Methylene blue, a nitric oxide inhibitor, prevents haemodialysis hypotension

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Abstract

Background. Plasma nitric oxide (NO) levels have been found to be high in haemodialysis (HD) patients, especially in those prone to hypotension in dialysis. The aim of the study was to prevent dialysis hypotension episodes by i.v. administration of methylene blue (MB), an inhibitor of NO activity and/or production.

Methods. MB was given i.v. in 18 stable HD patients with hypotensive episodes during almost every dialysis, in 18 HD patients without hypotension during dialyses, and in five healthy controls. MB was given as a bolus of 1 mg/kg bodyweight followed by a constant infusion of 0.1 mg/kg bodyweight lasting 210 min until the end of the dialysis session and only as a bolus on a non-dialysis day. Systolic and diastolic blood pressures (BP) were measured at 10-min intervals during HD sessions with or without MB and on a non-dialysis day with MB.

Results. In hypotension-prone patients, MB completely prevented the hypotension during dialysis and increased both systolic and diastolic BP on non-dialysis days. In normotensive patients, MB increased BP during the first hour of dialysis and for 90 min on the non-dialysis day. The BP in the healthy controls remained unchanged. Plasma and platelet NO$_2$ + NO$_3$ (stable metabolites of NO) levels were determined. The NO$_2$ + NO$_3$ generation rate in the first post-dialysis day was calculated. The plasma and platelet NO$_2$ + NO$_3$ were higher in the hypotensive group than in the normotensive dialysis group. The generation rate of nitrates was higher ($P < 0.01$) in the hypotensive group ($1.21 \pm 0.13$ mmol/min and $0.74 \pm 0.16$ after MB) than in the normotensive patients ($0.61 \pm 0.11$ mmol/min and $0.27 \pm 0.14$ after MB). No side-effects were recorded.

Conclusions. MB is an efficient therapy in the prevention of dialysis hypotension.

Keywords: haemodialysis; hypotension; methylene blue; nitric oxide

Introduction

Dialysis hypotension remains one of the most frequent intradialytic complications, although important improvements have been made in the dialysis techniques. The incidence of symptomatic hypotensive episodes during the haemodialysis (HD) sessions is high in the patients who have low or normal blood pressure and in those with large interdialytic weight gain [1,2]. The fluid removal during dialysis with its haemodynamic consequences (hypovolaemia, fall in circulating filling pressure and in cardiac output) is considered to be an important cause of these symptomatic hypotensive episodes [3]. However, in many patients without hypovolaemia, moderate or severe hypotensive episodes occur during HD and are reasonably explained by high venous capacity for the normal blood volume and/or an inappropriately low peripheral arterial resistance [4]. Beasley and Brenner [5] suggested that nitric oxide (NO), a very potent endogenous vasodilator, may be blamed for these hypotensive events.

It was found that NO production may increase significantly during HD [6–20]. Increased NO generation between and during dialysis sessions may result in significant and rapid vasodilatation, i.e. hypotension.

The aim of the present study was the prevention of the intradialytic hypotensive episodes by the intravenous administration of methylene blue (MB), an agent known to inhibit the vasodilatory effect of NO [21,22].
Subjects and methods

Subjects

The study protocol was approved by the Helsinki Committee and patients and volunteers all gave their informed consent.

Twenty nine patients (aged 29–69 years) with end-stage renal failure on maintenance HD participated in the study. The patients had been on dialysis for 3–21 years.

Fourteen had chronic glomerulonephritis, four polycystic kidney disease, two NIDDM, one analgesic nephropathy, and eight different tubulointerstitial nephropathies. Although none had severe congestive heart failure, class 1 or 2 NYHA CHF patients were not excluded from the study. Patients with adrenal insufficiency, chronic salt and/or volume depletion, pericardial effusion or constriction, or infections were not included. None of them received antihypertensive drugs or any other agents known to influence the blood pressure.

Patients with active coronary heart disease (as assessed by clinical symptoms, ECG and myocardial function—perfusion as measured by Tc-MIBI imaging) or on nitrate therapy were also excluded because of the potential reduction in coronary blood flow due to NO inhibition [23].

