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Effects of D-Polymannuronic Sulfate on Serum Nitric Oxide Levels and Plasma Endothelin-1 and Angiotensin Ⅱ Contents in Renovascular Hypertensive Rats (Series Ⅱ)*

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Abstract The effects of D-polymannuronic sulfate (DPS) on the serum nitric oxide levels and plasma Endothelin-1 and Angiotensin II contents were studied in renovascular hypertensive rats [two-kidney one clip, Goldblatt (2-K 1C)]. In the prophylactic experiment in which the effects of DPS given orally at doses of 12.50, 25.00 and 50.00 mg/kg once daily for five weeks simultaneously with the initiation of the establishment of renovascular hypertensive model were evaluated. Serum nitric oxide (NO) was determined with nitric oxide kit while plasma Angiotensin II (Ang II) and Endothelin-1 (ET-1) were measured by radioimmunoassay. Captopril (14 mg/kg), an Angiotensin II inhibitor, was served as a positive agent. The results showed that DPS could produce a dose-dependent effect on increased serum nitric oxide levels and decreased Endothelin-1 contents, whereas the inhibitory effect of DPS on plasma Angiotensin II contents was not found in a dose-dependent manner. It implicates that the mechanisms underlying the antihypertensive effects of DPS might be associated with its actions on increasing the synthesis or release of NO and decreasing the production of Ang II and ET-1 in vivo.

Key words marine D-polymannuronic sulfate (DPS); antihypertensive effect; angiotensin II; endothelin-1; renovascular hypertensive rats

D-polymannuronic sulfate (DPS), a kind of marine sulfated polysaccharides extracted from brown algae with some special technique of fractionation and chemical modification, bears a certain similarity in structure and pharmacological activity to heparin. Our previous work has confirmed that DPS could produce antihypertensive effects via sublingual vein injection and gastrointestinal injection in rats with renovascular hypertension[1]. However, the mechanisms underlying the antihypertensive effects of DPS have not been understood yet. Currently, there is a general agreement that the misbalance between endogenous vasodilators (i.e. nitric oxide) and endogenous vasoconstrictors such as Endothelin-1 (ET-1) as well as Angiotensin II (Ang II) plays an important role in the genesis and development of hypertension[2]. And it is documented that the antihypertensive effect of heparin on spontaneously hypertensive rats (SHR) and renovascular hypertensive rats (RHR) is due to

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its inhibition on rat aortic vascular smooth muscle mediated by the overproduction of nitric oxide (NO) released from endothelial cells[3]. As we mentioned above, DPS bears a similarity in chemical structure to heparin, this raises the possibility that the antihypertensive activity of DPS may be related to the participation of NO. Therefore, the mechanisms by which the effect of DPS on the decrease in blood pressure are discussed partially here.

1 Material and Methods

1.1 **Drugs and reagents** D-polymannuronic sulfate (DPS) was provided by the Marine Drugs and Food Institute, Ocean University of Qingdao. Captopril was purchased from Jinan Dongfeng Pharmaceutical Factory, Shangdong. Nitric oxide kit, Endothelin-1 and Angiotensin II kit were the products of Academy of Military Medical Sciences, Beijing.

1.2 **Animals and experimental design** Male Wistar-Kyoto (WKY) rats (n = 84) weighing 180 ± 27 g were obtained from the Experimental Animal Center of Qingdao (Certificate NO. 980201). The rats were housed for one week to be accustomed to our controlled environment before the experiments. In the prophylactic experiment, the rats were randomly divided into six groups (14 in each group). Group I: sham-operation; Group II: renovasular hypertensive model; Group III: 50 mg/kg; Group IV: 25 mg/kg; Group V: 12.50 mg/kg; Group VI: captopril 14 mg/kg. In the prophylactic experiment, the effects of DPS given orally once a day for five weeks simultaneously with the initiation of the establishment of renovascular hypertensive model were evaluated.

1.3 **Establishment of renovascular hypertensive model in rats** The rats were anesthetized intraperitioneally with 40 mg/kg sodium pentobarbital and placed on dorsal position. Under sterile conditions, the left renal artery was exposed, isolated and constricted as described by Li et al[4]. Penicillin at a dose of 1×10^5 IU was used to prevent the rats from infection. There was a rise in the blood pressure for most of the operated rats after three weeks. From four or five weeks, the blood pressure in these rats came into a steady state. Only the rats with systolic blood pressure over 21.3 kPa (60 among 84 rats) were selected for the following experiment.

