The Role of Fasudil and Ang (1-7) in PAN Induced Nephropathy in rats

Rehab E. Abo El gheit, Marwa N. Emam

Medical Physiology Department, Faculty of Medicine, Tanta University

Abstract

Background/Aim: : Nephrotic syndrome (NS) is a potentially life-threatening state characterized by heavy proteinuria, hypoalbuminemia and edema. The underlying pathological mechanisms are diverse and have not been fully elucidated. Several molecular processes have been implicated in its pathophysiology including the Rho-GTPases and renin-angiotensin (RAS) systems. We evaluated whether ROCK inhibition , through fasudil and RAS interfering through Mas agonist, AVE 0991 could have renoprotective effect, and whether it is comparable to well settled renoprotection of type-1 angiotensin II (AT1) receptor blocker, Losartan in Puromycin aminonucleoside (PAN)-induced nephropathy rat model.

Materials & methods: Fifty adult male albino rats were divided into five groups (10 rats each), control (received 0.9 % NaCl). PAN- nephrotic (PAN – injected rats received 0.9 % NaCl), Fasudil treated PAN (30 mg/kg /d), Losartan treated PAN (10 mg/kg/ d) and AVE 0991 treated PAN (3 mg/kg /d) group. All treated groups received treatment orally, from day 7 to day 14 of PAN injection.

RESULTS: PAN injection induced marked renal dysfunction indicated by significant ($P<0.05$) proteinuria, hypoalbuminaemia, reduction in creatinine clearance and elevated serum creatinine associated with significant hyperlipidemia and increase in Tumor necrosis factor $-\alpha$ (TNF-α), C-reactive protein (C-RP) and urinary transforming growth factor -β_1 (TGF-β_1). Treated groups with either fasudil or AVE0991, presented with significant improvement in kidney functions and lipid profile with parallel significant reduction in inflammatory and fibrogenic cytokines that was similar to the renoprotection observed with Losartan.

CONCLUSION: These data demonstrate that (RAS) and Rho-kinase pathways are involved in renal damage of NS, and their intervention could provide renoprotection and represent a novel therapy for NS.

Corresponding author: Marwa N. Emam, Medical Physiology Department, Faculty of Medicine, Tanta University, Egypt

Mob.: 01224089068,   Email : maroo_drmaroo@yahoo.com

INTRODUCTION

NS is a clinical condition with a high morbidity and mortality that often leads to end-stage renal failure with a high risk of cardiovascular complications due to severe hyperlipidemia (1).

At present, no promising therapy is available due to lack of understanding of signaling culprits involved in the pathogenesis of nephrosis (2). Unfortunately, these therapies only retard its progression, but do not prevent or reverse the renal function loss, indicating the need for additional therapeutic approaches (3).

RhoA, a member of the Ras superfamily of small GTP-binding proteins, and its down-stream effectors Rho associated kinases (ROCKs) are known to act as molecular switches controlling several critical cellular functions (4).

Accumulating evidence suggests that Rho/ (ROCK) signaling pathways may play important roles in kidney biology, which probably have homeostatic functions under normal physiological conditions, appear to be most highly activated under conditions of inflammation and injury and up-regulated in NS (5).

Fasudil is the first-generation Rho/ROCK inhibitor (6). It is the only Rho-kinase inhibitor practically available for long-term *in vivo* use (7). Fasudil is mainly metabolized to an active metabolite (hydroxyfasudil) when administered *in vivo*, this metabolite still retains activity with more selective sustained action than its parent drug on Rho-kinase. It specifically inhibits RhoA kinase activity by competing for ATP binding. It is mainly excreted by the kidney (8).

The new biologically active angiotensin (1–7) (Ang1-7), a metabolite of angiotensin I and II, is considered the most pleiotropic component of the (RAS), reported as a counter regulatory mediator of Ang II (9). It was detected at different nephron segments at both cortical and medullary regions of the kidney, acting at both glomerular and tubular sites, producing complex renal effects (10).

AVE 0991 is the first nonpeptide orally active analog of Ang (1-7), Mas receptor agonist, which has a 10-fold higher affinity for Mas receptor than Ang (1-7), AVE 0991 mimics Ang (1-7) effects in several organs including kidneys (11).

At present, the concept of the RAS as a dual axis system: one axis represented by angiotensin-converting enzyme (ACE)/AngII/AT1R and the other by ACE2/Ang-(1–7)/Mas has been settled (12). The balance between their activation plays an important role in the biological functions of different systems and an imbalance in these opposing pathways toward the ACE/AngII/AT1R axis predispose to many cardiovascular and renal diseases (13).

