24 h, $P<0.0001$) as compared with baseline values. As there was a large SD of proteinuria relative to the mean, the proteinuria amount was logarithmically transformed to a normally distributed variable. In the statistical analysis of the transformed values, the proteinuria amount remained stable throughout the study in the ISDN and placebo groups, whereas it was significantly reduced in patients treated with nicorandil. Urinary ET-1 levels were significantly reduced in nicorandil-treated patients, whereas plasma ET-1 levels remained stable throughout the study.

### Correlation

To identify determinants of the decrease in urinary protein excretion, a multivariate analysis was performed (Table 3). Multivariate regression analysis with log proteinuria as dependent variable and age, sex, changes in mean BP and urinary ET-1, and the presence or absence of nicorandil as independent variables was performed to investigate the effect of nicorandil on proteinuria. The use of nicorandil and urinary ET-1 were the factors significantly related to proteinuria regression ($P<0.0001$, adjusted $r^2=0.35$).

The linear regression models in the nicorandil-treated group showed that changes in ET-1 correlated with urinary protein excretion [change in urinary ET-1 (%) = 1.03* change in log proteinuria (%) – 1.21, $r=0.69$, $P<0.0001$, Fig. 3]. No significant correlation

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**Table 3 Multivariate analysis of independent factors for regression of proteinuria (model adjusted $r^2 = 0.35$)**

| Factor | Standardized β coefficient | 95% CI | *P* |
|---|---|---|---|
| Treatment assignment [nicorandil (+) vs. (−)] | 0.315 | 3.08–8.15 | <0.0001 |
| Urinary ET-1 (per additional %) | 0.453 | 0.14–0.27 | <0.0001 |
| Sex (male vs. female) | −0.043 | −3.02 to 1.58 | 0.54 |
| Age (per additional year) | −0.020 | −0.11 to 0.14 | 0.78 |
| Mean blood pressure (per additional mmHg) | 0.004 | −0.20 to 0.22 | 0.96 |

CI, confidence interval; ET-1, endothelin-1.

was observed between changes in proteinuria and hemodynamics (data not shown).

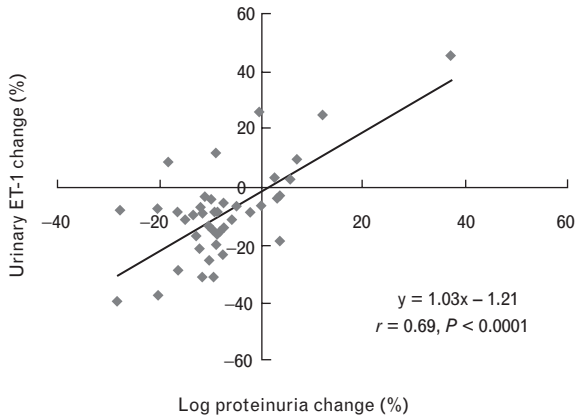
## Discussions

Our study is the first demonstration of the beneficial effects of nicorandil, but not ISDN, on proteinuria through the attenuation of $K_{ATP}$ channel-dependent ET-1 in patients with well controlled hypertension already treated with angiotensin II receptor blockers in a nitric oxide-independent manner. Indeed, our results were consistent with the findings of Zhao *et al.* [24], showing that the effect of nicorandil on modulating endothelial junctions was due to its effect on ET-1 via the activation of $K_{ATP}$ channels.

The effects of nicorandil and ISDN on proteinuria were significantly different, implying that $K_{ATP}$ channel activation is necessary for attenuating proteinuria. The beneficial effect of a $K_{ATP}$ channel agonist on proteinuria was supported by three lines of evidence:

(1) Oral nicorandil administered in addition to standard antiproteinuric therapy to hypertensive patients offers significant additional benefit by reducing proteinuria. Previous studies have shown that individual variability in antiproteinuric response to renin–angiotensin blockade is large, ranging from zero to complete remission of proteinuria [25]. Full remission of proteinuria is seldom obtained with drugs that antagonize the renin–angiotensin system alone, particularly if therapy is started late [26]. Thus, multidrug intervention has been proposed. The addition of $K_{ATP}$ channel agonists to the background therapy of angiotensin II receptor blockers led to a further striking reduction in proteinuria even in the late phase of hypertension with a mean duration of 15 years.

