Antihypertensive effects of \(D\)-polymannuronic sulfate and its related mechanisms in renovascular hypertensive rats

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KEY WORDS antihypertensive agents; nitric oxide; angiotensin II; endothelin-1; renovascular hypertensive rats

ABSTRACT

AIM: To investigate the antihypertensive effects of \(D\)-polymannuronic sulfate (DPS), a kind of sulfated polysaccharide, and the underlying mechanisms in renovascular hypertensive rats (RHR). METHODS: Used two-kidney one clip (Goldblatt, 2-K IC) method to produce RHR model. DPS was given iv or ig for 5 wk with the initiation of establishment of RHR. Serum nitric oxide (NO) was determined with NO kit; plasma angiotensin II (Ang II) and endothelin-1 (ET-1) were measured by radioimmunoassays. RESULTS: In acute therapeutic experiments, DPS markedly reduced systolic blood pressure (SBP) and diastolic blood pressure (DBP) dose-dependently and decreased heart rate (HR) with reduction in arterial blood pressure. In the prophylactic experiments, DPS prevented the rise in SBP and DBP in a dose-dependent manner. The hypertensive potency of DPS 50 mg/kg is comparable to that of captopril (14 mg/kg). Moreover, DPS elevated serum NO contents and lowered plasma concentrations of Ang II and ET-1. CONCLUSION: The antihypertensive activities of DPS might be involved both in increasing the generation of nitric oxide and in decreasing the production of angiotensin II and endothelin-1 in vivo.

INTRODUCTION

Although heparin is well recognized as a potent anticoagulant agent clinically, much more attention has been focused on its antihypertensive action with nonanticoagulant pharmacological activities in recent years. It is documented that heparin injected subcutaneously in dose of 200 – 300 IU daily causes continuous decrease in blood pressure in spontaneously hypertensive rats (SHR) as well as in renovascular hypertensive rats (RHR), characterized by an marked reduction in total peripheral resistance and increase in cardiac output. Furthermore, the antihypertensive action has also been demonstrated in the stroke prone spontaneously hypertensive rats and kidey-mass-ablation hypertensive rats\(^{11}\). Although the hypertensive mechanisms of heparin are not yet fully understood, several reports infer its mechanisms to be associated with inhibiting the proliferation of aortic smooth muscle cells (SMC) and increasing the release of nitric oxide (NO) from endothelial cells in rats\(^{22}\).

\(D\)-polymannuronic sulfate (DPS), a kind of sulfated polysaccharide extracted from brown algae with specific means of fractionation and chemical modification, bears certain similarity in structure and pharmacological activity to heparin such as inhibition of the proliferation of aortic SMC, which raises the possibility that DPS might exert antihypertensive activities. In our experiment, a model of renovascular hypertension of rat was used to evaluate the antihypertensive activities of DPS\(^{3-5}\).

MATERIALS AND METHODS

Drugs and Reagents DPS (\(M, 4000\)) was provided by Marine Drug & Food Institute, Ocean University of Qingdao. Captopril was purchased from Jinan Dongfeng Pharmaceutical Factory, Shandong; while heparin sodium (12 500 U/2 mL) from Changzhou Pharmaceutical Factory, Jiangsu. Nitric oxide, endothelin-1, and angiotensin II kits were products of Chinese Academy of Military Medical Sciences, Beijing.

Animals and Experimental design Male Wistar-Kyoto (WKY) rats (\(n = 130\)) weighing 180 g \(\pm 27\)
g were obtained from Experimental Animal Center of Shandong (Grade II, Certificate No 900201). In acute therapeutic experiments in which the effects of single injection of DPS were studied rats were randomly divided into seven groups (eight in each group); Group I: sham-operated; Group II: renovascular hypertensive model; Group III: DPS 25 mg/kg; Group IV: DPS 12.5 mg/kg; Group V: DPS 6.2 mg/kg; Group VI: DPS 3.1 mg/kg; Group VII: DPS 1.6 mg/kg. In the prophylactic experiment, the effects of DPS ig once a day for 5 wk simultaneously with the initiation of the establishment of renovascular hypertensive model was evaluated. Rats were randomly divided into six groups (ten in each group); Group I: sham-operated; Group II: renovascular hypertensive model; Group III: DPS 50 mg/kg; Group IV: DPS 25 mg/kg; Group V: DPS 12.5 mg/kg; Group VI: captorplil 14 mg/kg. Rats were housed for one week to be accustomed to our environmental conditions before experiments.