Losartan is a selective nonpeptite (AT1) receptor antagonist, has been applied in medical treatments of a variety of cardiovascular and renal diseases (14).

The observation that NS is frequently associated with over activation of RAS/Rho systems has drawn the attention to the possible interplay between the RAS and Rho axes. Such possibility was prompted by clinical trials and experimental studies (15).

The present study aimed to investigate whether Mas receptor activation with AVE 0991 and Rho Kinase inhibition by Fasudil, would have renoprotective effect in PAN induced NS, and

whether this effect was relevant to the well-known renoprotection elicited by AT1 receptor blocker, Losartan treatment, that has been used widely as first-line therapy to reduce the progression of chronic renal diseases, with well-documented effectiveness.

MATERIALS AND METHODS

Experimental Animals:

All animal experiments were undertaken with approval of Ethical Animal Research Committee of Tanta University. Fifty adult male albino rats weighing (200-250g) were purchased from Faculty of Science (Tanta University). The animals were housed at temperature 22±2 ◦C and 12 h dark/light cycle throughout the study.

Induction of experimental nephrotic syndrome:

NS was induced by single intravenous injection of 70 mg/kg body weight of the puromycin amino nucleoside (PAN) (Sigma) diluted in 0.9% saline (16). For consistency, day 0 was taken as the day of PAN injection. PAN -induced nephrosis is a well-described model of proteinuria, resembling the functional and morphological aspects of human minimal change disease or early stage focal segmental glomerulosclerosis (17).

Experimental Protocol:

Rats were randomly divided into five groups (10 rats each). ***Control group*****:** received an equivalent volumes of sterile vehicle (0.9 % NaCl solution). *(PAN) nephrotic group:* PAN – injected rats received an equivalent volume of sterile vehicle (0.9 % NaCl solution). ***Fasudil treated PAN group****:* Fasudil (Sigma Aldrich) is given orally at a dose of 30mg/kg/d, after PAN – injection. ***Losartan treated PAN group:*** Losartan

is given orally at a dose of 10mg/kg/ d, after PAN – injection. ***AV0991 treated PAN group:*** AV0991 (Aventis Pharma Deutschland, Frankfurt, Germany) is given orally at a dose of 3 mg/kg /d, after PAN – injection.

For the experiment, urine was collected from the day prior to PAN injection and on days 2 and 7 after injection. All treatment intervention started, after stabilization of proteinuria i.e. from the day (7) and continued until the end of experiment (till day 14), as an attempt to mimic the real clinical situation i.e. patients presented with some degree of injury, but not full blown renal damage. Previous studies have shown that in (PAN) rat model, daily urinary protein excretion was dramatically elevated on day 5, reaching peak on day 9 and gradually return to normal level from day 15 -27 (2).

Biochemical assay:

At the end of the study period, rats in each group were individually housed in metabolic cage for 24h urine collection. Total urine volume was measured; one ml was collected from the 24 h urine sample, and used for measurement of total proteinuria and Creatinine clearance.

Collection of Blood Sample:

Rats were decapitated. Blood was collected and centrifuged at 1000 rpm and sera stored at -20°C till biochemical analysis.

The following biochemical parameters were investigated:

Serum Creatinine was measured according to the alkaline picrate method (18). **Serum albumin** was measured according to method of Doumas (19). **Serum total cholesterol** was measured by

enzymatic methods by using diagnostic kit (Biodiagnostic, Egypt) according to the method of Allain et al (20). **Serum triglycerides** were measured by enzymatic methods by using commercially available diagnostic kit (Biodiagnostic, Egypt) according to the method of Fossati and Prencipe (21). **Total Urinary protein concentration** was measured using the method of Bradford (22). **Tumor necrosis factor–α (TNF-α)** was measured according to method of aderka (23) using TNF-α enzyme immunoassay kit (Biosciences, Egypt). **C-reactive protein (C-RP)** was measured by ELISA-based test kits according to Kindmark and Jean (24). **Urinary TGF-β1.,** since TGF-β1 has been considered a potential biomarker of renal tissue fibrosis (25), this cytokine was measured in 24-hour urine samples. Level of TGF-β1 in the urine was assessed by ELISA according to method of Tsakas (26). Sample of the urine were collected in metabolic cages and stored at -20ºC. Until refrigeration, 10 mL of commercial protease inhibitor cocktail (Sigma Aldrich) were added at urine sample. Results were expressed as relative units of cytokine per mg of urinary creatinine.

