Exploring the Clinical Relevance of Providing Increased Removal of Large Middle Molecules

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Abstract

Dialysis technologies have continued to advance over recent decades; however, these advancements have not always been met with improved patient outcomes. In part, the high morbidity and mortality associated with dialysis have been attributed to a group of uremic toxins, which are described as "difficult to remove." With a new generation of hemodialysis membranes now making meaningful clearance of these molecules possible, it is an apt time to review the clinical relevance of these middle molecules. Our review describes the developments in membrane technology that enable the removal of large middle molecules (molecular mass >15 kD) that is limited with high-flux dialysis membranes. Of the known 58 middle molecules, a literature search identified 27 that have molecular mass >15 kD. This group contains cytokines, adipokines, hormones, and other proteins. These molecules are implicated in chronic inflammation, atherosclerosis, structural heart disease, and secondary immunodeficiency in the literature. Single-center safety and efficacy studies have identified that use of these membranes in maintenance dialysis populations is associated with limited loss of albumin and increased clearance of large middle molecules. Larger, robustly conducted, multicenter studies are now evaluating these findings. After completion of these safety and efficacy studies, the perceived clinical benefits of providing clearance of large middle molecules must be assessed in rigorously conducted, randomized clinical studies.

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Introduction

ESKD is accompanied by a constellation of symptoms, functional system impairments, and accelerated disease processes related at least in part to the retention of a large number of solutes that are normally excreted or metabolized by the kidney. These solutes have become known as uremic toxins, and they are defined as molecules that accumulate in kidney impairment and have an adverse biologic effect (1). They can be broadly classified into three groups: small water-soluble molecule, middle molecule, and protein-bound solutes. Of these three groups, the small water-soluble molecules are most efficiently removed by established dialysis technologies. However, the clearance of many middle molecules and protein-bound solutes is limited using current dialysis strategies (2).

Middle molecules have a broad range of molecular mass from 500 to 60,000 D (1). Historically, the size of the middle molecules has been a barrier to their removal with dialysis membranes. The current high-flux membranes were principally designed to remove β2-microglobulin, an 11.8-kD middle molecule. The development of dialysis technologies and the improved ability to regulate fluid loss across the dialysis membrane have enabled an increase in the porosity of the membranes.

Suspicion that the inadequate removal of middle molecules has played a large part in the morbidity and mortality of patients on dialysis has driven developments in dialysis technology and clinical dialysis practice aimed at providing increased removal of these molecules. However, the clearance of middle molecules with molecular mass >15 kD is low using standard high-flux membranes (3). Therapy using convection in addition to diffusion, hemodiafiltration (HDF), has now become an established method for increasing the clearance of middle molecules (4). To date, regulatory constraints and a lack of conclusive evidence showing the benefit of HDF have resulted in sporadic uptake of the technology.

Recent advances in membrane technology have now enabled the development of a new generation of dialysis membranes, the medium-cutoff (MCO) membranes, which allow the removal of middle molecules up to approximately 50 kD, surpassing even the clearance range provided by HDF (5–7). Unlike the earlier high-cutoff (HCO) membranes, which were used in myeloma kidney, the MCO membranes have significantly less albumin loss (7–9).

The purpose of this review is threefold. First, it will describe the development of dialysis membranes to allow the removal of large middle molecules without albumin loss. Second, it will identify the large middle molecules that can now be removed by the MCO membranes, namely those with molecular mass above 15 kD, and assess their clinical relevance to patients on dialysis. Third, it will evaluate the clinical experience to date with these membranes and determine what should occur before they are accepted as a new therapeutic option for ESKD.

Adapting Hemodialysis Membranes to Remove Large Middle Molecules

The hollow fiber hemodialysis membrane is made by a process of spinning the membrane polymer through a solvent. This process of spinning results

in a semipermeable membrane with a very thin inner separation layer that acts as the functional surface of the membrane and a wider supportive matrix. The pores within these membranes are nonuniform and have a "bell-shaped" distribution of size from small to large (Figure 1). To prevent significant albumin loss, in high-flux dialyzers, the largest of these pores is still smaller than albumin.

To increase the size of molecules removed by a membrane, the sizes of the pores needs to be increased by moving the distribution of the pores to the right, which occurred with the HCO membranes. Although the HCO membranes were able to remove larger molecules, such as the free light chains-κ and -λ in myeloma kidney, this creation of larger pores resulted in the loss of albumin due to the nonuniformity of the pores (7).

