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

*Biljana Shirgoska *, Marija Soljakova **, Liljana Simjanovska***, Rozalinda Isijanovska[#] and Trajan Balkanov [##]*

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 intavenous 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 dicreases, the level of NO increases. *Acta Medica Medianae 2005;44(1): 5–9*.

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

Clinic for Ear, Nose and Throat Surgery, Skopje*
Clinic for Anestesiology, Reanimation and Intensive Care, Skopje**
Research Center for Genetic Engineering and Biotechnology, Skopje***
Institute for Epidemiology, Skopje[#]
Institute for Pharmacology, Skopje[##]

Correspondence to: Biljana Shirgoska
Clinic for Ear, Nose and Throat Surgery
6b Moskovska Street
1000 Skoplje Macedonia
Phone: +389 23 147 347; +389 23 147 217
E-mail: m_shirgoski@yahho.com

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 anesthesia.
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;

I group: 15 patients who received intravenous infusion of SNP as a hypotensive agent during general anaesthesia.

II group: 15 patients who received general anaesthesia without hypothensive 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 Fentanil (0.005 mg/kg) i.v. The intubation was facilitated by 5 mg/kg Tiopental and 0.5 mg/kg Rocuronium. Fentanil and Rocuronium were added during the operation. Anaesthesia was performed with O_2 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 dug.
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% naphthylethylenediamine dihydrochloride with 1% sulphanilamide in 5% phosphoric acid in equal proportions. The two solutions can be stored separately as stocks at 40C. 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

Demorgaphic data of groups are presented in Table 1.

Table 1. Characteristics of examined patients

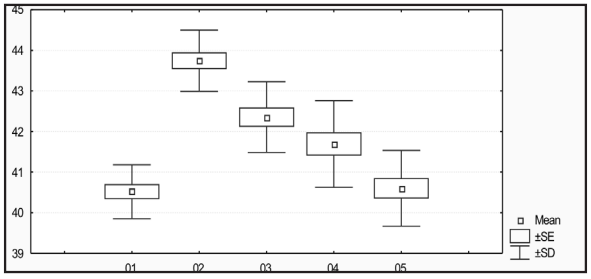
Group	Gender	Age	n
I	10 M (66%) 5 F (34%)	38,9 ± 11,9	15
II	3 M (22%) 12 F(78%)	39,9 ± 8,9	15

The results from measurement of values of NO, MAP and HR show that the type of anesthesia is hypotensive:

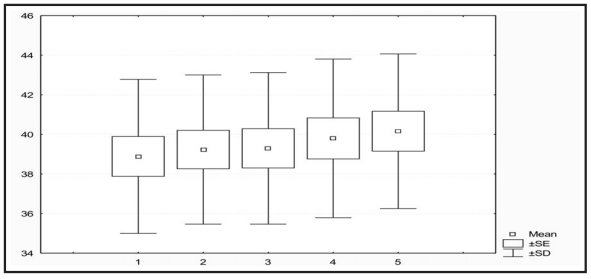
1. MAP between 60 and 70 mmHg at II, III and IV time interval (Table 2 and Graphic 1b).

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

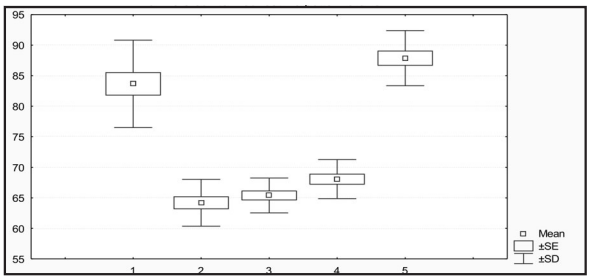
Time interval	NO		MAP		HR	
	x	± SD	x	± SD	x	± SD
I	40,52	0,66	83,66	7,2	96,07	10,1
II	43,74	0,75	64,2	3,8	67,7	6,4
III	42,36	0,88	65,4	2,8	69,13	5,5
IV	41,69	1,07	68,06	3,2	72,8	6,8
V	40,6	0,93	87,87	4,5	84,93	9,9



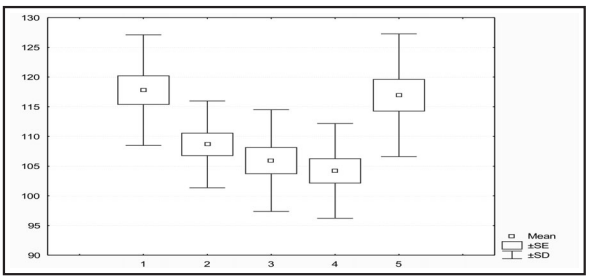
Graphic 1a . Plasma values of NO in the first group