Statistical analysis:

All values were expressed as mean ± SD. Statistical analysis was performed by GraphPad Prism software, release 4.0 (GraphPad Software, San Diego, CA). The data obtained from various groups were statistically analyzed using unpaired t test followed by one-way ANOVA. The *P* value < 0.05 was considered to be statistically significant.

RESULTS

Effect of PAN injection, on kidney functions and lipid profile in experimental animals:

All experimental animals injected with PAN developed nephropathy characterized by marked renal injury as reflected by heavy proteinuria and hypoalbuminemia. As shown in table (1) & figure (1), PAN injection, resulted in significant increase in proteinuria with significant reduction in serum albumin versus control group. Compared to control group, serum creatinine was significantly elevated together with significant reduction in creatinine clearance in PAN induced nephrotic group.

As shown in table (2) & figure (2), PAN administration caused marked hyperlipidemia, where there was significant elevation in both total cholesterol and triglyceride levels compared to control group.

Effect of PAN injection, on fibrogenic and inflammatory biomarkers in experimental animals:

PAN injection resulted in significant increase in C-RP, TNF-α and in urinary TGF-β$_1$ compared to control group (table 3 & figure 3).

Effect of ROCK inhibition and Mas receptor activation on kidney functions in PAN induced nephrotic rats:

As shown in table (1) & figure (1), treatment with Rho Kinase inhibitor, fasudil had marked beneficial effects on PAN-induced renal dysfunction. There was significant reduction in urinary protein excretion associated with significant improvement in serum albumin compared with PAN induced nephrotic rats.

Indeed, serum creatinine was significantly decreased and creatinine clearance was significantly improved in fasudil treated nephrotic versus PAN induced nephrotic group.

Parallel experiments were carried out with the AT1 receptor antagonist, Losartan and Mas receptor agonist, AVE 0991 which had similar protective effects on renal parameters, to those of fasudil, suggesting cross talk between Rho/ (ROCK) signaling pathways and the RAS and their possible participation in pathogenesis of NS.

Effect of ROCK inhibition and Mas receptor activation on lipid profile in PAN induced nephrotic rats:

Fasudil treatment resulted in marked improvement in dyslipidemia (table 2 & figure 2),

there were significant reduction in total cholesterol and triglyceride level compared to nephrotic group. Comparable results obtained with both Losartan and AVE 0991 as shown in table (2) & figure (2), there was significant reduction in nephrotic hyperlipidemia.

Effect of RhOK inhibition and Mas receptor activation on fibrogenic and inflammatory biomarkers in PAN induced nephrotic rats:

Fasudil treatment of PAN induced nephrotic rats resulted in significant reduction in C-RP, TNF-α and urinary TGF-β_1 levels (table 3 & Figure 3). These results were similar to those obtained with Losartan and AVE0991 treatment with significant reduction in fibrogenic and inflammatory parameters.

Table (1): Effect of Rho Kinase inhibitor, Fasudil (30 mg/kg), Losartan (10 mg/kg) and Mas receptor agonist, AVE 0991 (3 mg/kg), on kidney functions in PAN induced nephrotic rats.

Group parameter	control group	PAN nephrotic group	Fasudil treated PAN group	losartan treated PAN group	AVE0991 treated PAN group
Urinary proteins (mg/24h)	7.83±2.99	110.02±24.42[a]	68.59±26.74[b]	71.899±21.536[b]	73.37±16.56[b]
serum creatinine (mg/dl)	0.45±0.212	0.91±0.16[a]	0.66±0.18[b]	0.71±0.15[b]	0.72±0.17[b]
Creatinine clearance (ml/min)	1.39±0.20	0.58±0.10[a]	0.86±0.10[b]	0.84±0.11[b]	0.81±0.14[b]
Plasma albumin (µg/L)	2.551± 0.47	1.566±0.41[a]	2.252±0.48[b]	2.135±0.32[b]	2.107±0.46[b]

[a] $P < 0.05$; versus control group. [b]$P < 0.05$; versus PAN - induced nephrotic group . All values are expressed as mean ± SD of 10 rats in each group.

Table (2): Effect of Rho Kinase inhibitor, Fasudil (30 mg/kg), Losartan (10 mg/kg) and Mas receptor agonist, AVE 0991 (3 mg/kg), on lipid profile in PAN induced nephritic rats.

