SODIUMNITROPRUSSIDE AS A HYPOTENSIVE AGENT DURING GENERAL ANAESTHESIA AND PLASMA LEVEL OF NO

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Key words: plasma level of NO, sodium nitroprusside (SNP) hypotensive anaesthesia, middle ear surgery

Introduction

Deliberate hypotension has been used since 1948 for the control of operative bleeding. The goals of deliberate hypotension are: to reduce blood loss and blood transfusions, to improve operating conditions and to decrease operating time. Direct NO donors are pharmacological agents with either nitroso or nitrosyl functional group. In contrast to organic nitrates, which require metabolism for activity, these agents spontaneously release NO.

Three common members of this class are:
1. NO gas,
2. sodium nitroprusside (SNP),
3. sodium trioxodinitrate (Angeli’s salt).

NO gas is freely soluble in physiological solutions. Because of its short half-life and rapid reactivity toward molecular oxygen, its use has been limited to the inhalation therapy because of pulmonary vascular disorders, with limited success.

In SNP, NO is coordinated as a nitrosyl group liganded to iron in a square bipyramidal complex and is released spontaneously at physiological pH from the parent compound.

Angeli’s salt have not been tested in human subjects.

The aim of this study is:
1. To determine plasma level of NO if we use SNP as a hypotensive agent during general anaesthesia.
2. To determine the correlation between MAP and plasma level of NO.

Material and methods

30 ASA physical status I patients were divided in two groups;
1 group: 15 patients who received intravenous infusion of SNP as a hypotensive agent during general anaesthesia.

The study included 30 patients who received general anaesthesia due to chronically inflammatory disease of the middle ear.

The aim of the study was determination of plasma level of NO if we use sodium nitroprusside (SNP) as a hypotensive agent and the correlation between plasma level of NO and mean arterial pressure (MAP).

Material: 30 patients were divided in two groups: 15 patients received general anaesthesia with continuous intravenous infusion of sodium nitroprusside as a hypotensive agent and 15 patients received general anaesthesia without hypotensive agent.

The level of MAP during hypotensive anaesthesia was between 60 and 70 mmHg (8-9kPa).

Method: enzymatic method according to Conrad.

The results have shown that general anaesthesia with continuous intravenous infusion of SNP produces higher plasma level of NO than plasma level of NO in patients who received general anaesthesia without hypotensive agent.

The type of correlation between NO and MAP is negative, which means that if the level of MAP decreases, the level of NO increases. Acta Medica Medianae 2005;44(1): 5–9.

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II group: 15 patients who received general anaesthesia without hypotensive agent.

Study inclusion criteria:
1. adult patients (18-60),
2. ASA I,
3. without drugs which contain Nitrogen preoperatively,
4. without disorders of protein metabolism.

Study exclusion criteria:
1. severe hypotension, MAP<50 mmHg,
2. HR greater than120/min,
3. impossibility to achieve the hypotensive level of MAP after 20-minute continuous infusion.

Anesthetic Protocol:
Patients were premedicated with 2 mg Midazolam intravenously, and after 10 minutes they were introduced into anaesthesia with Fentanyl (0.005 mg/kg) i.v. The intubation was facilitated by 5 mg/kg Thiopental and 0.5 mg/kg Rocuronium. Fentanyl and Rocuronium were added during the operation. Anaesthesia was performed with O2 and air, 35%:65%. The neuromuscular block was reverted with Prostigmine and Atropine at skin closure.

We started with SNP started at 0.5 µg/kg/min and increased by 0.5 µg/kg/min every 2 minutes until the desired blood pressure (BP) was obtained. Maximum dose of SNP is 10 µg/kg/min. In fact, it was the dose of SNP that we reached while we were increasing the dose every 2 minutes, but we couldn’t reach the desirable BP for 20 minutes of continuous infusion. In those cases we excluded intravenous infusion of SNP.

MAP was maintained at the level between 60 and 70 mmHg (8-9 kPa).

Monitoring:
1. Mean arterial pressure.
2. Hart rate.
3. EKG.
4. Peripheral pulse oximetry.

Method
In order to eliminate the influence of nitrogen from food, all patients had to be subjected to a 12-hour-protein starvation.

Clinical values and levels of NO were measured in 5 time intervals:
T1. 10 minutes before induction.
T2. 10 minutes after starting with SNP infusion.
T3. 30 minutes after starting the study drug.
T4. 60 minutes after starting the study drug.
T5. 10 minutes at recovery room.

Determination of MAP was made indirectly, by using the non-invasive method for blood pressure determination.

Plasma level of NO was measured using the method according to Conrad. It is an enzymatic measurement of the stable end-products of NO metabolism, nitrate and nitrite.