Eighteen of these patients (group 1, age 56 ± 9 years, on dialysis for 3–11 years) had blood pressures ranging between 90 and 110/65–70 mmHg before dialysis and had intra-dialytic hypotensive episodes in at least four of the previous six HD sessions not responsive to a fluid challenge of 250 ml normal saline.

A dialysis hypotension episode was defined as a sudden reduction in systolic blood pressure of 20 mmHg or more in the first 2 h of dialysis. We chose this definition in order to minimize the effect of the fluid removal on the blood pressure, known to be more prominent in the second half of dialysis [24].

HD patients had a pre-set fluid removal of 1000 ml in the first 2 h of HD with or without MB. The mean weight gain was similar before the two study dialysis sessions with or without methylene blue; 1.9 ± 0.7 and 2.05 ± 0.65 kg respectively.

Various procedures had been used before the study in order to alleviate the hypotension and the symptoms: sequential ultrafiltration and dialysis, high (150 mEq/l) initial Na concentration in the dialysate with progressive reduction of the concentration during dialysis, dialysate cooling, and volume administration (hypertonic saline solutions). All these had partial and inconsistent effect on the prevention of the hypotensive episodes. This group was considered as dialysis hypotension-prone patients.

The remaining 11 dialysis patients (group 2, age 54 ± 7 years, 3–21 years on dialysis) were normotensive (blood pressure 135–140/80–85 mmHg) and had very rare symptomatic hypotensive episodes (less than one in 2 months). The mean weight gain was similar before the two study dialysis sessions with or without MB (2.07 ± 0.6 and 2.12 ± 0.6 kg respectively). Five healthy volunteers (group 3 age 49 ± 10 years) participated in the study.

All patients received erythropoietin treatment. Patients and volunteers had no G6PD deficiency. MB is reported to produce haemolysis in these subjects [25].

The dialysis efficiency, documented by Kt/V, was 1.35 ± 0.70 in the normotensive group and 1.27 ± 0.50 in the hypertensive group. The dialysers used were Fresenius F6 or Sure Flux 1.3 m² hollow-fibre dialysers. Blood flow ranged from 250 to 350 ml/min and was the same for the patients throughout the study. Bicarbonate (35 mEq/l) dialysis solution with a Na concentration of 140 mEq/l was used. The desired ultrafiltration necessary to achieve the target weight reduction was divided equally over the entire dialysis session.

MB administration

All HD patients underwent three tests, two during two consecutive HD sessions (one dialysis without MB and one with MB administration), and the third test on a non-dialysis day. The normal volunteers underwent one test. All tests lasted 240 min. MB was given as an i.v. bolus (1 mg/kg bw) over 5–6 min, in 30 ml normal saline on the non-dialysis day and in the normal volunteers.

The bolus of MB during the dialysis was given immediately before HD in a peripheral vein and not through the fistula, and was followed by a continuous infusion of 0.1 mg/kg bw in a total of 200 ml normal saline solution throughout the entire dialysis session. Each HD lasted 210 min followed by a 30-min post-dialysis observation period.

The blood pressure and heart rate were measured by an automatic recording device every 10 min during all tests. ECG was recorded before and after dialysis and monitored during dialysis.

Blood samples were drawn in all tests before, at 105 min, at 210 min (end of dialysis) and at 240 min (end of the test).

Laboratory determinations

Haemoglobin, haematocrit, reticulocytes, LDH, bilirubin, and methaemoglobin were determined by standard laboratory methods.

Nitrite (NO₂) and nitrate (NO₃), the stable metabolites of NO, were measured as previously described [26]. Briefly, after reduction of NO₂ to NO₃ with nitrate reductase (prepared from E. coli in our laboratory) the NO₂ was determined spectrophotometrically with Griess reaction. The NO₂ + NO₃ generation rate was calculated from the values obtained at the end of dialysis and those determined after 24 h and expressed in μmol/min according to the formula

\[
G = \frac{(V2 × C2) - (V3 × C3)}{1440}
\]

in which V2 volume of distribution of NO₂ + NO₃ (as for urea), C2 is the NO₂ + NO₃ plasma concentration immediately after dialysis, V3 × C3 is the volume of distribution and NO₂ + NO₃ plasma concentration after 24 h [27].