Group parameter	control group	PAN nephrotic group	Fasudil treated PAN group	losartan treated PAN group	AVE0991 treated PAN group
Total cholesterol (mg/dl)	84.43±14.55	137.40±36.15[a]	104.39±16.65[b]	106.03±10.64[b]	105.24±9.95[b]
Triglyceride (mg/dl)	77.63±11.14	143.53±27.85[a]	107.39±15.72[b]	114.96±26.40[b]	110.37±15.94[b]

[a] $P < 0.05$; versus control group. [b]$P < 0.05$; versus PAN - induced nephrotic group . All values are expressed as mean ± SD of 10 rats in each group.

Table (3): Effect of Rho Kinase inhibitor, Fasudil (30 mg/kg), Losartan (10 mg/kg) and Mas receptor agonist, AVE 0991 (3 mg/kg), on fibrotic and inflammatory markers in PAN induced nephrotic rats.

Group parameter	control group	PAN nephrotic group	Fasudil treated PAN group	losartan treated PAN group	AVE0991 treated PAN group
Urinary TGF-β1 (R.U/mg creatinine)	61.57±16.38	206.97±66.48[a]	115.07±32.94[b]	120.08±33.44[b]	112.56±24.84[b]
C-RP (mg/dl)	0.20±0.06	0.31±0.07 [a]	0.24±0.040[b]	0.25±0.038[b]	0.246±0.040[b]
TNF-α (ng/ml)	4.75±0.94	7.40±1.39 [a]	5.99±1.17[b]	6.20±1.14[b]	6.16±0.73[b]

TGF-β1., Transgrowth factor –β1, C-RP.,c-reactive protein ,TNF-α., Tumor necrosis factor –α

[a] $P < 0.05$; versus control group. [b] $P < 0.05$; versus PAN - induced nephrotic group

All values are expressed as mean ± SD of 10 rats in each group.
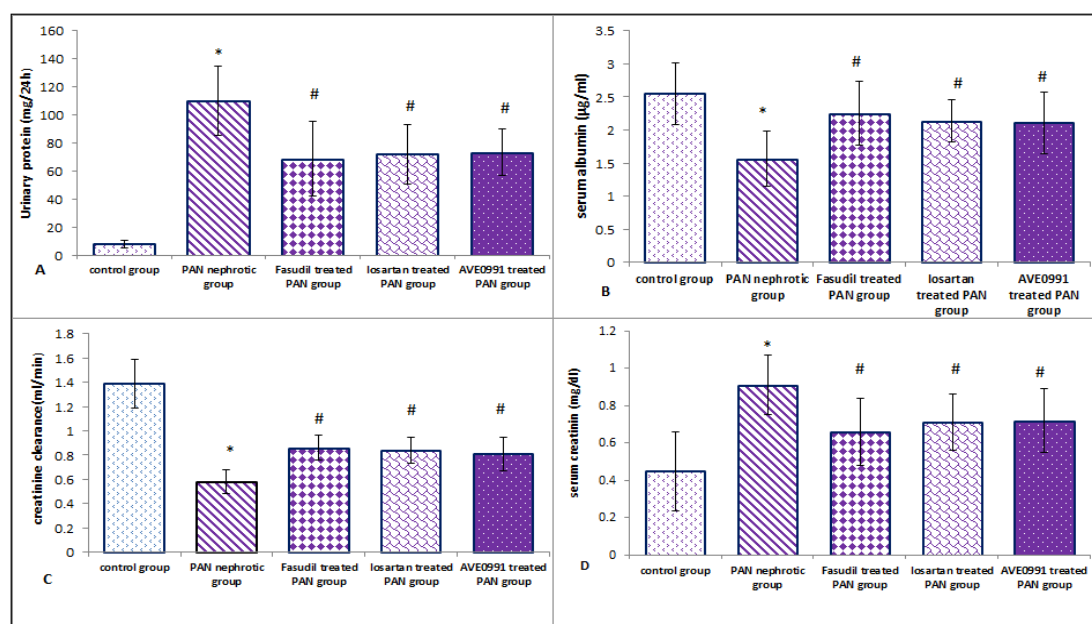

Figure (1): Effect of the treatment with Rho Kinase inhibitor, Fasudil, the Mas receptor agonist, AVE 0991 and the AT1 receptor blocker, Losartan on PAN induced renal injury. Data are means ± SD. ($n = 10$). * $P < 0.05$; versus control; # $P < 0.05$; versus PAN- nephrotic group.

DISCUSSION

The major findings of the present study can be summarized as follows: (i) treatment with fasudil, significantly improved renal function parameters, reduced urinary protein loss with improved creatinine clearance and lipid profile in rat model of PAN-induced nephrosis (ii) The renoprotective actions of fasudil were very similar to those produced by Losartan and AVE 0991 (iii) The renoprotection obtained in our study was associated with parallel reduction in urinary level of the fibrogenic cytokine, TGF-β₁ and inflammatory biomarkers, TNF-α, C- RP.