1.4 **Measurement of arterial blood pressure and heart rate** Before the rats were randomly divided into the designed groups, the arterial blood pressure and heart rate of the experimental animals were measured by a tail-clip device (made by Hunan Medical University, Changsha). In the prophylactic experiment, the effects of DPS at doses of 12.5, 25.00 and 50.00 mg/kg given orally once a day for five weeks simultaneously with the initiation of the establishment of renovascular hypertensive model. And the RHR without DPS administration were given orally in the same amount of saline as for DPS, the arterial blood pressure and heart rate of rats in each experimental group were measured respectively by a tail-clip device, prior to administration and after the third week and the sixth week.

Mean arterial pressure (MAP) was calculated routinely.

1.5 **Determination of serum NO contents and ET-1 and Ang II contents in plasma** After DPS was given orally to RHR for five weeks, 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 radioimmunoassay, respectively.

1.6 **Statistical analysis** Data for the experiment were represented as mean ± SEM in Dunnett's test.

2 Results

2.1 **Effects of DPS on serum NO contents in RHR** As shown in Figure 1, the effects of DPS on serum NO contents in RHR were also assessed. Serum NO contents in the RHR without DPS administration (43.65±5.03 μmol L^{-1}) were decreased by 26% as compared with that of the sham operated rats (57.70±2.56 μmol/L). DPS increased the release of serum NO contents in RHR dose-dependently, compared to that of RHR without DPS administration ($p < 0.01$). In addition, the degree of increasing the release of serum NO contents (49.90±5.28μmol/L) in RHR with DPS administration (50 mg/kg) was as great as in the RHR with captopril administration (56.35±4.75μmol/L).

2.2 **Effects of DPS on plasma Endothelin-1 contents in RHR** The effects of DPS in plasma ET-1 contents were investigated in RHR. A dramatic reduction in plasma ET-1 contents in RHR with DPS administration given orally once a day for five weeks was elucidated compared to that of the RHR without DPS administration, characterized by a dose-dependent manner. But the degree to the decrease in plasma ET-1 contents in the RHR with DPS administration was less than that of RHR with captopril administration (Figure 2).

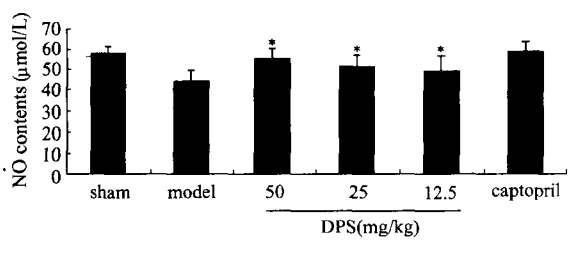

Fig. 1 Effects of DPS on serum No contents in rats of renovascular hypertension. DPS was given orally at doses of 12.50, 25.00 and 50.00 mg/kg once daily for five weeks. Ten rats in each group. Data are represented as mean±SEM. *P<0.01 vs model.

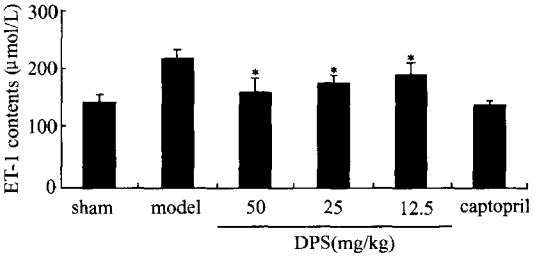

Fig. 2 Effect of DPS on plasma ET-1 contents in renovascular hypertensive rats. DPS was given orally at doses of 12.50, 25.00 and 50.00 mg/kg once daily for five weeks. Ten rats in each group. Data are represented as mean ±SEM. *P<0.01 vs model.

2.3 **Effects of DPS on plasma Ang II contents in RHR** The effects of DPS on plasma Ang II contents were evaluated in RHR. In the RHR with DPS administration, the reduction in plasma Ang II contents (254.27 ± 8.50 μmol/L) was significant as compared with that of the RHR without DPS administration (464.41 ± 5.02μmol/L), but not dosedependently. The extent to the reduction in plasma Ang II contents in the RHR with DPS administration was less than that of RHR with captopril administration, being similar to that of a decrease in plasma Ang II contents in vivo (Figure 3).

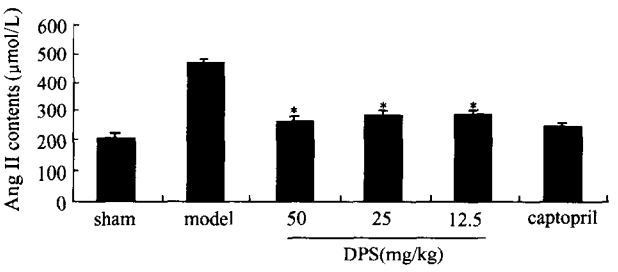

Fig. 3 Effects of DPS on plasma Ang Ⅱ contents in renovascular hypertensive rats. DPS was given orally at doses of 12.50, 25.00 and 50.00 mg/kg once a day for five weeks. Ten rats in each group. Data are represented as mean±SEM.
*P<0.01 vs model.