Establishment of renovascular hypertensive model in rats Rats were anesthetized with 40 mg/kg sodium pentobarbital intraperitoneally and placed on dorsal position. Under sterile conditions, the left renal artery was exposed, isolated, and constricted as described by Li et al.[6]. Benzylpenicillin 1 x 10^6 IU was used to prevent infection. There was a rise in blood pressure in most of the operated rats after 3 wk. After 4 wk, blood pressure in these rats became steady. Only the rats with systolic blood pressure over 160 mmHg (116 out of 130 rats, 1 mmHg = 133.3 Pa) were used for the following experiments.

Measurement of arterial blood pressure and heart rate Before the rats were randomly divided into desired groups, the baseline of arterial blood pressure and heart rate of the experimental animals were measured by a tail-clip method (Human Medical University, Changsha). In acute experiments, after renovascular hypertension was induced by 2-K IC method in Wistar rats for one month, the effects of single administration of DPS were investigated. A heparinized catheter (containing 2 % heparin, 2500 units) was implanted into the carotid artery in reanesthetized RHR, and the other end of the catheter was connected to a pressure sensor on Physiological Parameter Recorder (Shimadzu, Japan). When blood pressure remained steady for 10 min, DPS 1.6, 3.1, 6.2, 12.5, and 25 mg/kg, was administered via sublingual vein, and DPS-untreated group was injected with the same volume of saline. The arterial systolic blood pressure, diastolic blood pressure and heart rate were measured prior to and 5, 10, 15, 20, 30 and 120 min after drug treatment. ECG was monitored throughout the experiments.

In prophylactic experiments, the effects of DPS 12.5, 25, and 50 mg/kg ig once a day were measured for 5 wk on arterial blood pressure and heart rate in RHR by a tail-clip method, prior to and after the third and the sixth week, respectively. Mean arterial pressure was calculated routinely.

Determination of serum NO contents and plasma ET-1 and Ang II concentrations After DPS was administered ig to RHR for 5 wk, rats were decapitated and blood samples were taken out for the determination of serum NO contents by NO kit, while plasma ET-1 and Ang II were measured by radioimmunoassays.

Statistical analysis Data were expressed as x ± s and analyzed with t test.

RESULTS

Therapeutic effects of DPS on arterial blood pressure and heart rate in RHR In acute therapeutic experiments, DPS 1.56, 3.13, 6.25, 12.50, 25.00 mg/kg was administered via sublingual vein. DPS markedly reduced arterial systolic blood pressure and diastolic blood pressure in a dose-dependent fashion and significantly decreased heart rate with reduction in arterial blood pressure. The maximal hypertensive effect of DPS was observed at 25 mg/kg within 5 min [SBP decreased from (176 ± 6) to (65 ± 9) mmHg; DBP from (148 ± 8) to (43 ± 4) mmHg], and the antihypertensive activities of DPS at doses ranging from 6.2 to 25 mg/kg were characterized by a rapid onset (within 1 min) and long-lasting duration (approximately 30 min), while heart rate was restored earlier than arterial blood pressure (Fig 1).

Prophylactic effects of DPS on arterial blood pressure and heart rate in RHR In prophylactic experiments, SBP and DBP in DPS-untreated RHR were significantly higher than in sham group, indicating that the renovascular hypertensive model was established successfully. DPS, given ig to RHR once a day for 5 wk, significantly lowered arterial SBP and DBP in RHR dose-dependently, and DPS at dose of 50 mg/kg showed a similar potency as compared with that of captorplil at a dose of 14 mg/kg (Tab 1). While there was no significant difference in heart rate observed either prior to or after administration (data not shown).