Multiple mechanisms have been suggested to explain the protective role of Rho-kinase inhibitor, fasudil in the nephrotic diseases. Among them the effect of fasudil on renal microvascular tone ,where fasudil dilates both afferent and efferent arterioles with prominent action on afferent arterioles, mediating glomerular hemodynamics changes involving autoregulation of glomerular
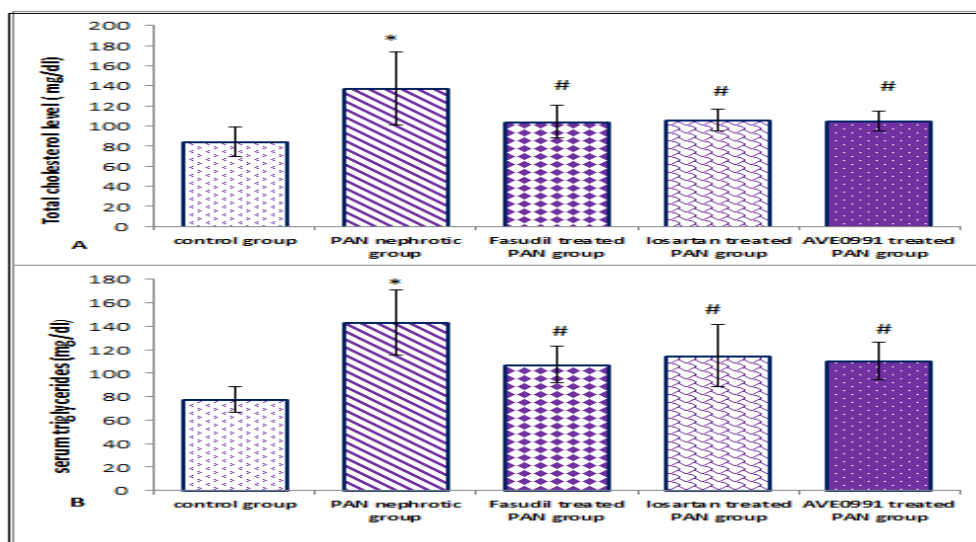
Figure (2): Effect of the treatment with Rho Kinase inhibitor, Fasudil, the Mas receptor agonist, AVE 0991 and the AT1 receptor blocker, Losartan on lipid profile in PAN induced nephrotic rats. Data are means ± SD ($n = 10$). * P < 0.05; versus control; # P < 0.05; versus PAN- nephrotic group.
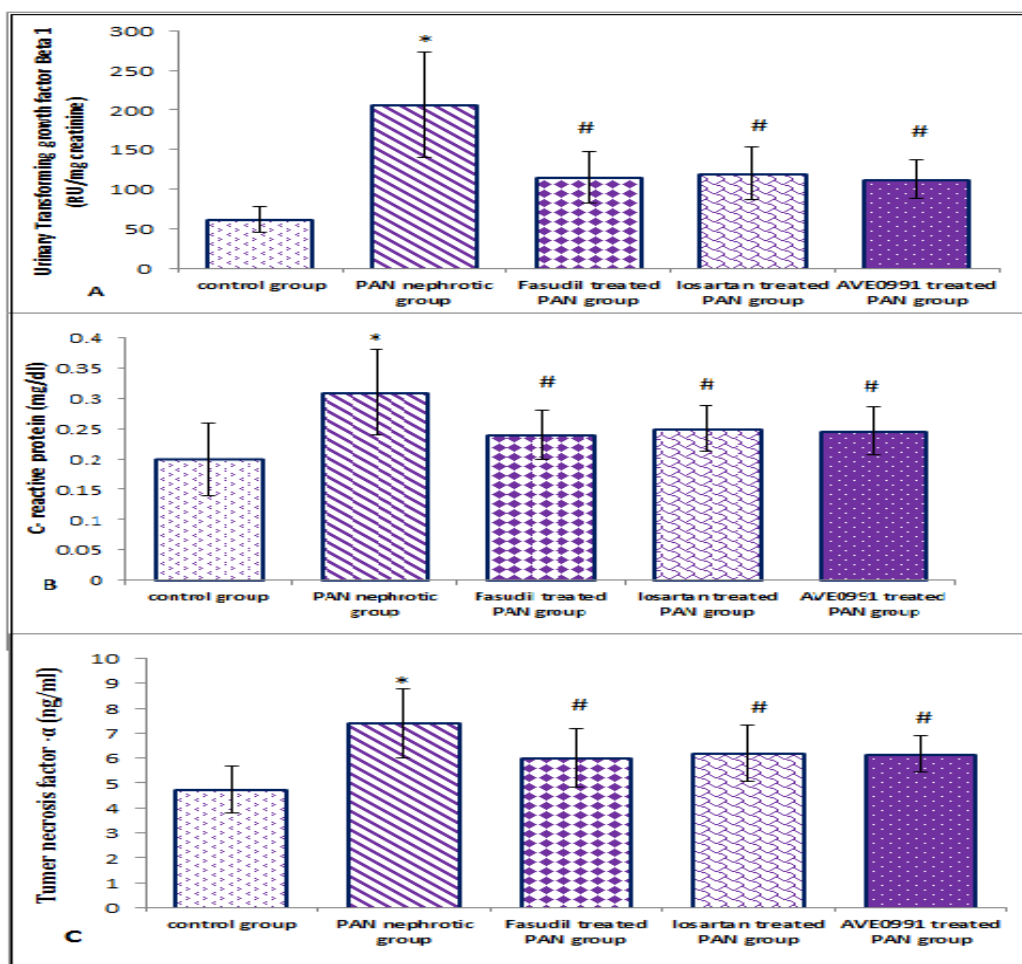

