Circulating tetrahydrobiopterin concentrations in patients with septic shock

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Nitric oxide synthase requires tetrahydrobiopterin for its activity. In animal models of sepsis, changes in circulating tetrahydrobiopterin concentrations precede increases in nitrate. We measured plasma tetrahydrobiopterin and nitrate concentrations on three consecutive days in 10 patients with septic shock and 10 critically ill control patients. Total nitrate concentration was measured after reduction of nitrite to nitrate. Tetrahydrobiopterin concentrations were measured using HPLC. The median (range) APACHE II score was 22 (13–27) in the patients with septic shock and 25 (7–28) in the control group. The nitrate concentration was significantly higher in patients with septic shock than in controls ($P=0.01$) on all days but did not change with time. Tetrahydrobiopterin concentrations were highest in the patients with septic shock on day 1 only ($P=0.037$). In the seven patients with renal failure, both nitrate and tetrahydrobiopterin concentrations tended to be higher than in the 13 patients without renal failure. The nitrate concentration correlated with tetrahydrobiopterin concentration on day 1 only ($P=0.05$). In patients with septic shock, both tetrahydrobiopterin and total nitrate concentrations were higher than those in critically ill controls but were increased mainly in patients with renal failure. In summary, tetrahydrobiopterin concentration increases during septic shock, in line with increases in nitrate concentration. However, as for nitrate, concentrations...
Nitric oxide is synthesized from L-arginine by the action of nitric oxide synthase (NOS) and NOS enzymes require tetrahydrobiopterin for catalytic activity. Nitric oxide is important in the maintenance of vasodilator tone and arterial pressure and it has been suggested that cytokine-mediated circulatory shock is caused by activation of the inducible isoform (type II) of NOS. In biological systems, nitric oxide decomposes to nitrite and nitrate. Cytokine-mediated increases in concentrations of nitrite/nitrate are found in cell cultures. Circulating nitrite and nitrate concentrations also increase in patients with sepsis and increased NOS activity in leucocytes from these patients has been demonstrated. However, nitrite and nitrate are excreted by the kidney, so plasma and urine concentrations of these compounds are highly dependent upon renal function. Tetrahydrobiopterin bioavailability has an important role in the regulation of nitric oxide production. In an animal model of sepsis, increased circulating biopterin concentrations preceded increases in nitrate and mirrored changes in arterial pressure. The authors suggested that tetrahydrobiopterin concentrations in plasma may be a more helpful biochemical marker of the onset of septic shock than nitrite/nitrate concentrations. We investigated the use of tetrahydrobiopterin concentrations as an early biochemical marker of septic shock, compared with plasma nitrite/nitrate concentrations.

Methods

Blood samples from indwelling arterial lines were obtained on day 1, and 24 and 48 h later. Blood was centrifuged and frozen at −80°C. Plasma total nitrate concentration was measured spectrophotometrically using Greiss reagent after reduction of nitrite to nitrate. Tetrahydrobiopterin concentrations were measured using high-performance liquid chromatography (HPLC) after selective oxidation with iodine. Samples were added to acid iodine solution containing 0.5% iodine and 1% potassium iodide in 0.2 M trichloroacetic acid (tube A) and to 2 M sodium hydroxide and alkaline iodine solution (1% iodine and 2% potassium iodide in 0.1 M sodium hydroxide) (tube B) and incubated for 1 h at room temperature in the dark. To the alkali tube (A) only, 1 M hydrochloric acid was then added and both were centrifuged at 10 000 g. Excess iodine was removed by adding freshly made 1% ascorbic acid. Under acid conditions, all pterins are converted to fluorescent biopterin, whilst under alkali conditions, tetrahydrobiopterin is converted to non-fluorescent material, with only biopterin detected. Tetrahydrobiopterin can therefore be measured as the difference between biopterin concentration under acid and alkali conditions, using HPLC with fluorescent detection.

Biopterins were separated on a C18 Techsil reverse phase column (HPLC Technology, Macclesfield, Cheshire, UK) with 10% HPLC-grade methanol in water as mobile phase, and calibrated against biopterin which had also undergone acid/alkali oxidation. Biopterin was detected fluorimetrically (excitation at 350 nm, emission at 440 nm). The accuracy of the technique was 78.3 (77.0–79.8)% in recovery experiments (n=30), using biopterin 30 ng ml⁻¹ in the presence of bovine serum albumin 1 mg ml⁻¹ to mimic plasma protein content. The within-run and between-run precision of the assay was 1.8% and 8.1%, respectively (n=10).

Statistical analysis

Results are expressed as median (range). Statistical analysis was performed using Microsoft Analyse-it statistical add-in for Excel for Windows. Data sets were compared using Mann Whitney U-test/Friedman analysis of variance, and correlations were assessed using Kendall’s rank correlation. A P value of <0.05 was considered statistically significant.

Keywords: pharmacology, nitric oxide; shock; kidney, function; enzymes, nitric oxide synthase

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Increased production of nitric oxide in response to activation of the type II isoform of NOS to cytokines has been suggested to be responsible for the hypotension of septic shock.\textsuperscript{2} The first step in the synthesis of tetrahydrobiopterin, which is rate limiting for nitric oxide production by type II NOS, involves the enzyme GTP cyclohydrolase I, which is co-induced by the same mediators as those which induce type II NOS synthesis. Inhibitors of GTP cyclohydrolase I downregulate type II NOS mRNA, protein and activity. GTP cyclohydrolase activity and hence tetrahydrobiopterin have important roles in the regulation of nitric oxide release. In both rabbits and rats treated with live \textit{Escherichia coli}, increases in tetrahydrobiopterin concentration preceded increases in nitrate concentration.\textsuperscript{5}

Although tetrahydrobiopterin concentration was increased in patients with septic shock, the increases may have been due entirely to the contribution of reduced renal clearance. A previous study of 21 intensive care patients found that release of D-erythro-neopterin from macrophages tended to be greatest in patients who ultimately died.\textsuperscript{6} Although both tetrahydrobiopterin and total nitrate concentrations were higher in the patients with septic shock than in non-infected critically ill controls, we found no differences between survivors and non-survivors, although clearly our small pilot study was not designed to show such a difference.

In summary, tetrahydrobiopterin concentration is increased during septic shock, in line with increases in nitrate concentration. However, as with nitrate, concentrations seem to be affected by renal function. Measurement of tetrahydrobiopterin concentrations is extremely technically demanding and time consuming and offers no advantage over measurement of nitrate concentrations.

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