Effect of DPS on serum NO contents, plasma ET-1 contents and Ang II contents in RHR Serum NO contents [(44 ± 6) μmol/L] in DPS-untreated

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RHR were decreased by 26% as compared with that of sham operated rats [(58 ± 4) μmol/L]. DPS increased the release of serum NO contents in RHR dose-dependent compared to DPS-untreated group (P < 0.01). In addition, the increase in the release of serum NO contents [(50 ± 6) μmol/L] in DPS-treated RHR (50 mg/kg) was as great as in captopril-treated RHR [(56 ± 6) μmol/L]. A dramatic reduction in plasma ET-1 contents in DPS-treated RHR, ig once a day for 5 wk, was observed in a dose-dependent manner compared to that in DPS-untreated group. But the decrease in plasma ET-1 contents in the DPS-treated group was less than that in captopril-treated RHR. In DPS-treated RHR, the reduction in plasma Ang II contents [(254 ± 18) μmol/L] was significant compared with that in DPS-untreated RHR [(464 ± 15) μmol/L], but there was no dose-dependent relationship. The reduction in plasma Ang II contents in DPS-treated RHR was less than that in captopril-treated
RHR (Fig 2).

Tab 1. Prophylactic effects of DPS (po) on mean arterial pressure (MAP) in renovascular hypertensive rats. *n* = 10 rats. *x ± s*. "*P < 0.01 vs model."

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Before treatment</th>
<th>3rd wk</th>
<th>6th wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>saline</td>
<td>94 ± 4</td>
<td>94 ± 5</td>
<td>94 ± 6</td>
</tr>
<tr>
<td>Model</td>
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<td>94 ± 4</td>
<td>155 ± 6</td>
<td>167 ± 8</td>
</tr>
<tr>
<td>III</td>
<td>50</td>
<td>94 ± 4</td>
<td>114 ± 5</td>
<td>117 ± 4</td>
</tr>
<tr>
<td>IV</td>
<td>25</td>
<td>94 ± 4</td>
<td>130 ± 6</td>
<td>137 ± 7</td>
</tr>
<tr>
<td>V</td>
<td>12</td>
<td>94 ± 4</td>
<td>136 ± 7</td>
<td>144 ± 5</td>
</tr>
<tr>
<td>Captopril</td>
<td>14</td>
<td>94 ± 4</td>
<td>114 ± 5</td>
<td>116 ± 6</td>
</tr>
</tbody>
</table>

RHR were pretreated with DPS ig at doses of 12.5, 25, and 50 mg/kg once a day for 5 wk. MAP were measured prior to and at the third and the sixth week of DPS administration, respectively.

DISCUSSION

In this study, the effects of DPS on blood pressure in renovascular hypertensive rats were systemically investigated. The results indicated that DPS exerted antihypertensive activities in a dose-dependent fashion in RHR, characterized by a significant reduction in both SBP and DBP.

Generally, the evaluation of efficiency of antihypertensive drugs is based upon their capacity of lowering either diastolic blood pressure, or systolic blood pressure, or both. And traditional methods for measuring the efficiency of antihypertensive drugs have been focused on reduction in DBP, because of the importance of reducing DBP according to epidemiological and clinical studies. However, recent evidence has highlighted the role of SBP...
in the pathogenesis of hypertension and subsequent importance in assessing the efficiency of potent antihypertensive drugs. The hypotensive effects by DPS on both SBP and DBP makes it a potentially promising candidate as a new chemical entity for antihypertensive.