Figure (3): Effect of the treatment with Rho Kinase inhibitor, Fasudil, the Mas receptor agonist, AVE 0991 and the AT1 receptor blocker, Losartan on fibrotic and inflammatory cytokines in PAN induced nephrotic rats. Data are means ± SD. (n = 10). * P < 0.05; versus control; # P < 0.05; versus PAN- nephrotic group.

filtration rate and renal blood flow , through myosin light chain phosphatase inhibition, which promotes phosphorylation of myosin light chains and smooth muscle contraction . Moreover fasudil could modify renal Rho-kinase activity probably through decreased mechanical stress (27).

The renal protective effect of fasudil , may also be explained by decreasing the loss of glomerular nephrin expression at both the mRNA and protein levels, maintaining the integrity of the glomerular filtration barrier. It is proved through several *in vivo* studies, in human and animal that nephrin level is decreased in NS (28). Hidaka T et al demonstrated previously that fasudil reversed AngII induced the down-regulation of nephrin expression in podocytes (29).

Fasudil could preserve podocyte viability via stabilization of particular a slit diaphragm proteins, so attenuating progressive podocyte injury, recorded in NS (30).

The antioxidant effects of fasudil reported previously in hypercholesterolemic rats (31).The antioxidant effect of fasudil could be attributed not only to direct inhibition of ROS production through down-regulation of NADPH oxidases(32), but also indirectly through inhibition of oxidative stress – induced expression of pro-inflammatory cytokines and adhesion molecules (33) .

Kanda et al., (34) documented anti-proliferative, anti- inflammatory effects of fasudil through up-regulated expression of a cyclin-dependent kinase inhibitor, p27kip1 in renal tissues which safeguards against inflammatory injury and counter abnormal cell growth.

Fasudil, could obliterate many injury promoting effects associated with Rho- signaling cascades, that proved to be implicated in nephrosis, through decreasing the expression of TGF-β mRNA (35) , that upregulated significantly in the renal cortex of PAN models(36), and suppress both extracellular matrix accumulation and α-SMA expression (37) and its associated signal pathways mediated TGF-β effect(38).

Recent findings derived from targeting ROCK inhibition , recorded the potential role of fasudil as antiapoptotic in CsA induced nephropathy model, through modulation of mitogen-activated protein kinase (28),and as anti-inflammatory in the unilateral ureteral obstruction model, mediated by inhibition of macrophage migration and proliferation (39) .

The renoprotective effect of fasudil, associated with improved lipid profile in PAN nephrosis, supporting the fact that Rho-kinase constitutes a critical determinant acting as a key mediator for cross-talk between metabolic and hemodynamic abnormalities in renal diseases. Statins that proved to inhibit the biological actions of Rho, reported as first-line agents in the management of nephrotic dyslipidemia, through inhibiting the early step in the cholesterol synthesis (30). Kikuchi Yet al., (40) reported improved dyslipidemia with fasudil, at least in part, through the improved adipocyte differentiation.

The favorable effect of fasudil may be augmented by inhibition of its elimination in nephrosis as its metabolite; hydroxyl fasudil is mainly excreted by the kidney (8).