To enable the clearance of larger middle molecules with molecular mass between 15 and 60 kD without the loss of albumin, the distribution of the pores within the dialysis membranes had to fundamentally change to a tighter distribution (Figure 1). The MCO dialyzers use this new distribution of pores (10). In clinical practice, these membranes should provide effective clearance of large middle molecules without excessive albumin loss.

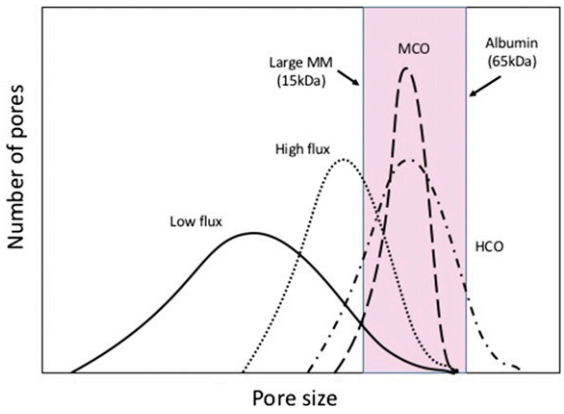

Figure 1. | Medium cut-off membranes provide clearance of large middle molecules without albumin loss. Schematic of pore size distribution in dialysis membranes. As membranes have been developed to allow the removal of large middle molecules (MMs) without albumin loss, the distribution of the pore sizes has had to be "tightened." The pink bar represents the distribution of large MMs before albumin is lost. The solid line indicates low flux, the dotted line indicates high flux, the dot-dash line indicates high cutoff (HCO), and the large dashed line indicates medium cutoff (MCO).

Identification of Large Middle Molecules

The EuTox database (http://www.uremic-toxins.org/DataBase.html), associated publications (8), and a Medline search were used as the starting points to generate a list of uremic toxins that can be classified as middle molecules, which were then applied to a literature search algorithm. The accumulated literature was then reviewed to determine the clinical relevance of the middle molecules as uremic toxins in relationship with the following broad groups: cardiovascular disease, chronic inflammation, secondary immunodeficiency, protein-energy wasting, and cachexia. The review was confined to those middle molecules with molecular mass >15 kD, above which clearance by high-flux dialysis membranes is reduced and clearance is increased by MCO membranes (5) (Table 1). In addition to the middle molecules previously described, we identified two novel uremic toxins with molecular mass >15 kD: Pentraxin-3 (PTX3) and Visfatin. Protein-bound solutes are clinically relevant but were not assessed, because they cannot be removed by the MCO membranes.

Classification and Biologic Summary of Larger Middle Molecules

The literature review identified 27 middle molecules with molecular mass >15 kD, the largest of which was 52 kD (Table 2). Although advanced oxidative protein products are "large" middle molecules (8), with molecular mass ranging from 60 to 600 kD, they would not be removed by the new MCO membranes, and therefore, they were not reviewed. For the purpose of this review, some groups of the large middle molecules identified have been summarized into a single entity for convenience, such as advanced glycosylation end products (AGEs).

The serum levels of the identified middle molecules range from <1.5- to >200-fold higher in individuals receiving dialysis or with advanced CKD compared with those with normal kidney function. The majority of the molecules can be measured using commercially available assays, with most using an ELISA-based assay (Table 2). The molecules identified have diverse biologic roles; for the purposes of this review, the molecules were grouped into four broad functional groups (cytokines [n=5], adipokines [n=4], immune-related proteins [n=8], and growth factors and hormones [n=4]) and other molecules (n=6). In Table 2, the molecular mass, usual biologic role, and possible adverse effects in uremia are described for each molecule.

The Clinical Relevance of Large Middle Molecules as Uremic Toxins

To determine the clinical relevance of providing increased clearance of these large middle molecules, their involvement in the following processes was assessed: cardiovascular disease, secondary immunodeficiency, protein-energy wasting, cachexia, and chronic inflammation.

Accelerated Atherosclerotic Cardiovascular Disease

Patients with CKD and especially those reliant on maintenance dialysis are subject to a substantially elevated risk of cardiovascular disease and cardiovascular mortality compared with the general population. Many of the large middle molecules are involved in progressive atherosclerosis (Figure 2), and serum concentrations are correlated with both rates of cardiovascular disease and overall survival (Table 3).

