Clearance of middle molecules during haemodialysis and haemodiafiltration: new insights

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Patients with end-stage kidney disease exhibit the retention of a large variety of solutes that may affect a range of biological functions and, for instance, contribute to elevation of cardiovascular risk and modulate inflammatory responses. Such uraemic retention solutes can be classified on the basis of molecular size and protein binding as reviewed recently by the European Uraemic Toxin Work Group (EUTOX) [1]. Serum levels of uraemic retention solutes will reflect a complex interplay of changes in production or generation, reduced renal clearance and adsorption or clearance during renal replacement therapy, but could also be affected by protein binding or non-renal (e.g. hepatic) clearance. The recent EUTOX review demonstrated that new studies using state-of-the-art methodology show major differences in the concentration of some retention solutes from original reports. As nephrologists consider how to adjust dialysis therapy regimes to improve clinical outcomes, it is important to carefully consider the biological significance of elevated levels of any solute and how it may be influenced by the mode or dose (or intensity) of dialysis. For many years, attention has focused on high-molecular-weight toxins or middle molecules and their effects in dialysis patients or changes with dialysis therapy.

β₂-Microglobulin (β₂M) is commonly measured as a representative middle molecule because its concentration is elevated significantly in end-stage kidney disease patients, it can be readily measured in serum, and it is readily cleared by high-flux membranes. Large randomized clinical trials have used either the clearance of β₂M or serum β₂M concentrations to define high-flux haemodialysis [2, 3] or haemodiafiltration [4]; however, these trials have failed to demonstrate that the use of high-flux membranes generally enhance patient survival in haemodialysis or haemodiafiltration therapies, although there may be benefits in certain patient subgroups [3–5]. These results were disappointing; many nephrologists expected more clear evidence of the importance of middle molecule removal in determining patient outcomes. The reasons why these large clinical trials did not more clearly demonstrate improved patient survival remain incompletely understood.

One possible reason is that the trial interventions did not provide clearances of middle molecules that were large enough to improve patient survival. Mean predialysis serum β₂M concentrations during the follow-up phase were 33.5 mg/L in the high-flux group during the HEMO study [6] and 26.4 mg/L in the haemodiafiltration group during the CONTRAST study [4], but these concentrations are still more than 10-fold higher than in individuals with normal kidney function. Furthermore, although the absolute levels of serum β₂M were not reported in the primary outcome paper from the MPO study, serum levels of β₂M for patients treated with high-flux membranes increased during the follow-up because incident patients participated in that study [3] and residual kidney function presumably dropped over time. In that study, the increase in serum β₂M concentration in patients treated by high-flux membranes was less than that treated by low-flux membranes; nevertheless, serum levels of β₂M, and likely of other middle molecules, continued to increase above normal levels during the follow-up in patients treated by high-flux membranes. Although improvements to high-flux membrane performance continue to be achieved [7–9], it is increasingly difficult to develop membranes to remove substantially higher amounts β₂M without also permitting clinically significant albumin loss [10, 11]. Therefore, it is unlikely that substantial increases in β₂M clearance by improvements in high-flux membrane design will be forthcoming in the near future. One approach for substantially increasing middle molecule removal is to increase haemodialysis treatment time, such as with frequent nocturnal haemodialysis [12], and this high-dose haemodialysis therapy is associated with improved patient survival [13–15].

A second possible reason for the observed lack of benefit of increased β₂M clearance on patient outcomes is poor understanding of the consequences of inflammation on middle molecule toxicity. Certainly, middle molecules can be pro-inflammatory, and certain putative uraemic toxins have been shown to be associated with inflammation [16]. For example, several secondary post hoc analyses of HEMO study outcomes demonstrated that the mean cumulative serum β₂M concentrations, but not dialyser clearance of β₂M, were associated with all-cause
mortality [6]. When these serum β2M concentrations were evaluated for cause-specific mortality, it was reported that serum β2M concentration was associated with a higher infectious, but not cardiac, death rate [17]. This observation together with the lack of an effect of dialyser β2M clearance suggests the possibility that generation of β2M also contributes significantly to poor clinical outcomes. Subsequently, Okuno et al. [18] examined the association between serum β2M concentrations and haemodialysis patient survival at one hospital in Osaka, Japan. These investigators reported that all-cause and non-cardiovascular mortality was higher in the group with high-serum β2M concentration; these findings were independent of patient vintage, diabetes, malnutrition and chronic inflammation as assessed by serum C-reactive protein levels. In contrast to the HEMO study cohort, all of these Japanese patients were treated using high-flux dialyzers (also without dialyser reuse) and therefore had comparable dialyser β2M clearance; thus, variability in serum β2M concentrations in this cohort was largely due to differences in generation, not clearance, of β2M. These clinical data therefore also suggest that higher generation of β2M in end-stage kidney disease is associated with higher patient mortality. These findings do not specifically implicate β2M generation as causative; instead, higher generation of β2M may be a marker of other pathological processes.