Oral administration of the Mas receptor agonist, AVE 0991 significantly improved renal function parameters, improved dyslipidemia.Several studies have shed more light on the underlying molecular mechanisms behind the effect of AVE 0991, including NO synthase phosphorylation,

phospholipase A2 stimulation and consequently release of arachidonic acid for prostanoid production and finally, prostacyclin-mediated production of cAMP and activation of cAMP-dependent protein kinase, leading to increase NO, prostaglandins production and bioavailability (41).

AVE0991, could have antiproliferative , anti-inflammatory effect, that was explained by down regulating the mitogen-activated protein kinase cascade, particularly p38 MAPK phosphorylation(42) ,COX (cyclooxygenase)-2 reduction (43), or by modulating ROS production through inhibited NADPH oxidase expression, contributing to the renoprotection (44) .

Our data regarding decreased urinary TGF-β1 level with AVE 0991 administration, support an anti-fibrogenic role for ACE2-Ang-(1–7)-Mas receptor axis at renal tissue, that could attributed either to decrease TGF-β levels or expression in rat proximal tubular cells . The renoprotective effect of AVE 0991, previously reported by Silveira et al.,(10), Giani et al.,(45) and Barroso et al., (46).

The beneficial effect of AVE0991, on dyslipidemia in our model could be attributed to the Ang-(1–7)-induced lipolysis via a Mas/PI3K/eNOS signaling pathway (9,47).

The renoprotective action of Losartan, recorded previously in nephrotic model (48), clearly involve multiple pathways including preserved glomerular nephrin expression (49), antiproliferative effect through prostaglandins stimulation and cAMP production (50), anti-inflammatory effect , through reduction of pro-inflammatory cytokines asTNF-α, IFN-γ, suppressed expression of inflammatory genes (51) and increase of the anti-inflammatory cytokine, IL-10 and decreases leukocyte endothelial cell interactions (52).

The proximal renal tubule is innervated by renal sympathetic nerve termini, whose subsequent catecholamine release in turn stimulates α2 / β1 receptors. AT1 receptors are located at the nerve terminals. Losartan is also able to attenuate activation of the intrarenal renin-angiotensin system by inhibiting renal sympathetic outflow (53) .

Extensive evidence suggests a direct link between the RAS and TGF-β, concluding TGF-β1 as Ang II downstream (54) . Reduced urinary TGF-β1 level observed with losartan , reflected the antifibrotic role that could be explained by reduced the renal   TGF-β mRNA expression with inhibition of the Smad2 pathway(55), as suggested previously by Crowley et al(56). Another possible antifibrotic TGF-β-independent mechanism could be elicited by Losartan, is blocking the Ang II-stimulated phosphorylation and activation of ERK1 and ERK2 and MAPK activity (57).

The antiproteinuric effect of Losartan relies on the decrease in glomerular pressure due to their preferential vasodilatory effect on the glomerular efferent arterioles, rather than on their antihypertensive effect per se, However direct podocyte protection can't be excluded (58) .

The improvement in nephrotic proteinuria and hypoalbuminemia observed with Losartan, is expected to reduce hyperlipidemia since those conditions contribute independently to the impaired lipoprotein catabolism in NS (59). In addition, Losartan may have a direct lowering effect on plasma triglyceride concentration, even though direct effects on intrinsic mechanisms resulting in NS cannot be ruled out (2), which could contribute to the overall effect of Losartan .

Several works demonstrated AT1 receptor blockers, including Losartan, associated with

altered balance between Ang II and Ang-(1–7), with increased endogenous Ang-(1-7) level by as much as 25-fold (60) .Moreover, the Losartan effects were blunted in mice with genetic deletion of Mas receptor (61),supported by the immunohistochemical data ,which have shown a similar distribution for Ang-(1–7), ACE2 and Mas within the kidney, placing the key components together for activation (10) .

Taken together, these findings indicated that ACE2/Ang-(1–7)/Mas receptor axis activation participate in the renoprotection triggered by Losartan, and AVE0991 (through Mas) and Losartan have many overlapping actions.

The results obtained with fasudil were comparable to those of Losartan and AVE0991.This is supported by the fact that AT1 receptors activation has been linked to Rho activation (62). The expression of Rho-kinase itself is accelerated by ang II (63). More importantly, Rho-kinase is substantially involved in the vascular effects of ang II (8). Indeed, some of the beneficial effects of AT1 receptors blockers in disease processes may be mediated by decreasing ANG2-dependent Rho activation .So it is possible that some of the beneficial effects of ROCK inhibition in the present study are mediated by antagonizing downstream effectors of ANG2-dependent Rho activation. So several lines of evidence assumed that Rho-kinase inhibitors could cover the pharmacological effects of many conventional drugs, including statins, (ACE) inhibitors, AT1 receptor blockers (64).