Elevated levels of the proinflammatory cytokines IL-18, TNF-α, IL-6, and IL-1b are all involved with cardiovascular disease. IL-6 is an upstream inflammatory cytokine that plays a central role in propagating the downstream inflammatory response responsible for the development of atherosclerosis. This causative role of IL-6 has been suggested by the recognition that individuals with a variant in

Table 1. Summary of middle molecules (*n*=59)

Removed by High Flux (<15 kD)	Molecular Mass, kD	Removed by HDF (15–24.9 kD)	Molecular Mass, kD	Not Currently Removed (>25 kD)	Molecular Mass, kD
Methionine-enkephalin	0.5	Clara cell protein	15.8	Hyaluronic acid	25
Glutathione	0.6	Leptin	16	β-Trace protein	26
Angiotensin A	0.8	Myoglobin	17	Soluble TNF receptor-1	27
δ–Sleep-inducing peptide	0.8	TNF-α	17	Adiponectin	30
Dinucleoside polyphosphates	1	Soluble TNF receptor-2	17	FGF-23	32
Substance P	1.3	IL-1β	17.5	α1-Microglobulin	33
Motilin	2.7	FGF-2	18	VEGF	34.2
Orexin B	2.9	IL-10	18	YKL-40	40
Atrial natriuretic peptide	3	Retinol binding protein	21.2	Pentraxin-3	40.2
Desacylgherlin	3.2	Prolactin	22	α1-Acid glycoprotein	43
Vasoactive interstinal peptide	3.3	κ-Ig light chain	22.5	AGEs	45
Calcitonin	3.4	Complement factor D	23.75	λ-Ig light chain	45
Gherlin	3.4	IL-18	24	Visfatin	55
β-Endorphin	3.4	IL-6	24.5	AOPPs	>60
Orexin A	3.5				
Calcitonin gene–related peptide	3.7				
Cholecystokinin	3.8				
Endothelin	4.2				
Neuropeptide Y	4.2				
SIAM-1	4.2				
Adrenomedullin	5.7				
Osteocalcin	5.8				
IGF-1	7.6				
IL-8	8				
Parathyroid hormone	9.5				
Guanylin	10.3				
β2-Microglobulin	11.8				
Uroguanylin	12				
Resistin	12.5				
Cystatin C	13.3				
Degranulation inhibiting protein[a]	14.1				

Thirty-one molecules had molecular mass under 15 kD, and therefore, they can be removed by high-flux dialysis. Fourteen molecules had molecular mass between 15 and 25 kD, and therefore, they can be removed by HDF. Fourteen molecules had molecular mass >25 kD. HDF, hemodiafiltration; FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor; AGE, advanced glycosylation end product; AOPP, advanced oxidative protein products.
[a]Degranulation inhibiting protein corresponds to angiogenin.

the IL-6 receptor that impairs classic IL-6 signaling have a decreased risk for coronary heart disease (11). Similarly, IL-1b has been described to be pathologically involved in the progression of atherosclerosis (12).

Serum concentrations of IL-18 are associated with plaque burden and instability, and in large populations, they have been shown to been independently predictive of cardiovascular outcomes (13). Animal models have identified that infusion of IL-18 leads to plaque formation (14). TNF-α alters endothelial and vascular smooth muscle cell function as well as increases expression of adhesion molecules on vascular endothelium, leading to vascular dysfunction and atherosclerosis.

Other inflammation-related middle molecules have also been associated with cardiovascular disease (Table 2). PTX3 has been linked to unstable plaque in coronary and carotid arteries, raising suspicion that PTX3 may play a causative role in this process (15). There is evidence that atherogenic lipids can induce PTX3 in vascular smooth muscle cells (16) and that PTX3 may have prothrombotic effects by amplifying tissue factor expression in endothelial cells and monocytes in response to inflammatory stimuli as well as impairing nitric oxide production (17,18).

Other middle molecules that have been linked with cardiovascular disease include β-trace protein (BTP) in patients on incident dialysis (19). Serum BTP correlated with severity of coronary disease (20), and BTP mRNA is most strongly expressed in early atherosclerotic plaque lesions (21). This again does not clarify if BTP is a protective or damaging factor in this process. However, knockout of the gene in mice susceptible to diet-induced diabetes and atherosclerosis led to accelerated glucose intolerance, insulin resistance, nephropathy, and aortic thickening (22).

Prolactin levels have also been associated with increased risk of cardiovascular and all-cause mortality in the general population (23) and cardiovascular events and all-cause mortality in CKD/dialysis populations (24). The mechanism is not clear, but prolactin is now thought to have diverse effects on lipids and blood vessel function, suggesting a plausible pathway.

