Protective Effects of Aliskiren, Enalapril and Valsartan on Hypertension Target Organs in Spontaneously Hypertensive Rats

Kalina Gjorgjievska1, Maja Slaninka-Miceska1, Gordana Petrucevska2

1Department of Preclinical and Clinical Pharmacology and Toxicology, Medical Faculty, University “Ss Cyril and Methodius”, Skopje, Republic of Macedonia; 2Department of Pathology, Medical Faculty, University “Ss Cyril and Methodius”, Skopje, Republic of Macedonia

Abstract
Aim: Aliskiren is the first orally active renin inhibitor approved for treatment of essential hypertension. There are as yet no clinical data regarding the ability of aliskiren to prevent clinical end-points or reduce end-organ damage over and above the presumed effects of blood pressure reduction. The aim of this study was to find out if aliskiren has a potential to improve renal function and if it has a protective effect on hypertension target organs: (kidney, heart and aorta).

Materials and Methods: The study was conducted on 64 SHR rats (male and female), 20 weeks old, weighing from 250-300 g. Animals were divided into 4 groups and treated with aliskiren (100 mg/kg bw/24 h), enalapril (10 mg/kg bw/24 h) and valsartan (10 mg/kg bw/24 h). Effects of investigated drugs were evaluated by urinalysis and histopathological analysis.

Results: Diuresis was significantly increased in the group of animals treated with enalapril and valsartan. Effect on diuresis with aliskiren was mild and statistically insignificant. No significant difference in urine pH between treated and control group of SHR rats was noted. Albuminuria was decreased in animals treated with enalapril and valsartan. Aliskiren after 28 days of treatment had a statistically insignificant effect on albuminuria in SHR rats. Only certain changes of wall thickness of arteries and arterioles were noted that led to improved perfusion and function of the kidneys more pronounced in rats treated with valsartan and enalapril. Effects of aliskiren on target organs were inferior compared to valsartan and enalapril.

Conclusion: In SHR experimental model aliskiren has minor effects on hypertension target organs when compared to enalapril and valsartan.

Introduction
The renin - angiotensin system (RAS) is a key regulator of blood pressure and body fluid volume, acting primarily via the effects of angiotensin II (Ang II). Formation of Ang II involves two main steps: renin cleaves angiotensinogen to form Ang I, and then Ang I is converted into Ang II by angiotensin-converting enzyme (ACE). RAS activity is the major underlying determinant of many pathological states, because Ang II increases blood pressure and aldosterone levels and has direct growth-promoting effects on cardiac, vascular and renal tissue that contribute to end-organ damage [1].

RAS inhibitors such as ACE inhibitors and AT1-
receptor blockers have proven to be highly successful treatments for disorders such as hypertension [2], heart failure [4], left ventricular dysfunction after myocardial infarction [3, 5, 6], and renal conditions [7-9]. Renin inhibitors would similarly be expected to lower blood pressure and provide organ protection benefits because they target the first step of the RAS, and thereby block the pathway at its source. Aliskiren is the first orally active renin inhibitor approved for treatment of essential hypertension [10]. There are as yet no clinical data regarding the ability of aliskiren to prevent clinical end points or reduce end organ damage over and above the presumed effects of blood pressure reduction per se. Although there are supportive data from animal studies, it is also unknown whether aliskiren will prove to be superior, equal, or inferior to ACE inhibitors or AT1-receptor blockers in terms of end organ protection [11].

The aim of this study was to determine if aliskiren has a potential to improve renal function and if it has protective effect on hypertension target organs: kidney, heart and aorta. Effects of aliskiren were compared with effects of enalapril (ACE inhibitor) and valsartan (AT1-receptor blocker) when given in spontaneously hypertensive (SHR) rats.