The Griess Reaction: The concentration of nitrate plus nitrite in samples is measured by two-step procedure. The nitrate is first converted to nitrite using either enzymatic conversion. There are two enzymatic methods for the conversion of nitrate to nitrite:
1. nicotinamide adenine dinucleotide phosphate (NADPH)-dependent nitrate reductase from Aspergillus species
2. formate-nitrate reductase prepared from Escherichia coli.

Griess reagent needs to be prepared by mixing of 0.1% naphthylethylene diamine dihydrochloride with 1% sulphanilamide in 5% phosphoric acid in equal proportions. The two solutions can be stored separately as stocks at 40°C. The stocks and Griess reagent need to be protected from light.

We add 100 µl of the solution containing nitrate reductase and 10 µM NADPH to a sample-100 µl plasma (centrifuged before at 15000 c/min) in a cuvette and incubated for 20 min at room temperature. This reduces the nitrate to nitrite. The absorbance of the reaction product is read at 450 nm.

We created a standard curve by plotting the mean absorbance for each standard on the y-axis against the nitrite concentration on the x-axis. Because our samples were diluted, we multiplied the concentration read from the standard curve by the dilution factor. We calculated the concentration of nitrite corresponding to the mean absorbance from the nitrite standard curve. (2,5)

The referent values of plasma level of NO by this method are 40.33 µMol/l.

Results

Demographic data of groups are presented in Table 1.

Table 1. Characteristics of examined patients

<table>
<thead>
<tr>
<th>Group</th>
<th>Gender</th>
<th>Age</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>10 M (66%) 5 F (34%)</td>
<td>38,9 ± 11,9</td>
<td>15</td>
</tr>
<tr>
<td>II</td>
<td>3 M (22%) 12 F (78%)</td>
<td>39,9 ± 8,9</td>
<td>15</td>
</tr>
</tbody>
</table>

The results from measurement of values of NO, MAP and HR show that the type of anaesthesia is hypotensive:
1. MAP between 60 and 70 mmHg at II, III and IV time interval (Table 2 and Graphic 1b).

Table 2. Plasma values of NO, values of MAP and HR in the first group

<table>
<thead>
<tr>
<th>Time interval</th>
<th>NO ± SD</th>
<th>MAP ± SD</th>
<th>HR ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>40,52 ± 0,66</td>
<td>83,66 ± 7,2</td>
<td>96,07 ± 10,1</td>
</tr>
<tr>
<td>II</td>
<td>43,74 ± 0,75</td>
<td>64,2 ± 3,8</td>
<td>67,7 ± 6,4</td>
</tr>
<tr>
<td>III</td>
<td>42,36 ± 0,88</td>
<td>65,4 ± 2,8</td>
<td>69,13 ± 5,5</td>
</tr>
<tr>
<td>IV</td>
<td>41,69 ± 1,07</td>
<td>68,06 ± 3,2</td>
<td>72,8 ± 6,8</td>
</tr>
<tr>
<td>V</td>
<td>40,6 ± 0,93</td>
<td>87,87 ± 4,5</td>
<td>84,93 ± 9,9</td>
</tr>
</tbody>
</table>
2. Plasma level of NO were higher than referent values at II, III and IV time interval (Table 2 and Graphic 1a).

Pearson’s coefficient of correlation between MAP and NO shows negative correlation which means that if the values of MAP decrease, the values of NO increase (Table 3). The reason for hypotension and vasodilatation is high plasma level of NO.

Table 3. Pearson’s coefficient of correlation between NO and MAP in the first group

<table>
<thead>
<tr>
<th>Time interval</th>
<th>Pearson's coefficient of correlation (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>-0.17</td>
</tr>
<tr>
<td>II</td>
<td>-0.19</td>
</tr>
<tr>
<td>III</td>
<td>-0.46</td>
</tr>
<tr>
<td>IV</td>
<td>-0.38</td>
</tr>
<tr>
<td>V</td>
<td>0.06</td>
</tr>
</tbody>
</table>

There are no significant differences between plasma levels of NO, middle values of MAP and HR in the second group. These results are presented in Table 4 and Graphics 2a, 2b and 2c.

There is a positive correlation between NO and MAP in the second group, (Pearson’s coefficient of correlation) (Table 5). That means if the values of NO decrease, the values of MAP decrease too.

Middle values of NO between two groups are presented at Table 6. There are significant differences between the second and third time interval of plasma level of NO (10 minutes and 30 minutes after starting with SNP infusion).

Discussion

Nitric oxide mediates multiple physiological and patophysiological processes in the cardiovascular system. Pharmacological compounds that release NO have been useful tools for evaluating the pivotal role of NO in cardiovascular physiology and therapeutics. These agents constitute two broad classes of compounds, those that release NO or one of its redox congeners spontaneously and those that require enzymatic metabolism to generate NO (4).