AGE levels correlate with cardiovascular disease and mortality in patients on dialysis (25). Tissue accumulation of AGEs can contribute to cardiovascular disease by cross-linking with other molecules, causing structural changes and inducing inflammation in the heart and blood vessels (26).

Table 2. Classification, levels in ESKD, and methods of measurement of large middle molecules (n=28)

Molecule (Alternative Names)	Group	Size, kD	Usual Biologic Function	Possible Adverse Effects in Excess or Uremia	Relative Increase in Dialysis or Advanced CKD	Commercially Available Test System and Manufacturer
IL-18	Cytokine	18	Proinflammatory; induction of TH1 response and IFN-γ production	Proatherogenic; increased amyloid-β production	Approximately twofold higher	ELISA
IL-6	Cytokine	21–28	Diverse proinflammatory actions; neutrophil attraction; monocyte and T cell recruitment; induction of fever	Proatherogenic; sarcopenia and wasting; anorexia	Two- to fivefold higher	ELISA (Invitrogen)
IL-1β	Cytokine	17.5	Proinflammatory; upregulation of IL-6 and systemic inflammation	Proatherogenic; contributes to systemic inflammation	Approximately twofold higher	ELISA (R&D Systems)
IL-10	Cytokine	18	Anti-inflammatory actions; downregulation of macrophage activity and proinflammatory cytokine production	Diminished anti-infectious immune function (may have beneficial effects on CVS disease)	~1.5-Fold higher	ELISA
TNF-α	Cytokine	17	Upregulation of immune response, induction of fever	Enhanced protein catabolism, anorexia, and muscle protein breakdown	Four- to fivefold higher	ELISA (R&D Systems)
Adiponectin	Adipokine	30	Modulates glucose regulation and fatty acid oxidation	Unknown	Two- to threefold higher	ELISA (R&D Systems)
Visfatin (Nicotinamide phosphoribosyltransferase)	Adipokine	52	Intracellularly involved in NAD biosynthesis; extracellularly stimulates angiogenesis and endothelial cell proliferation	Proinflammatory cytokine effects; angiogenic effects, promotion of vascular smooth muscle cell proliferation	Three- to sixfold higher	ELISA (R&D Systems)
Leptin	Adipokine	16	Regulates appetite and body energy stores	Possible contribution to anorexia and protein-energy wasting	Three- to fourfold higher	RIA
Retinol binding protein 4	Adipokine	21.2	Delivers retinol from liver to peripheral tissues	Inhibition of leukocyte chemotaxis and function	Three- to fourfold higher	ELISA (R&D Systems)
Soluble TNF receptor 2 (p55)	Immune-mediated protein	17	Binds to and limits TNF-α activity	May increase circulating TNF-α $t_{1/2}$ to prolong cytotoxic effects	Three- to tenfold fold higher	
κ-Ig light chains	Immune-mediated protein	22.5	Unknown	Inhibit leukocyte chemotaxis, apoptosis, and function	Two- to 16-fold higher	Nephelometric (Binding Site)
Complement factor D (C3 proactivator convertase)	Immune-mediated protein	24	Component of alternative complement pathway; humoral defense	Overactivity of complement system	Four- to 17-fold higher	ELISA (R&D Systems)
Soluble TNF receptor 1 (p75)	Immune-mediated protein	27–30	Binds to and limits TNF-α activity	May increase circulating TNF-α $t_{1/2}$ to prolong cytotoxic effects	Three- to tenfold higher	

Table 2. (Continued)