Materials and Method

The study was conducted on 64 SHR rats (male and female), 20 weeks old, weighing from 250-300 g. SHR rats were used at the Department of Preclinical and Clinical Pharmacology and Toxicology, Medical Faculty, Skopje. Animals were kept in cages, under controlled light and temperature, fed with a normal rat chow and had a free access to tap water. Animals were divided into 4 groups and treated according to the following dosage regimen: -1st group (n=16) received aliskiren (100 mg/kg bw/24 h); -2nd group (n=16) received enalapril (10 mg/kg bw/24 h); -3rd group (n=16) received valsartan (10 mg/kg bw/24 h); and -4th group (n=16): control (vehicle).

Drugs were administered orally by a gastric tube for a period of 4 weeks (28 days), once in the morning. Protective effects of the investigated drugs were evaluated by urinalysis and pathohistology.

Urine was collected in metabolic cages in 24-hour intervals for determination of the following parameters: diuresis (24 hour urine), pH analysis and albuminuria before and after 28 days of treatment with the investigated drugs. pH was measured by using urine test strips (pH Combi-Sceen®, Analyticon Biotechnologies AG) and albuminuria was determined turbidimetrically at 340 nm (Albumin, Cobas Integra systems, Roche).

After 28 days of treatment animals were sacrificed and kidney, aorta and heart were removed and immersed in formaldehyde for histopathological analysis. Fixed tissues were embedded in paraffin. Sections were stained with haematoxylin-eosin and evaluated using microscope Olimpus Bx41 (software for picture analysis Olimpus Cell-A).

Statistical analysis

Data were expressed as mean ± SD. Student’s unpaired and paired t – test and one way analysis of variance ANOVA were used as appropriate. P values < 0.05 were considered as statistically significant.

Results

Urnalysis

After 28 days of treatment diuresis was significantly increased in the group of animals treated with enalapril (46 %) (p<0.01) and valsartan (25 %) (p<0.05).

Table 1: Effect of aliskiren, enalapril and valsartan on diuresis after 28 days of treatment vs. baseline levels.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline</th>
<th>28 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.44 ± 1.14</td>
<td>7.53 ± 1.34</td>
</tr>
<tr>
<td>Aliskiren (100 mg/kg)</td>
<td>7.35 ± 3.33</td>
<td>7.8 ± 3.38</td>
</tr>
<tr>
<td>Valsartan (10 mg/kg)</td>
<td>7.21 ± 2.05</td>
<td>9 ± 2.05</td>
</tr>
<tr>
<td>Enalapril (10 mg/kg)</td>
<td>7.31 ± 1.18</td>
<td>10.8 ± 3.01**</td>
</tr>
</tbody>
</table>

* p>0.05; ** p<0.01.

Effect on diuresis in animals treated with aliskiren was mild (6%) and statistically insignificant (p>0.05) (Table 1, Figure 1).
Before and after 28 days of treatment there was no significant difference in urine pH between treated (enalapril, valsartan and aliskiren) and control group of SHR rats (Table 2, Figure 2).

Table 2: Effect of aliskiren, enalapril and valsartan on urine pH after 28 days of treatment vs. baseline levels.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline</th>
<th>28 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.53 ± 0.02</td>
<td>6.48 ± 0.26</td>
</tr>
<tr>
<td>Aliskiren</td>
<td>6.51 ± 0.35</td>
<td>6.54 ± 0.41</td>
</tr>
<tr>
<td>Valsartan</td>
<td>6.4 ± 0.30</td>
<td>6.35 ± 0.32</td>
</tr>
<tr>
<td>Enalapril</td>
<td>6.55 ± 0.28</td>
<td>6.6 ± 0.37</td>
</tr>
</tbody>
</table>

As shown in Table 3, after 4 weeks of treatment, a significant decrease of albuminuria (p<0.01) was noted in the group of SHR rats treated with enalapril compared to the control group (-12.5%). In animals treated with valsartan decrease of albuminuria was also significant (p<0.01) but in a lesser degree compared to enalapril (-10.4%). After 28 days of treatment Aliskiren had a statistically insignificant effect on albuminuria (-1.67%) in SHR rats.