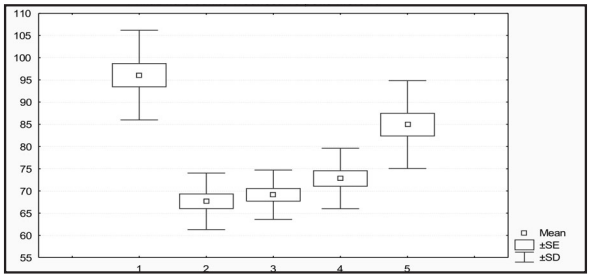
Graphic 2a. Values of NO in the second group



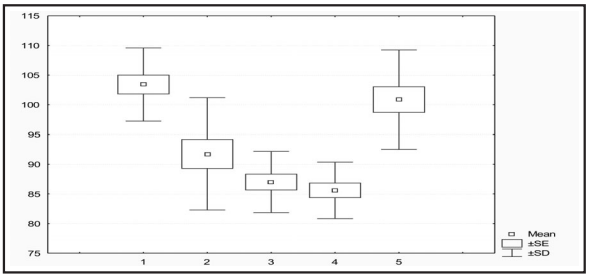
Graphic 1b . Plasma values of MAP in the first group



Graphic 2b. Values of MAP in the second group



Graphic 1c . Plasma values of HR in the first group



Graphic 2c. Values in the HR in the second group

2. Plasma level of NO were higher than referent values at II,III and IV time interval (Table 2 and Graphic 1a)

Pearoson'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 (Table3). The reason for hypotension and vasodilatation is high plasma level of NO.

Table 3. Pearoson's coefficient of correlation between NO and MAP in the first group

Time interval	Pearoson's coefficient of correlation (r)
I	- 0,17
II	- 0,19
III	- 0,46
IV	- 0,38
V	0,06

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, (Pearoson's coefficient of correlation) (Table5). 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.

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

Time interval	NO		MAP		HR	
	x		x		x	
	38,89	3,89	117,8	9,3	103,4	6,17
II	39,24	3,77	108,67	7,3	91,73	9,44
III	39,29	3,83	105,93	8,56	87,0	5,16
IV	39,79	4,0	104,2	7,97	85,6	4,76
V	40,16	3,9	116,93	10,33	100,86	8,36

Table 5. Pearoson's coefficient of correlation between NO and MAP in the second group

Time interval	Pearoson-s coefficient of correlation - (r)
I	0,64
II	0,68
III	0,62
IV	0,36
V	0,53

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

Time interval	NO-SNP		NO-normotensive		t-test	
	x	±SD	x	±SD		
I	40,52	0,66	38,89	3,9	p	0,1217
II	43,74	0,75	39,24	3,8	p	0,0001*
III	42,35	0,87	39,3	3,8	p	0,0052*
IV	41,69	1,07	39,8	4,0	p	0,088
V	40, 6	0,93	40,16	3,9	p	0,6741

* significant differences

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.

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

1. DeWitt JB, Marrone JR, Kaye AD, Keefer LK, Kadowitz PJ. Comparison of responses to novel nitric oxide donors in the feline pulmonary vascular bed; European Jurnal of Pharmacology 430(2001) 311-2.
2. Lincoln J, Hoyle CHV, Burnstock G. Nitric oxide in health and disease, Cambridge University Press; 1997.
3. Koyama K, Yamada T, Takeda J. Effects of nicardipine, nitroglycerin, and nitroprusside on left ventricular diastolic function during enflurane anesthesia. J Cardiothorac Vasc Anesth 1994;8:404-9.
4. Ignarro LJ, Napoli C, Loscalzo J. Nitric Oxide Donors and Cardiovascular Agents Modulating the Bioactivity of Nitric Oxide; Circulation Res 2002;90:21-8.
5. MichaelA.Titheradge:Nitric Oxide Protocols, Humana Press Inc, Totowa, New Jersey,1998. pp 83-93.
6. Lustik SJ, Papadacos PJ, Jackman KV, Rubery PT, Kaplan KL, Chhibber AK. Nicardipine versus Nitroprusside for Deliberate Hypotension during Idiopathic Scoliosis Repair. Journal of Clinical Anesthesia 2004; 16:25-33.
7. Testa LD, Tobias JD. Pharmacologic drugs for controlled hypotension. J Clin Anesth 1995; 7:326-37.

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.

Tip korelacije između NO i SAP-a je negativan, što znači da ukoliko nivo SAP-a opada, nivo NO raste. *Acta Medica Medianae 2005; 44 (1):5–9.*

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