Statistics

Mean ± SD, two-way analysis of variance with repeated measurements, Student’s t-test was used to assess statistical significance. P < 0.05 was considered significant.

Results

The effect of MB on blood pressure (Figures 1 and 2). The administration of MB to healthy volunteers did not change the blood pressure. In the normotensive dialysis patients, who had no dialysis hypotension, MB administration resulted in significantly higher systolic and diastolic blood pressure compared with the values during a dialysis session without MB (P < 0.001 by two-way analysis of variance with
repeated measurements). The highest values after MB were measured during the first 90 min of dialysis.

In the dialysis patients with hypotensive episodes during the HD session without MB, both systolic and diastolic pressure decreased significantly after the first hour of dialysis.

In the same group of patients, the decrease in blood pressure was prevented during the dialysis session when MB was administered. Both systolic and diastolic curves during the dialysis with MB were very significantly different from those of the dialysis session without MB.

MB administration on a non-dialysis day resulted in an increase in both systolic and diastolic blood pressure in all dialysis patients for at least 180 min.

The changes in heart rate did not attain statistical significance in any group of dialysis patients and volunteers during the dialysis sessions or on a non-dialysis day.

**Side-effects**

Three patients complained of slight pain at the injection site during the MB administration. In four dialysis patients, a bluish discoloration of lips was observed, without any complaint. No other side-effects were recorded. No ECG changes were recorded. No signs of haemolysis and/or methaemoglobinaemia were found.

After the end of dialysis and termination of the MB administration, the blood pressure decreased to the predialysis values and the patients remained asymptomatic.

**Plasma nitrates (Table 1)**

The patients with dialysis hypotension had higher nitrates levels than those with normal blood pressure but this difference did not reach statistical significance. The nitrates levels decreased significantly during the dialysis in both groups with or without MB treatment. The nitrates generation rate in the first post-dialysis day was higher in the dialysis-hypotensive patients (1.21 ± 0.13 µmol/min) compared to the normal blood-pressure patients (0.61 ± 0.11 µmol/min) *P* < 0.01. Furthermore, the nitrates generation rate in the first post-dialysis day following a HD session with MB was higher in the dialysis-hypotensive patients compared to the normotensive patients (0.74 ± 0.16 vs 0.27 ± 0.14 µmol/min, *P* < 0.01).
<table>
<thead>
<tr>
<th>Time (min)</th>
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<th>Healthy controls</th>
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<tr>
<td>HD + MB</td>
<td>0</td>
<td>55.1 ± 58.8 98.54 ± 4</td>
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<tr>
<td>105</td>
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<td>210</td>
<td>25.7 ± 11.1*</td>
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<td>66.4 ± 39.2 87.2 ± 65.5</td>
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<tr>
<td>105</td>
<td>28.1 ± 23*</td>
<td>39.6 ± 26.7*</td>
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<tr>
<td>210</td>
<td>30.5 ± 32.2*</td>
<td>36.1 ± 19.4*</td>
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<td>-HD + MB</td>
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<tr>
<td>210</td>
<td>45.7 ± 23.4</td>
<td>57.8 ± 47 35.4 ± 9.9</td>
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</table>

Mean ± SD; NO$_2$ + NO$_3$ in µmol/l; HD, haemodialysis; −HD, non-HD day; MB, methylene blue; *P < 0.05 (at least vs the predialysis respective values).

Discussion

The major finding of the present study is the prevention of intradialytic hypotension by the continuous i.v. administration of MB. MB is known to block cGMP [21,22], thus inhibiting the action of the NO resulting from the increased NO synthase (NOS) activity. It also has the ability to scavenge NO [28], as well as to inhibit NO synthesis [29]. MB has been given for many years for clinical purposes without serious side-effects [25,30,31]. The previously described side-effects of MB include: haemolysis and skin eruption due to photosensitivity nitric oxide, one of the most potent endogenously-produced vasodilator molecules, seems to be a very good candidate to cause intradialytic hypotension. Several reports suggest that NO production is enhanced in HD patients [6–20]. The exposure of healthy blood to HD membranes, mainly to cuprophane, markedly enhanced the expression of mRNA encoding for inducible NOS by a line of murine endothelial cells [6,18].

