Clear Correlation of Tetrahydrobiopterin with Nitric Oxide Bioavailability in Continuous Ambulatory Peritoneal Dialysis

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Nitric oxide (NO) plays diverse physiological roles including vasodilatory and anti-atherogenic actions [1]. In continuous ambulatory peritoneal dialysis (CAPD), NO has been thought to regulate permeability of the peritoneal membrane and suppress angiogenesis in the peritoneum [2–4]. Thus, abnormal production and/or metabolism of NO would lead to peritoneal dysfunctions. Previous studies suggested that changes in NO bioavailability induce the dysfunction of peritoneal membranes [3, 5]. Recently, we demonstrated that NO exists in spent dialysates at measurable levels over a wide range [6].

Tetrahydrobiopterin (BH₄) is a cofactor for NO synthase (NOS) and plays an important role in maintaining the NOS activity. Depletion of BH₄ would result in NOS uncoupling and NOS-derived superoxide release [1, 7, 8]. Yokoyama et al. [9] reported that a significant positive correlation between creatinine clearance and the BH₄/BH₂ ratio (BH₂ = biopterin, an oxidized form of BH₄) in the patients with chronic renal failure. Yamamizu et al. [10] demonstrated the involvement of BH₄ deficiency in endothelial dysfunction in rats with chronic renal failure. Recently, we also reported the possible involvement of BH₄ in the NO production rate of hemodialysis (HD) patients [11]. However, there

have been no investigations on the roles of NO and BH₄ in CAPD. Therefore, we measured both NO and BH₄ concentrations in spent dialysates of CAPD patients to investigate the relation between the two.

The NO concentration was measured by an NO sensor [6, 12] and BH₄ and BH₂ concentrations were measured fluorometrically by the HPLC-based method [13]. This study protocol was approved by the Ethical Committee of Kawasaki Medical School. Written informed consent was obtained from each patient.

Both NO and BH₄ in the spent dialysates (dwelling time about 5 h) showed diverse distribution among the patients (n = 28; NO, 0.5–11.8, 4.7 ± 0.6 nmol/l; BH₄, 0.6–17.0, 3.8 ± 0.7 ng/ml). There was a positive correlation between NO and BH₄ (fig. 1; $p < 0.001$). This result suggests that NO is produced in dependence on BH₄ availability. Similarly to BH₄, BH₄/BH₂ also showed diverse distribution (2.3–122.3, 42.2 ± 6.8). There was a positive correlation between NO and BH₄/BH₂ (fig. 2; $p < 0.002$). This result may also sup-

Fig. 1. Relation between nitric oxide (NO) and tetrahydrobiopterin (BH₄) concentrations.

Fig. 2. Relation between nitric oxide (NO) concentration and tetrahydrobiopterin/biopterin (BH₄/BH₂) ratio.

port the dependence of NO availability on BH₄.

Recently, we reported the plasma BH₄ concentrations and the BH₄/BH₂ ratios for the HD patients and the healthy volunteers [11]. The BH₄ concentration in the spent dialysates of the CAPD patients in the present study (3.8 ± 0.7 ng/ml) was nearly at the same level as that of healthy volunteers (3.0 ± 0.2 ng/ml) and that of HD patients before HD (3.8 ± 0.6 ng/ml). The BH₄/BH₂ ratio of the CAPD patients (42.2 ± 6.8) seems higher than that of the HD patients (12.7 ± 7.4 before HD; 16.1 ± 10.3 after 4-hour HD) but almost at the same level as that of the healthy volunteers (31.4 ± 15.2). Thus, it is implied that CAPD is a milder therapy (similar to the healthy conditions) than HD in terms of

BH₄ bioavailability and resultant NO bioavailability.

A wide distribution of BH₄ levels was observed (fig. 1). Although the exact reason for the distribution is not clear, some possible mechanisms may be inferred. GTP cyclohydrolase I, one of the key enzymes for the BH₄ synthesis, may be adversely affected by high oxidative stress [7]. BH₄ itself has reducing capacity and thus may be oxidized to BH₂ by high oxidative stress. Therefore, depending on the oxidative stress levels, BH₄ bioavailability may differ among the patients studied.

In case of depletion of BH₄, NOS uncoupling is induced and causes release of superoxide from NOS [14]. This may lead to detrimental effects on the peritoneal membrane. In our preliminary studies, we

observed lower NO production in cultured mesothelial cells with increasing glucose content (increasing oxidative stress) in culture solutions [15]. A recent report demonstrated the effectiveness of oral supplementation of BH₄ on recovery of endothelial dysfunction in the rats of chronic renal failure [10]. Thus, in case of BH₄ deficiency, exogenous BH₄ intake may be beneficial to maintenance of peritoneal functions.

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