**Fig. 3**




Correlation of the change (%) between log proteinuria and urinary endothelin-1 in the nicorandil-treated patients ($r = 0.69$, $P < 0.0001$, $n = 43$). A greater decrease of urinary endothelin-1 release was associated with greater improvement of proteinuria. ET-1, endothelin-1

(2) The administration of nicorandil, but not ISDN, was associated with reduced proteinuria. Nicorandil, the only $K_{ATP}$ channel agonist in clinical use, has an opening effect on the K channel that activates $K_{ATP}$ channels and induces nitric oxide release in the same way as nitrates. The nitric oxide signaling pathway has been linked to the opening of mitochondrial $K_{ATP}$ channels [27], making it difficult to separate the two components of the dual mechanism of action of nicorandil. In this study, nicorandil treatment showed a reduction in proteinuria; however, ISDN, a nitric oxide donor, did not provide this renoprotective effect. This different biological effect implied that activation of $K_{ATP}$ channels plays a role in attenuating proteinuria. There were differences in the downstream pathway between nicorandil and ISDN. First, previous studies have shown that nicorandil enhanced endothelial nitric oxide synthase (eNOS) expression by activating $K_{ATP}$ channels [28]. In contrast, ISDN suppressed eNOS expression through cyclic GMP-mediated negative feedback regulation by nitric oxide [29]. eNOS has been shown to play a role in the pathogenesis of albuminuria. Epidemiological studies have shown that eNOS variant was positively correlated with a higher prevalence and a higher degree of albuminuria [30]. Second, long-term use of low potencies of organic nitrates such as ISDN leads to formation of the nitric oxide/superoxide reaction product, peroxynitrite, and causes endothelial dysfunction [31]. Endothelial dysfunction was associated with the severity of albuminuria [32].

(3) Our clinical data suggest a pathophysiological link between proteinuria and renal synthesis of ET-1. ET-1 has been consistently implicated to play a pivotal role in the pathogenesis of proteinuria. Transgenic mice overexpressing the ET-1 promoter have been shown to develop renal glomerular and

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tubular fibrosis [33]. Selective or nonselective blocking of endothelin receptors reduced proteinuria [15]. The patients in this study had glomerular and tubular dysfunction assessed by significantly higher excretion of IgG and retinol-binding protein compared with normal controls, implying that urine protein excretion can result from a loss of the glomerular barrier function and a decrease of tubular protein reabsorption. This is consistent with a previous report [34] of impaired glomerular and tubular function in hypertensive patients with proteinuria. Glomerular function remained unchanged during the treatment. A larger reduction in urinary retinol-binding protein excretion after nicorandil administration implied an improvement of tubular properties. Thus, nicorandil-induced reduction in proteinuria is mediated by improvement in tubular reabsorption rather than modifications in glomerular function.

The mechanisms by which nicorandil affects the proteinuria remain undefined. However, hemodynamic factors can be excluded. SBP and DBP were similar in the three groups at baseline and during follow-up, providing evidence that nicorandil attenuated urinary protein excretion through a hemodynamics-independent mechanism. The in-vivo administration of $K^+$ channel openers such as cromakalim and levcromakalim effectively reduces the BP of hypertensive patients and spontaneously hypertensive rats [35,36], which was not consistent with our stable hemodynamics throughout the study in nicorandil-treated patients. The discrepancy could be due to differences in protocols, patient population, and periods of treatment. In fact, our results were consistent with the Impact Of Nicorandil in Angina (IONA) trial findings [37], showing that there were no significant hemodynamic changes with administration of nicorandil even up to a dose of 40 mg/day. Our results lend further support to the findings from animal studies suggesting that the benefits of nicorandil may be independent of its hemodynamic or vasodilatory effects [38].

