The role of hydrogen sulfide system in the pathogenesis of renovascular hypertension in rats

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Objective To investigate the role of hydrogen sulfide (H₂S) synthases / H₂S pathway in the pathogenesis of renovascular hypertension. Methods Wistar rats were subdivided into 4 groups: (1) 2-kidney, 1-clip (2K-1C group, n=7), (2) control (n=7), (3) sham (n=7), and (4) 2K-1C plus sodium hydrosulfide (NaHS) (NaHS-treated group, n=7). The systolic blood pressure (SBP) was measured by a tail-cuff method using a pulse transducer once a week. Four weeks later, all rats were killed and the concentration of plasma hydrogen sulfide (H₂S), the activity of the H₂S synthases in the kidneys on both sides, the plasma angiotensin concentration, and the left-to-whole heart weight ratio were measured. Results The SBP was significantly increased in the 2K-1C group (185.4±14.0 mmHg) comparing with those in the sham group (112.9±6.5 mmHg) or the NaHS-treated group (134.8±9.5 mmHg) (both P<0.01). At 4 weeks, the angiotensin concentration in the plasma was increased in the 2K-1C and NaHS-treated group, comparing with the control and the sham group (306.9±7.0 pg/ml and 240.7±13.2 pg/ml vs 122.6±25.4 pg/ml and 125.9±10.5 pg/ml, respectively, both P<0.05). The plasma H₂S concentration and the activity of the H₂S synthases in the left kidney were decreased in the 2K-1C group comparing with those in the sham and the control groups. There was no difference of the activity of the H₂S synthases in the right kidneys among the 4 groups. The left-to-whole heart weight ratio was increased in the 2K-1C and the NaHS-treated group comparing with that in the sham and natural control groups. Conclusion Dysfunction of the H₂S synthases/H₂S pathway was involved in the 2K-1C-induced renovascular hypertension in rat. Exogenous administration of H₂S donor can attenuate the development of hypertension. These findings suggest that the H₂S synthases/H₂S pathway participates in the pathogenesis of renovascular hypertension. (J Geriatr Cardiol 2008; 5: )

Key Words angiotensin; hypertension; renal; blood pressure; hydrogen sulfide.

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Hypertension is the major risk factor of cerebrovascular disease, coronary heart disease and renal dysfunction. Renal arterial stenosis, mostly induced by atherosclerosis, eventually leads to renovascular hypertension. The incidence of renovascular hypertension gets higher and higher. At the present time, the renin-angiotensin-aldosterone system (RAAS) hyperexcitation is widely recognized as the key point in the mechanism of the renovascular hypertension. Meanwhile, published data have shown that vasodilator nitric oxide (NO) decreases in the 2K-1C renovascular hypertension and the increase of heme oxygenase-1 (HO-1, endogenous carbon monoxide (CO) synthases) level may ameliorate 2K-1C renovascular hypertension. As NO and CO, hydrogen sulfide (H₂S) was only recognized as a kind of toxicological gas for a long time, but in the recent years it was found to have important physiological functions and had been known as the third neurotransmitter. In the previous study, we had discovered that H₂S may induce relaxation not only in the rat aorta but also in the resistance mesenteric artery bed of rats. We had found that endogenous H₂S system was involved in both the maintenance of basal blood pressure and the development of hypertension, including the L-NAME-induced hypertensive, the spontaneous hypertension, hypoxic pulmonary hypertension and high blood flow induced pulmonary hypertension in rats. In these experiments, exogenous H₂S donor could exert beneficial effect on these types of hypotensions. These studies suggest that the H₂S synthases/H₂S pathway participates in some types of hypotensions. However, it is unknown whether the H₂S synthases/H₂S pathway is involved in the pathogenesis of renovascular hypertension. It is very important to find out the mechanism of the renovascular hypertension and to prevent and cure it. The aim of this study is to observe the role of the H₂S synthases/H₂S pathway in the renovascular hypertension.
Materials and methods

The renovascular hypertension model of 2 kidney 1 clipped (2K-1C)

The 2K-1C hypertensive rat model was made according to Li with modification.12 In brief, male Wistar rats(180-220g) were anesthetized with 3% sodium pentobarbital (Abbott) (30mg/kg). The left kidney was exposed and the renal artery was carefully dissected free of the renal vein. The renal artery was then partially occluded by placing a silver clip with an internal diameter of 0.23 mm on the vessel. Then juxtapose acupuncture needle (D=0.2mm) and blood vessel, ligate them with operating silk thread, then draw acupuncture needle to result in left renal artery stenosis partly but no occlusion completely, while the right renal artery remains intact. The wound was closed with a running 3-0 silk suture Sham operated rats underwent identical surgical procedures, except that a clip was not applied to the renal artery.