Conclusion: Based on the overall promising studies and the results obtained from the current investigation, we may conclude that RAS / RHO systems have important implications in NS and

constitute an important determinant as a central molecule linking several underlying pathological process. So ROCK inhibition or Ang-(1–7)/Mas activation, could obliterate many injury promoting effects associated with underlying signaling cascades and could be a good candidate for treating NS and its complications, but further researches should be done to strengthen our results.

In any case, new opportunities and questions emerge to resolve the complexity of renal nephrosis and discovery of possible new drugs directed to intracellular signal transduction pathways such as AVE0991 and fasudil that may be combined with the classical treatment schemes for more effective therapy.

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الملخص العربى

دورالفازوديل والانجسيوتنسين (1-7) في الإعتلال الكلوي المستحدث عن طريق حقن البوروميسين في الفئران

الخلفية العلمية والهدف من البحث:تعد المتلازمة الكلوية حالة ربما تهدد الحياة و تتميز بكثرة البروتين فى البول ، نقص البومين الدم و الإستسقاء. وعلى الرغم من أن الآليات المرضية الكامنة وراء هذه المتلازمة متنوعة و لم يتم توضيحها بالكامل، إلا أن العديد من العمليات الجزيئية قد أدخلت لتفسير الفيزيولوجيا المرضية منها أنظمة الروكاينيز و الرينين أنجيوتنسين. فى هذه الدراسة قمنا بتقييم ما اذا كان تثبيط نظام الروكاينيز من خلال تناول الفازوديل والتدخل في نظام الرينين أنجيوتنسين من خلال تناول (AVE0991) الناهض لمستقبلات الماس ، له تأثير واقى للكلى و ما إذا كان هذا التأثير يضاهي التأثير المتعارف عليه لمثبط المستقبلات 1 للأنجيوتنس 2 (لوسارتان) ، فى الفئران المصابة بالمتلازمة الكلوية عن طريق حقن البوروميسين.

طرق البحث: تم إجراء البحث على خمسين من ذكورالفئران البيضاء وقد تم تقسيمهم إلى خمس مجموعات تتكون كل منها من 10فئران.

1. **المجموعة الضابطة:** تم اعطائها محلول ملح بتركيز 0.9 ٪ ،
2. **المجموعة المصابة بالمتلازمة الكلوية** : تم حقنها بالبوروميسين بجرعة ٧٠ مجم/ كجم من وزن الجسم، عن طريق الوريد ،
3. **المجموعة المصابة بالمتلازمة الكلوية و المعالجة بالفازوديل** بجرعة ٣٠مجم/كجم من وزن الجسم يوميا،
4. **المجموعة المصابة بالمتلازمة الكلوية و المعالجة باللوسارتان** بجرعة ١٠ مجم/كجم من وزن الجسم،
5. **المجموعة المصابة بالمتلازمة الكلوية و المعالجة ب AVE0991** بجرعة ٣ مجم/كجم من وزن الجسم يوميا.

تم إعطاء الأدوية لجميع المجموعات عن طريق الفم ، من اليوم السابع و حتى اليوم الرابع عشر من حقن البوروميسين.

نتائج البحث: نتج حقن البوروميسين عن خلل ملحوظ في وظائف الكلى ذا دلالة إحصائية كما يستدل عليه بزيادة البروتين فى البول، نقص البومين الدم، نقص تصفية الكرياتينين، ارتفاع الكرياتينين فى مصل الدم مصحوباً بإرتفاع ـ ذا دلالة إحصائية ـ في مستوى كل من الدهون فى الدم، عامل نخر الورم ألفا فى الدم، بروتين سى التفاعلى و زيادة عامل النمو التحويلي للبول بيتا ١. وأظهرت المجموعات المعالجة سواء بالفازوديل أو ب AVE0991 تحسن ـ ذا دلالة إحصائية ـ فى وظائف الكلى ، مستوى الدهون فى الدم و انخفاض مواز في مستوى السيتوكينات الالتهابية و التليفية، و جاءت هذه التغيرات مماثلة لتأثير اللوسارتان الواقى للكلى.

الخلاصة: نستنتج من هذه الدراسة أن كل من مسارات الروكاينيز و الرينين أنجيوتنسن تساهم في إعتلال الكلى الناتج عن المتلازمة الكلوية وأن التدخل بتهيئة هذه المسارات يمثل حماية للكلى ، و بالتالي يمثل طريقة جديدة لعلاج المتلازمة الكلوية.