Table 3: Effect of aliskiren, enalapril and valsartan on albuminuria after 28 days of treatment vs. baseline levels.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline</th>
<th>28 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (100 mg/kg)</td>
<td>0.46 ± 0.04</td>
<td>1.43 ± 0.26</td>
</tr>
<tr>
<td>Aliskiren (100 mg/kg)</td>
<td>0.45 ± 0.01</td>
<td>1.41 ± 0.25</td>
</tr>
<tr>
<td>Valsartan (10 mg/kg)</td>
<td>0.44 ± 0.04</td>
<td>1.28 ± 0.18*</td>
</tr>
<tr>
<td>Enalapril (10 mg/kg)</td>
<td>0.43 ± 0.06</td>
<td>1.25 ± 0.16*</td>
</tr>
</tbody>
</table>

*p<0.01.

Pathohistological changes

Histomorphological analysis of tissue samples of aorta, heart and kidneys in the control and treated group of animals has revealed hypertension-related changes with vascular remodeling.

Discussion

The main goal of this study was to investigate if pharmacological interruption of the rate-limiting step by aliskiren would be as effective as interruption of the renin cascade by ACE inhibitors and AT1 receptor blockers in improving renal function and having a protective influence on hypertension target organs.

We determined protective effects in SHR rats by urinalysis and pathomorphology changes before and after treatment with aliskiren, enalapril and valsartan.

After 28 days of treatment diuresis was signifi-
significantly increased in the group of animals treated with enalapril and valsartan. Effect on diuresis in animals treated with aliskiren was mild and statistically insignificant. Before and after treatment there was no significant
difference in urine pH between treated and control group of SHR rats.

After 4 weeks of treatment in the group of SHR rats treated with enalapril and valsartan a significant decrease of albuminuria was noted compared to the control group. Aliskiren had insignificant effect on albuminuria in SHR rats.

After 28 days of treatment with the investigated drugs no significant effect on existing pathomorphology of the aorta and heart muscle was seen. However, certain changes of wall thickness of arteries and arterioles were noted that led to improved perfusion and function of the kidneys. These changes were more pronounced in rats treated with valsartan and enalapril. Effects of aliskiren were inferior compared to valsartan and enalapril treated groups.

The specificity of aliskiren for primate renin precludes the use of most animal models in which end organ effects of antihypertensive agents are commonly evaluated [12].

The development of double transgenic rats (dTGR), which express the human genes for both renin and angiotensinogen, has provided a suitable animal model in which to investigate the tissue protective effects of renin inhibitors [13, 14]. Pilz et al. [15] compared aliskiren and valsartan in preventing target organ damage in dTGR. Matched 6-week-old dTGR received no treatment, low-dose or high-dose aliskiren, or low-dose or high-dose valsartan. Untreated dTGR showed severe hypertension, albuminuria, and increased serum creatinine by week 7, and 100% mortality rate by week 9. In contrast, high-dose valsartan and both doses of aliskiren lowered BP, reduced albuminuria and creatinine levels, and resulted in 100% survival at week 9. Treatment with aliskiren and high-dose valsartan also reduced left ventricular hypertrophy (LVH); the magnitude of this effect was somewhat greater with high-dose aliskiren. In other renal protection studies using double transgenic rat model, aliskiren reduced renal inflammation and fibrosis as well as albuminuria [16]. In dTGR rats with diabetic nephropathy, aliskiren reduced albuminuria and other markers of renal damage, including gene expression of TGF-β and collagens III and IV [17]. When aliskiren was compared with ACE inhibitors or angiotensin receptor blockers (ARBs), the renal protective effects were approximately equal [15, 17].

In conclusion, in SHR experimental model aliskiren has minor effects on hypertension target organs when compared to enalapril and valsartan.

References