Animals and groups

The study was carried out in the PLA General Hospital. Male Wistar rats (180-220g) were randomly divided into natural control group (N=7), sham operated group (N=7), 2K-1C model group (N=7) and NaHS group. The rats in the NaHS group were intraperitoneally injected with NaHS(56.7 μmol/kg body weight), and the 2K-1C model group (N=7) and NaHS group were intraperitoneally injected with a mixture of N,N-dimethyl-N'-phenylenediamine dihydrochloride in 7.2mol/L HCl and 0.4 ml of 30mol/L FeCl3 in 1.2mol/L HCl were also added to the test tube for 20min of incubation at room temperature. The protein in the plasma was removed by adding 1ml of 10% trichloroacetic acid to the reaction mixture and centrifugation. The optical absorbance of the resulting solution at 670nm was measured with a spectrometer (Shimadzu UV 2100, Japan). The H2S concentration in the solution was calculated against the calibration curve of the standard H2S solution.

Measurement of activity of H2S synthases in the kidney tissues

Since there are two H2S synthases in the kidney tissue: cystathionine-β-synthases (CBS) and cystathionine-γ-lyase (CSE) , we used tissue H2S production rate as the activity of these two H2S synthases. The tissue H2S production rate was measured according to Stipanuk’s method.13 The kidney tissues were homogenized in ice-cold 50mmol/L potassium phosphate buffer (PH 6.8) immediately as soon as they were removed from rat body. Reactions were performed in a 25 ml Erlenmeyer flasks. The reaction mixture contained: 10 mmol/L L-cysteine, 2 mmol/L pyridoxal 5'-phosphate, 100 mmol/L potassium phosphate buffer (PH 7.4), and 10% (w/v) homogenates. Cryovial test tubes (2ml) were used as the center wells each contained 0.5ml of 1% zinc acetate as trapping solution. A filter paper of 2×2.5cm2 was put into the central well of the flask to increase the air/liquid contacting surface. The flasks were then flushed with N2 before being sealed with a double layer of parafilm. The catalytic reaction was initiated by transferring the flasks from an ice bath to a 37°C shaking water bath. After incubating at 37°C for 90min, the reactions were stopped by injecting 0.5 ml of 50% trichloroacetic acid. The flasks were sealed again and incubated in the shaking water bath for an additional hour at 37°C to ensure a complete trapping of H2S. The contents of the central well were then transferred to test tubes and mixed with 3.5 ml distilled water and 0.5 ml of 20mmol/L N,N,N-dimethyl-p-phenylenediamine dihydrochloride in 7.2 mol/L HCl. To each tube, 0.4 ml of 30mmol/L FeCl3 in 1.2 mol/L HCl was added immediately. After 20min of incubation at room temperature, the optical absorbance of the resulting solution at 670nm was measured with a spectrometer (Shimadzu UV 2100, Japan). The H2S concentration in the solution was calculated against the calibration curve of the standard H2S solution. For each sample, the measurement was done in duplicate. The H2S production was expressed in a unit of nmol/mg wet tissue·min⁻¹.

Determination of plasma angiotensin ΩΔ

Plasma angiotensin ΩΔ concentration determined by radioimmunoassay, confirmed strictly to the role offering by the radiate-immunity laboratory in the General Hospital of PLA.
Data analysis

Results were expressed as mean ± SE. Changes of blood pressure of the rats were analyzed using paired Student’s t test. Values of P < 0.05 were considered to be significant. For comparison of the differences among control and experimental groups, ANOVA followed by a post hoc analysis (Bonferroni test) was used by using SPSS 10.0 statistic analysis software.

Result

One week after the operation, the tail artery systolic pressure (ASP) began to increase in the 2K-1C model group and the NaHS treated group until the end of the experiment. However, no changes of ASP were found in the sham group. And the increase of ASP in the NaHS group was 70% lower than that in the 2K-1C model group (see Table 1).

Plasma H$_2$S concentration was decreased in the 2K-1C model group compared with control and the sham group. Plasma H$_2$S concentration in the NaHS group was significantly higher than that in the 2K-1C model group, and similar to that in control and sham group (Fig.1.).

The activity of H$_2$S synthases in the left kidney tissue was decreased in the 2K-1C model group compared with control and sham group, while there was no significant difference between the NaHS group and the 2K-1C group, although there was an increasing trend in the NaHS group. There was no significant differences of the activity of H$_2$S synthases in the right kidney among the four groups (see Table 2).