3 Discussion

In this study, the mechanisms underlying the antihypertensive effects of DPS were partially investigated.

Hypertension is generally regarded as a chronic cardiovascular disease of gradual progression. Although its pathological mechanism has not been fully understood, many studies reveal that the disturbance between endogenous vasodilators and vasoconstrictors plays an important role in the genesis of hypertension[2]. Since Furchgott firstly suggested the existence of an EDRF[5], and then with others, its identity as NO[6], many articles have provided strong evidence for this key mediator's role in vascular control. NO not only serves as an important locally-acting vasodilator but also acts as a central factor in the short-term and long-term regulation of multiple determinants of arterial blood pressure[7]. Impaired NO synthesis or endothelial dysfunction may, in some cases, be a result of hypertension, in others it may contribute to the initiation or perpetuation of this disorder[8]. Some reports have confirmed that NO generation is impaired in a variety of animal models of hypertension, especially in those at the advanced stage[9]. Consistent with the reports mentioned above, in the present study, serum NO contents in RHR without DPS administration were significantly lower than the sham, implicating the impairment of NO synthesis or release also exists in rats with renovascular hypertension in our experiment. In the prophylactic experiment where DPS was given orally to RHR for five weeks, an increase in serum NO contents was observed dose-dependently as compared with RHR without DPS administration, coinciding with the antihypertensive effects of DPS in a dose-dependent manner. Accordingly one of the possible mechanisms by which the antihypertensive effects of DPS in prophylactic therapy, we believe

could be associated with increases in the circulation of NO.

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 ample evidence demonstrating the tonic inhibition of renin secretion by endogenous NO, with the stimulation of renin release by NOS inhibitors. In addition, NOS inhibitors seem responsible for the 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[10]. Therefore, (a more likely understanding of the suppression of DPS on Ang II production in this experiment, though that on renin secretion not determined, might take NO-mediated suppression in renin-angiotensin activity into consideration.) Besides, the decreased Ang II levels were not accompanied by a dose-dependent manner, and we inferred, this might be affected by many factors, probably due to the complicated metabolism of marine sulfated polysaccharides.

The increment in Endothelin-1 (ET-1) production induced by the two-kidney one clip (2-K 1C) method was significantly reversed by DPS. The possible mechanisms responsible for the impact of DPS on inhibiting ET-1 formation may contribute to increased release of NO. Ample evidence has indicated that ET-1 and NO are formed by endothelial cells to function paradoxically on vascular smooth muscle cells[11]. 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[12]. Sufficient data indicate that the inhibition of NO formation with NO synthase inhibitor causes the elevation of blood pressure in rats, accompanied by a marked increase in plasma ET-1 levels[13]. In this respect, the suppression of ET-1 production by DPS contributed greatly to the increased formation of NO.

In conclusion, DPS is capable of producing a dose-dependent antihypertensive effect on arterial blood pressure in renovascular hypertehsive rats and its underlying mechanisms could be related to its actions on increase in the synthesis or release of NO and decrease in the production of ET-1 and Ang II in vivo.

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海洋硫酸多糖DPS对肾血管性高血压大鼠血清NO和血浆Ang II及ET-1含量的影响*

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摘 要 采用肾血管性高血压大鼠模型(两肾一夹型)观察海洋硫酸多糖DPS对肾血管性高血压大鼠血清中一氧化氮(NO)和血浆中血管紧张素II(Ang II)及内皮素-1(ET-1)含量的影响。DPS在肾血管性高血压大鼠造模第二天起分别以12.50, 25.00,50.00 mg/kg口服预防给药五周,每日给药一次。于给药前、给药后第三周和第六周分别测定动脉血压和心率。实验结束前,从每只大鼠取血6 mL,用试剂盒测定血清中NO的含量;用放射免疫法测定血浆中Ang II和ET-1的含量。血管紧张素转化酶抑制剂卡托普利(14 mg/kg)作为本实验阳性对照药。结果:DPS口服预防给药五周,可显著增加血清中NO的含量和降低血浆中ET-1的含量,且呈剂量依赖性;DPS亦能降低血浆中Ang II的含量,但未见剂量依赖性。结论:海洋硫酸多糖DPS对肾血管性高血压大鼠的降压作用机制可能与其促进体内NO生成或释放、降低Ang II和ET-1的含量有关。

关键词 海洋硫酸多糖(DPS);降压作用;血管紧张素II;内皮素-1;肾血管性高血压大鼠

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