There are two groups of NO donors:
1. Direct donors: SNP
2. Donors requiring metabolism: organic nitrate and nitrite esters (nitroglycerin)

SNP is the most popular agent because of its rapid onset, rapid offset, titratability, reliability and low cost. SNP decreases systemic vascular resistance which increases cardiac index despite a decrease in blood pressure.
Stewart J. Lustik et al. compare SNP with Nicardipine (calcium channel blocker) for deliberate hypotension (6). They compare coagulation, blood loss and transfusion requirements between patients receiving nicardipine and nitroprusside for deliberate hypotension. They concluded that both drugs were acceptable choices for obtaining the goals of deliberate hypotension, but they didn’t measure the level of NO. Our results show that there are significant difference between plasma levels of NO during the first and second time interval, between the first and third and between the first and fourth time interval, too. Plasma level of NO higher than 40.33 µMol/l in the first group of patients means that SNP is an adequate donor of NO.

**Table 4. Middle values of NO, MAP and HR in the second group**

<table>
<thead>
<tr>
<th>Time interval</th>
<th>NO</th>
<th>MAP</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>I</td>
<td>38.89</td>
<td>117.8</td>
<td>103.4</td>
</tr>
<tr>
<td>II</td>
<td>39.24</td>
<td>108.67</td>
<td>91.73</td>
</tr>
<tr>
<td>III</td>
<td>39.29</td>
<td>105.93</td>
<td>87.0</td>
</tr>
<tr>
<td>IV</td>
<td>39.79</td>
<td>104.2</td>
<td>85.6</td>
</tr>
<tr>
<td>V</td>
<td>40.16</td>
<td>116.93</td>
<td>100.86</td>
</tr>
</tbody>
</table>

**Table 5. Pearson’s coefficient of correlation between NO and MAP in the second group**

<table>
<thead>
<tr>
<th>Time interval</th>
<th>Pearson-r coefficient - (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.64</td>
</tr>
<tr>
<td>II</td>
<td>0.68</td>
</tr>
<tr>
<td>III</td>
<td>0.62</td>
</tr>
<tr>
<td>IV</td>
<td>0.36</td>
</tr>
<tr>
<td>V</td>
<td>0.53</td>
</tr>
</tbody>
</table>

**Table 6. Values of NO between two groups and t-test**

<table>
<thead>
<tr>
<th>Time interval</th>
<th>NO-SNP</th>
<th>NO-normotensive</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x ±SD</td>
<td>x ±SD</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>40.52</td>
<td>3.9</td>
<td>p 0.1217</td>
</tr>
<tr>
<td>II</td>
<td>43.74</td>
<td>3.9</td>
<td>p 0.0001*</td>
</tr>
<tr>
<td>III</td>
<td>42.35</td>
<td>3.9</td>
<td>p 0.0052*</td>
</tr>
<tr>
<td>IV</td>
<td>41.69</td>
<td>4.0</td>
<td>p 0.088</td>
</tr>
<tr>
<td>V</td>
<td>40.6</td>
<td>3.9</td>
<td>p 0.6741</td>
</tr>
</tbody>
</table>

There are no significant differences between plasma levels of NO in the second group of patients who received general anesthesia without a hypotensive agent.

**Conclusion**

To conclude, our study shows that SNP is a direct NO donor, which reacts with endothelial smooth muscle cells and the final effects are vasodilatation and hypotension. There is a negative correlation between MAP and NO, which means that if values of MAP decrease, the plasma levels of NO increase. It means that NO is the main reason for vasodilatation and hypotension.

**References**

SODIUMNITROPRUSID KAO HIPOTENZIVNI AGENS ZA VREME OPŠTE ANESTEZIJE I NIVO NO U PLAZMI

Biljana Shirgoska, Marija Soljakova, Liljana Simjanovska, Rozalinda Isijanovska i Trajan Balkanov

Ispitivanje je uključilo 30 bolesnika kojima je data opšta anestezija zbog hronične upale srednjeg uva.

Cilj ispitivanja je bio određivanje nivoa plazme NO ukoliko koristimo natrijum nitroprusid (NNP) kao hipotenzivni agens i korelacije između nivoa plazme NO i srednjeg arterijskog pritiska (SAP).

Materijal: 30 bolesnika je podeljeno u dve grupe: 15 je primilo opštu anesteziju sa kontinuiranom intravenoznom infuzijom natrijum nitroprusida kao hipotenzivnog agensa, dok je preostalih 15 bolesnika primilo opštu anesteziju bez hipotenzivnog agensa.

Nivo srednjeg arterijskog pritiska za vreme hipotenzivne anestezije je bio između 60 i 70 mmHg (8–9 kPa).

Metoda: enzimatična metoda po Konradu.

Rezultati su pokazali da opšta anestezija sa kontinuiranom intravenoznom infuzijom NNP-a dovodi do većeg nivoa plazme NO nego što je to kod bolesnika koji su primili opštu anesteziju bez hipotenzivnog agensa.


Ključne reči: nivo plazme NO, natrijum nitroprusid (NNP), hipotenzivna anestezija, operacija srednjeg uva