* P<0.05 vs control and sham groups
# P<0.05 vs the 2K-1C model group

Figure 1: Plasma H$_2$S concentration 4 weeks after the operation (umol/l)

Table 1  Changes of the rat tail artery blood pressure (mmHg, X±S)

<table>
<thead>
<tr>
<th></th>
<th>sham group(n=7)</th>
<th>2KIC group (n=7)</th>
<th>NaHS group (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-operation</td>
<td>108.3±2.6</td>
<td>111.3±7.5</td>
<td>112.1±5.3</td>
</tr>
<tr>
<td>1week</td>
<td>110.1±8.9</td>
<td>123.6±12.3</td>
<td>117.9±4.1</td>
</tr>
<tr>
<td>2week</td>
<td>112.2±3.2</td>
<td>147.5±7.5*</td>
<td>120.7±6.5*</td>
</tr>
<tr>
<td>3week r</td>
<td>113.6±2.4</td>
<td>160.5±7.9*</td>
<td>129.9±5.6*</td>
</tr>
<tr>
<td>4week</td>
<td>112.9±6.5</td>
<td>185.4±14.0*</td>
<td>134.8±9.5*</td>
</tr>
</tbody>
</table>

*P<0.01 VS sham group  P<0.01 VS 2KIC group

Table 2   The activity of H$_2$S synthases in the kidney 4 weeks after the operation (nmol·mg wet Tissue$^{-1}$·min$^{-1}$)

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>sham(n=6)</th>
<th>2KIC(n=7)</th>
<th>2KCI+NaHS(n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left kidney</td>
<td>4.049±0.914</td>
<td>5.267±0.826</td>
<td>2.140±0.480*</td>
<td>3.698±0.656</td>
</tr>
<tr>
<td>Right kidney</td>
<td>5.033±0.321</td>
<td>4.278±0.127</td>
<td>3.718±1.459</td>
<td>3.930±0.432</td>
</tr>
</tbody>
</table>

* P<0.05 vs control and the sham group

Discussion

Hypertension is a disease with a high incidence. There

Figure 2  plasma angiotensin○ concentration 4 weeks after operation

The plasma angiotensin concentration 4 weeks after the operation was increased in the 2K-1C group (compared with control and the sham group). There was no significant difference between the NaHS group and the 2K-1C model group (P > 0.05), although there was a decreasing tendency in the NaHS group.

The ratio of left ventricular weight/whole heart weight was higher in the 2K-1C model group than that in control and the sham group. There was no difference of the ratio between the NaHS group and the 2KIC group. There was a decreasing tendency of the ratio in the NaHS group compared with that in the 2K-1C model group (Fig.3).
are two types of hypertension, idiopathic hypertension and secondary hypertension. As to the idiopathic hypertension, the pathophysiology is not well known. Nowadays, more and more about it is getting into our knowledge. Among them, the kidney artery stenosis induced by atherosclerosis is an important etiological factor for almost 5-10% adult hypertensive patients. Therefore, more and more attention is paid to the renovascular hypertension. In the past, renin-angiotensin-aldosterone system (RAAS) hyperexcitation was widely recognized as the key mechanism of the renovascular hypertension. Recently, some studies had shown that nitric oxide (NO) decreased in the 2K-1C renovascular hypertension and the increase of heme oxygenase-1(HO-1), endogenous carbon monoxide (CO) synthases levels may ameliorate 2K-1C renovascular hypertension. Like NO and CO, hydrogen sulfide (H2S) was only recognized as a kind of toxicological gas for a long time. But in recent years it was found to have important physiological functions and had been known as the third gasotransmitter. In the previous study, it was discovered that H2S may induce relaxation not only in the aorta but also in the resistance mesenteric artery bed of rats. We had found that endogenous H2S system was involved in both of the maintenance of basal blood pressure and the development of hypertension, including the L-NAME-induced hypertensive, the spontaneous hypertension, hypoxic pulmonary hypertension and high blood flow-induced pulmonary hypertension in rats. In these experiments, exogenous H2S donor could exert beneficial effect on the hypertensive rats.

Upon the findings above, we assumed that the H2S synthases / H2S pathway might be associated with the renovascular hypertension. In this study, we found the following.

**Firstly**
the systolic blood pressure of rats in the 2K-1C model group elevated from the second week after the operation and elevated significantly at the fourth week after the operation. At the same time, we found that the plasma H2S concentration were depressed in the 2K-1C model rats at the fourth week after the operation, with the activity of the H2S synthases in the clipped kidney decreased. It suggested that there was a dysfunction of H2S synthases / H2S pathway in the 2K-1C renovascular hypertension. At the present time, we are still not sure if the plasma H2S concentration measured is the only representative for the activity of H2S synthases in the clipped kidney. It might represent the activity of the H2S synthases in the whole body, including the heart and the vessels throughout all of the body, besides in the clipped kidney. However, we think that the activity of H2S synthases in the clipped kidney is the important reason for the depression of the plasma H2S concentration. Therefore, we may say that, in the 2K-1C renovascular hypertensive rats, the decreased activity of the H2S synthases in the clipped kidney is one of the mechanisms in the pathogenesis of the renovascular hypertension. This is a new discovery, yet we are not clear why it dose occur and how it dose occur. The decrease of the activity of the H2S synthases may due to the renal ischemia, since we found no changes in the no-clipped kidneys.