In all probability the release of cytokines like IL-1β and TNFα by activated mononuclear cells triggers the activation of NOS. Noris et al. [9] found that platelets of uraemic patients produce increased amounts of NO, leading to hypotension during dialysis. It has also been suggested [32] that heparin promotes NO production by human vascular endothelial cells in culture, but in an in vivo study [8] in dialysis patients by the same group [7] it was found that heparin was not involved in NO production. These authors also found that NO production increased in patients who had a hypotensive episode during dialysis but not in those who remained normotensive during dialysis. NO production was negatively correlated with the mean blood pressure after dialysis.

However, the haemodynamic role of NO is still not clear because of the increased plasma concentration of inhibitors of NO like ADMA in end-stage renal disease [11,19,20,33]. These substances are dialysable, so the final effect of the end-stage uraemic plasma on NOS activity derives from the balance between inhibitors and activators. In a previous work (unpublished) we found significantly higher levels of NO$_2$ and NO$_3$ in the plasma of HD patients as compared with normal controls. These levels decreased progressively during dialysis, indicating that they were probably cleared.

Recently, direct NO measurements by the blood of dialysis patients by a NO-selective electrode confirmed the increased NO production during HD [8,13]. Calculation of the rate of nitrates generation showed that dialysis enhanced nitrates production. However, although the plasma NO$_2$ and NO$_3$ levels must be taken with some caution for establishing a true generation rate, our results on the first post-dialysis 24 h generation rate strengthen our findings.

NO may lower the blood pressure not only by its powerful vasodilatory effect or by opposing vasoconstriction, but also by its negative inotropic effect [34,35]. The MB administration in the dose we used had no effect on blood pressure and heart rate in normal controls. This was in contrast with the effect of another systemic NO inhibitor L-NMMA, given to healthy individuals [36]. However, in the dialysis patients, MB given on a non-dialysis day increased blood pressure in both of the studied groups. This may be explained by the lower total peripheral resistance due to an enhanced NO production in the hypotension-prone patients.

Our study has some limitations. Only patients without coronary heart disease were included, because of the possible implications of the systemic NOS inhibition on the coronary vascular bed [23]. In our patients, there were no signs or symptoms of cardiac ischaemia due to MB administration. Only a single dose of MB was used.

No pharmacokinetic data on MB were obtained during HD, and the decision to choose a continuous infusion of the substance during dialysis was empirical. Recently a method for MB quantitation was described in catfish tissue [37].

In a very recent publication, MB was given i.v. to five neonates with refractory neonatal hypotension in the same dose as used by us, with very good results [38].

MB had no significant effect of the blood levels of nitrates. The nitrate levels before dialysis were not different statistically in the patients with hypotension and those with normal blood pressure, as Yokokawa et al. [7] also found. However, in their study NO$_2$ + NO$_3$ levels measured after 4 h of dialysis did not change in the normotensive subjects, but were greatly increased in their hypotensive patients, while these levels decreased in our patients, as reported also other publications [8]. Yokokawa also found a decrease in cGMP blood levels instead of the expected elevation due to NO activity. We did not find significant differences in NO$_2$ + NO$_3$ levels measured during dialysis between the groups.
However, the nitrates generation rate values in the first 24 h after a dialysis session, were significantly higher in the hypotensive patients with or without MB treatment. After MB, the generation rate decreased in both groups. We found, as did Noris et al. [9,10], that dialysis patients produced more NO than healthy controls. Furthermore, the dialysis hypotension-prone patients have higher NO compared with the normotensive group. This high generation rate of nitrates and the effect of MB in this group of dialysis patients emphasized the role of NO in the pathogenesis of dialysis hypotension.

In conclusion, MB may be an efficient therapeutic agent for the prevention of dialysis hypotension. Since the completion of the study, nine hypotension-prone dialysis patients have received MB during HD once weekly at least six times, with effective prevention of dialysis hypotensive episodes, and with no side-effects. However, a long-term study is necessary to assess the safety of chronic MB administration in HD patients.

References


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