DPS promoted a significant increase in NO release in a dose-dependent manner. It appears likely that DPS exerted antihypertensive activity in RHR due to NO release. A rapidly evolving literature has provided compelling evidence for the role of this key mediator in vascular tone. NO not only serves as an important locally-acting vasodilator, but also acts as a central factor in the short- and long-term regulation of multiple determinants of arterial blood pressure. Impaired NO synthesis or endothelial dysfunction may be a result of hypertension in some cases, and may contribute to the initiation or genesis of this disorder in others. Some reports have confirmed the deficiency of NO in a variety of animal models of hypertension, especially those in advanced stage. Recent findings also indicate that NO exerts acute hypotensive effect in vivo primarily due to the decrement of systemic vascular resistance by increasing cyclic GMP and by inhibiting proliferation of vascular smooth muscle cells leading to a chronic antihypertensive effect. This may, at least in part, explain both rapid and persistent blood pressure-lowering effects exerted by DPS and its related underlying mechanisms.

Physiologically, basal NO synthesis serves to buffer the action of endogenous vasoconstrictors, such as angiotensin II (Ang II). This phenomenon is best recognized by the findings that renin secretion, the rate-limiting step in systemic angiotensin II production, is principally mediated by NO. In fact, there is mounting evidence demonstrating the tonic inhibition of renin secretion by endogenous NO, with stimulation of renin release by NOS inhibitors. In addition, NOS inhibitors seem responsible for enhancement of the vascular response to exogenous angiotensin II, which might explain, in great part, the close interaction between L-arginine-NO pathway and renin-angiotensin system. Therefore, a more likely understanding of the suppression of DPS on angiotensin II production in this paper, though renin secretion was not determined, might be by taking NO-mediated suppression in renin-angiotensin activity into consideration.

The increment in endothelin-1 (ET-1) production induced by two-kidney one clip (2-K 1C) method was significantly reversed by DPS. The possible mechanism responsible for the impact of DPS on inhibiting ET-1 formation may contribute to an increased release of NO. A line of evidence has indicated that ET-1 and NO are formed by endothelial cells to function simultaneously on vascular smooth muscle. NO not only opposes the vasoconstricting effects of ET-1, but also suppresses ET-1 gene expression and facilitates the termination of the action of ET-1. A wealth of data indicates that inhibition of NO formation by NO synthase inhibitors causes elevation of blood pressure in rats, accompanied by a marked increase in plasma ET-1 levels. In this respect, suppression of ET-1 production by DPS contributed greatly to the increased formation of NO.

As for captopril, its inhibitory effect on ET-1 production could be supported by recent data showing that captopril decreased the synthesis or release of ET-1 via its antagonizing effect on SMC proliferation and on Ang II production.

In conclusion, this is the first report to observe the dose-dependent antihypertensive effect of sulfated polysaccharide DPS on arterial blood pressure in renovascular hypertensive rats. And its underlying mechanisms could be associated to its actions on increasing the synthesis or release of NO and decreasing the production of angiotensin II and ET-1 in vivo.

REFERENCES

硫酸多糖 DPS 对肾血管性高血压大鼠的降压作用及其相关机制

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关键词 抗高血压药；一氧化氮；血管紧张素 II；内皮素-1；肾血管性高血压大鼠

目的：观察硫酸多糖（DPS）对肾血管性高血压大鼠的降压作用并对其机制进行初步探讨。方法：（1）急性降压实验：DPS 单次静注给药于大鼠，于给药前测定大鼠尾动脉血压及心率。（2）口服给药实验：在肾血管性高血压大鼠造模后第 2 天即开始口服给药，每日 1 次。于给药前、后第三周和第六周分别以大鼠尾动脉测压法测定动脉血压和心率。结果：在急性降压实验中，DPS 能够显著降低肾血管性高血压大鼠的收缩压和舒张压且其降压强度呈剂量依赖性，降压的同时伴有心率减慢。DPS 口服给药后 5 周，剂量依赖性抑制大鼠的收缩压和舒张压升高，DPS 50 mg/kg 的降压效果与卡托普利 14 mg/kg 相当。DPS 给药后 5 周，可显著增加血清中 NO 的含量和降低血浆中 ET-1 的含量；亦降低血浆中 Ang II 的含量。结论：硫酸多糖 DPS 对肾血管性高血压大鼠具有良好的降压作用，其降压机制可能与其促进体内 NO 生成或释放、降低 ET-1 和 Ang II 的含量有关。

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