**Secondly**
with H2S donor- NaHS, plasma H2S concentration in the 2K1C increased significantly compared with the 2K1C only group. And it is similar to that in the sham group. At the same time, the blood pressure decreased by 70% due to the addition of NaHS. It verified our hypothesis that exogenous H2S may decrease the renovascular hypertension just as in other types of hypertension model. Simultaneously, the activity of the H2S synthases in the clipped kidneys increased similarly with that in the natural control group. It is still not clear whether the increased activity of the H2S synthases in the clipped kidneys is due to exogenous H2S. Published data showed that exogenously applied NaHS might exert some sort of positive feedback on the gene expression and the subsequent enzymatic activity of CSE. In our study, we did not measure the gene expression of the CSE and CBS, but we measured the H2S concentration in the plasma and the activity of the H2S synthases in the clipped kidneys, which also suggested that exogenously applied NaHS might exert some sort of positive feedback on the enzymatic activity of CSE and /or CBS in the clipped kidneys. The same result had been observed by Yan and associates in the spontaneous hypertensive rats. They found that the upregulation of both CSE gene expression and enzymatic activity result in an increased endogenous H2S production and thus exert protective effect. In our study, NaHS, the donor of H2S, may be used in the human being as an antihypertension medicine in the near future.

**Thirdly**
the plasma angiotensin 2 weeks after the operation

*P<0.05 vs control and the sham group

Fig3 The ratio of left ventricular weight/whole heart weight

Published data showed that exogenously applied NaHS might exert some sort of positive feedback on the gene expression and the subsequent enzymatic activity of CSE. In our study, we did not measure the gene expression of the CSE and CBS, but we measured the H2S concentration in the plasma and the activity of the H2S synthases in the clipped kidneys, which also suggested that exogenously applied NaHS might exert some sort of positive feedback on the enzymatic activity of CSE and /or CBS in the clipped kidneys. The same result had been observed by Yan and associates in the spontaneous hypertensive rats. They found that the upregulation of both CSE gene expression and enzymatic activity result in an increased endogenous H2S production and thus exert protective effect. In our study, NaHS, the donor of H2S, may be used in the human being as an antihypertension medicine in the near future.
increased in the 2K-1C model group, which conformed to
that in the published data. It is right that RAAS plays an
very important role in the pathogenesis of the renovascular
hypertension. Although, there was no significant difference
between the NaHS group and the 2K-1C model group ($p>0.05$),
yet we noted a decreasing tendency of the plasma
angiotensin?concentration in the NaHS group (240.73±13.22
vs 306.92±7.03, $p>0.05$). There might be some relationship
between the angiotensin ε2and H$_S$ or between RAAS
system and H$_S$ synthases/ H$_S$ pathway, which needs fur-
ther study.

Finally

the left ventricle/whole heart weight ratio reflects the
left ventricular hypertrophy. It increased in the 2K-1C model
group at the 4th week. In this study, due to the damage to
the heart by 2K-1C process in the early phase of the ren-
ovascular hypertension, the blood pressure decreased at
first. There was no significant difference between the NaHS
group and the 2K-1C model group ($p>0.05$). However, we
noticed that there was a decreasing tendency if we pro-
long the NaHS-treating phase..

It is well known that the 2K-1C renovascular hyper-
tension is a rennin-angiotensin-aldosterone system (RAAS)
dependent hypertension.$^{12}$ Gaseous transmitters including
NO and CO have been demonstrated to play an important
role in the renovascular hypertension.$^{1,2,13}$ H$_S$ is the third
gaseous transmitter. They all have vasorelaxating effects,
although through various ways. NO and CO relaxate blood
vessel though the activation of GMP, but H$_S$ relaxates rat
aortic and mesenteric artery in a KATP channel dependent
manner, which is absolutely different from the way of NO
and CO. The three small molecular gases, NO, CO and H$_S$,
may form an unique interactive network, or form a very
complex interactive three-dimensional network. So there are
still many problems to be solved. For example, What will
happen if we use both inhibitor of CSE and inhibitor of CBS
in the 2K-1C rats and if we give NO, CO and H$_S$ donors
together in the 2K-1C rats ,what will happen? What will be
the probable effects of them on the RAAS in the kidney,
heart and circulation. We are looking forward to more ex-
periments to answer these questions, to get new methods
to prevent and cure the renovascular hypertension.

In conclusion, we observed the changes of endog-
енous H$_S$ system in the 2K-1C renovascular hypertensive
rats. It shows that the H$_S$ synthases/ H$_S$ pathway is one of
the important factors in the development of renovascular
hypertension. Exogenous H$_S$ donor exerted reverse
effect in the pathogenesis of the 2K-1C renovascular
hypertension.

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