The role of ET-1 in the kidney is dissociated from circulating ET-1. Urinary ET-1 and plasma ET-1 are two distinct functional systems, each of which is regulated by its own control mechanisms. Because most of the plasma ET-1 filtered through glomeruli is subject to degradation by neutral endopeptidase in the proximal tubule, ET-1 in urine is of renal origin [39]. In this study, although nicorandil did not reduce plasma ET-1 levels, renal ET-1 production assessed by urinary ET-1 excretion was significantly reduced, implying the importance of the effect of nicorandil on renal local ET-1.

Although the present study suggests that the mechanisms of the nicorandil-induced antiproteinuric effect may be related to attenuated ET-1 expression and multivariate analysis showed a statistically significant relationship, the correlation coefficient between the two variables was 0.69, indicating that ET-1 explained only 48% of the variation in the proteinuria regression. Therefore, nicorandil-induced attenuated proteinuria cannot be attributed solely to urinary ET-1 levels and this stresses the importance of other factors such as effects on free radicals and vascular endothelial growth factor (VEGF). First, blockade of free radicals alleviated the progression of proteinuria [40], and nicorandil was shown to inhibit production of free radicals [41]. Second, the antiproteinuric effect of nicorandil may also result from increased expression of *VEGF*. Previous studies have shown that nicorandil enhanced capillary and arteriolar densities by inhibiting the downregulation of *VEGF* gene expression [42]. Increased expression of *VEGF* has been shown to attenuate proteinuria [43].

There are some limitations in the present study that have to be acknowledged. First, overall withdrawal from randomized treatment was more frequent on nicorandil than on placebo, the absolute difference in withdrawal rates being about 11%. The reason for withdrawal was adverse events in 17% of the nicorandil group compared with 6% on placebo. This trend was evident from the first visit, and was mainly due to the increased incidence of headache in the nicorandil group. The higher withdrawal rate in the nicorandil group leads to a tendency to underestimate the outcome benefit. Second, although Hollenberg *et al.* [44] have shown that there was a dose-dependent renoprotective effect of valsartan in type 2 diabetic patients, the average dose of valsartan was 93 mg in our study, far below the 'optimal' dose. There were several reasons why we did not use the 'optimal' dose. Valsartan has a similar increase in peak plasma renin activity in humans if the dose is more than 80 mg/day [45], implying a similar degree of renin–angiotensin system blockade. Previous studies used the 'regular' dose (80 mg daily) [7,46]. Furthermore, the patient population of the Hollenberg *et al.* [44] study was different from ours. We excluded diabetic patients in this study. Whether the conclusions obtained from type 2 diabetic patients can be extrapolated to hypertensive patients remains unknown. The dose–response studies of valsartan in hypertensive patients with proteinuria are not available. Finally, proteinuria is improved by nicorandil, but not ISDN, implying that renal $K_{ATP}$ channels may play a role in proteinuria. However, this study did not indicate a direct relation between the activation of $K_{ATP}$ channels and proteinuria by administering $K_{ATP}$ channel antagonists such as glibenclamide for ethical consideration.

## Conclusion

The present study demonstrates that addition of nicorandil, but not ISDN, to standard antiproteinuric therapy was associated with further reduction of urinary protein excretion mediated by reducing $K_{ATP}$-dependent renal ET-1 production and improving tubular function in

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patients with well controlled hypertension. The proposed therapeutic approach could add to the available armamentarium for proteinuric patients in which angiotensin II receptor blockers alone fail to prevent progressive renal injury.

## Acknowledgements

This work was supported by grant number DOH97-TD-I-111-TM001 from Department of Health, Taiwan, Republic of China.

There are no conflicts of interest.

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