Factors that have been reported to increase generation of β2M in chronic kidney disease include the presence of an inflammatory state, metabolic acidosis and treatment by calcitriol [19, 20]. Chronic inflammation has received the bulk of attention, and data do support the importance of inflammation in enhancing the generation of β2M. For example, the use of ultrapure dialysate has been shown to reduce serum levels of C-reactive protein, cytokines and β2M [21–23]. Reduction in chronic inflammation using ultrapure dialysate appears to be important; however, other factors also likely contribute—note that both the intervention and control groups in the CONTRAST study used ultrapure dialysate [4].

A third possible reason is the poor understanding of the complex biology of middle molecules and thus the potential shortcomings of selecting a single molecule, such as β2M, to represent the entire class of molecules in question. Hyaluronan is another middle molecule [16] that has been studied in patients with chronic kidney disease and, in particular, peritoneal dialysis (PD). Hyaluronan is a component of the glycocalyx which forms a protective barrier around mesothelial cells, and peritoneal effluent levels of hyaluronan have been assessed as a surrogate marker for peritoneal inflammation [24]. PD fluid levels of hyaluronan may change over time, and randomized controlled trials have shown that patients using PD fluids with improved biocompatibility have reduced hyaluronan levels in peritoneal effluent compared with standard PD fluids [25–27]. The special communication in this issue by Goswami et al. confirms that hyaluronan is a middle molecule whose serum concentration is elevated in end-stage kidney disease and cannot be reduced to the normal range by maintenance haemodialysis [28–31]. Reduction in hyaluronan concentration in end-stage kidney disease may be important since its serum concentration is associated with higher patient mortality [32, 33]. The elevated hyaluronan concentrations in haemodialysis patients are likely due, in part, to impaired renal clearance, but as circulating hyaluronan is mainly removed by endothelial cells of the liver sinusoids, elevated blood levels will be largely due to increased generation, presumably as a result of activated inflammatory processes, or alternatively to decreased removal by dysfunctional endothelial cells.

Moreover, the work by Goswami et al. also describes an observation that may be relevant for future studies of other middle molecules in end-stage kidney disease; these investigators identified a reduction in serum high-molecular-weight hyaluronan during treatment by haemodiafiltration, but not by direct removal via the extracorporeal circuit. A novel clearance mechanism is suggested because our expectation is that clearance during extracorporeal treatment is only via the extracorporeal circuit (or possibly residual kidney function). Textbook knowledge teaches us the following: (i) dialysis membranes remove molecules largely based on diffusive processes that are size dependent, with small, not protein-bound, molecules removed faster than middle molecules because they diffuse more rapidly across the membrane; (ii) removal of middle molecules is expected to be limited when using low-flux membranes and it is only when using high-flux membranes with larger pores that significant amounts of middle molecules can be removed and (iii) addition of ultrafiltration, such as during haemodiafiltration, lessens that dependence on molecular weight by substantially increasing middle molecule clearance; nonetheless, small molecules are cleared more rapidly than middle molecules. Instead, the report by Goswami et al. suggests that there are yet undefined intracorporeal mechanisms of clearance of middle molecules during extracorporeal treatment.

Hyaluronan is a polymer of glucuronic acid alternating with N-acetyl glucosamine and is found in biology largely as a polymer with a very high-molecular weight (on the order of 10^6 Daltons); however, a large number of low-molecular-weight hyaluronan fragments could also be observed, especially during tissue injury and inflammation [34]. Because its chemical structure is quite simple, the activities of hyaluronan molecules depend largely on their size, with low-molecular-weight hyaluronan being removed faster than middle molecules because they diffuse more rapidly across the membrane; (ii) removal of middle molecules is expected to be limited when using low-flux membranes and it is only when using high-flux membranes with larger pores that significant amounts of middle molecules can be removed and (iii) addition of ultrafiltration, such as during haemodiafiltration, lessens that dependence on molecular weight by substantially increasing middle molecule clearance; nonetheless, small molecules are cleared more rapidly than middle molecules. Instead, the report by Goswami et al. suggests that there are yet undefined intracorporeal mechanisms of clearance of middle molecules during extracorporeal treatment.
of unanswered questions. Could it be that degradation of high-molecular-weight hyaluronan is product inhibited and the enhanced extracorporeal removal of the low-molecular-weight and pro-inflammatory hyaluronan drives enhanced degradation of the high-molecular weight and anti-inflammatory hyaluronan with little change in the concentration of low-molecular-weight hyaluronan? Or, is the reduction in high-molecular-weight hyaluronan due to changes in hyaluronan turnover or distribution? Is the purported enhanced removal of hyaluronan during haemodialfiltration a net pro-inflammatory or net anti-inflammatory event? Further, is the association between serum concentrations of β2M and low-molecular-weight hyaluronan due to inflammation or to residual kidney removal? These and other questions regarding the role of hyaluronan and inflammation and how they might alter middle molecule toxicity need further exploration. Toole [35] may indeed be correct that ‘hyaluronan is not just a goo.’

Conflict of interest statement. All authors are employees of Baxter Healthcare Corporation with ownership interests.

(See related article by Goswami et al. Paradoxical clearance of hyaluronan fragments during haemodialysis and haemodiafiltration. Nephrol Dial Transplant 2012; 27: 4420–4422.)

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Received for publication: 15.6.2012; Accepted in revised form: 4.9.2012