Molecule (Alternative Names)	Group	Size, kD	Usual Biologic Function	Possible Adverse Effects in Excess or Uremia	Relative Increase in Dialysis or Advanced CKD	Commercially Available Test System and Manufacturer
α1-Acid glycoprotein (Orosomucoid)	Immune-mediated protein	35–44	Anti-inflammatory acute-phase protein; suppresses local leukocyte activity and promotes immunosuppressive macrophage differentiation	Inhibition of leukocyte migration, contribution to secondary immunodeficiency	<1.5-Fold higher	Nephelometric (Siemens Healthcare)
Pentraxin-3	Immune-mediated protein	40	Opsonization and complement activation; modulates macrophage activity	Prothrombotic actions in endothelial cells; impaired NO production	Two- to sevenfold higher	ELISA (R&D Systems)
YKL-40 (Chitinase-3–like protein 1)	Immune-mediated protein	40	Regulates local inflammatory markers; other functions unclear	Contribution to upregulation of local tissue inflammation and fibrosis	Two- to fivefold higher	ELISA (Quidel)
λ-Ig light chains	Immune-mediated protein	45	Unknown	Inhibit leukocyte chemotaxis, apoptosis, and function	Two- to 18-fold higher	Nephelometric (Binding Site)
Vascular endothelial growth factor (Vascular permeability factor)	Growth factor	34	Promotes endothelial cell proliferation, migration, and differentiation; involved in cardiac adaptation to hypoxia and stretch	Involved in cardiomyopathy and left ventricular dysfunction	Approximately twofold higher	ELISA (Thermo Fisher)
Fibroblast growth factor 2 (Basic fibroblast growth factor)	Growth factor	18	Angiogenic growth factor; stimulates neovascularization; upregulates inflammatory cytokines and chemokines	Cardiac hypertrophy; contribution to local inflammation		ELISA (Thermo Fisher)
Fibroblast growth factor 23	Growth factor	32	Regulates phosphate homeostasis *via* effects on the sodium/phosphate cotransporter and kidney hydroxylation of vitamin D	Cardiac hypertrophy	>200-Fold higher	ELISA (R&D Systems)
Prolactin	Hormone	23	Primary role in mammary cell proliferation and reproductive function; diverse roles in immunomodulation *etc.*	Amplification of inflammatory cytokine response (IL-12 and TNF-α); increased CVS events	Two- to fourfold higher	ELISA (R&D Systems)
Clara cell protein (CC16)	Protein	15.8	Phospholipase-A2-inhibitor; immunosuppressive role in respiratory tract	Unknown	Approximately 30-fold higher	
α1-Microglobulin	Protein	33	Inhibitor of heme and neutrophil-induced oxidative damage; immuno-modulatory functions	Inhibition of leukocyte migration, chemotaxis, and IL-2 secretion	Approximately ninefold higher	ELISA (Abcam Nephelometric–Siemens Healthcare)
β-Trace protein (L-prostaglandin D2 synthase)	Protein	26	Catalyzes isomerization of precursor prostanoids to active forms	Observationally associated with atherosclerotic plaque	>35-Fold higher	Nephelometric (Siemens Healthcare)
Myoglobin	Protein	17	Oxygen carrier in muscle tissue	Increased oxidative stress	Threefold	RIA

Table 2. (Continued)

Molecule (Alternative Names)	Group	Size, kD	Usual Biologic Function	Possible Adverse Effects in Excess or Uremia	Relative Increase in Dialysis or Advanced CKD	Commercially Available Test System and Manufacturer
Hyaluronic acid (Hyaluronan)	Glycosaminoglycan	Variable	Formation of endothelial glycocalyx; structural role in extracellular matrix	Small species proinflammatory; innate immune system triggers; promotes endothelial dysfunction and damage	Five- to 16-fold higher	ELISA (Echelon Biosciences)
Advanced glycosylation end products	Other	<1–70	Unknown	Adverse structural effects; interactions with RAGE	Two- to 20-fold higher	ELISA (Cell Biolabs)

CVS, cardiovascular system; NO, nitric oxide; RAGE, receptor for advanced glycosylation end product.

Direct effect of AGEs *via* interactions with the receptor for AGE seems to cause endothelial dysfunction by inhibiting nitric oxide synthase and increasing expression of adhesion molecules.

The adipokine visfatin is also strongly upregulated in atherosclerotic plaque, and serum levels are higher in those with unstable rather than stable vascular disease. Visfatin may contribute to inflamed plaque by inducing inflammatory macrophage differentiation (27). Additionally, the adipokines adiponectin and leptin have been implemented in progressive atherosclerosis by contributing to the recruitment of macrophages and the formation of foam cells.

Contribution to Structural Cardiac Disease

Several growth factors have been linked to cardiac hypertrophy, with experimental animal studies implicating fibroblast growth factor 2 (FGF-2) and FGF-23 as having a direct causal role in this process. Gene knockout models suggest that FGF-2 is necessary for the development of cardiac hypertrophy in response to hypertension (28), and administration of FGF-23 caused left ventricular hypertrophy, which was attenuated with an FGF blocker in another series of experiments (29). There is also robust observational evidence in humans supporting a link between FGF-23 and left ventricular hypertrophy (29,30).

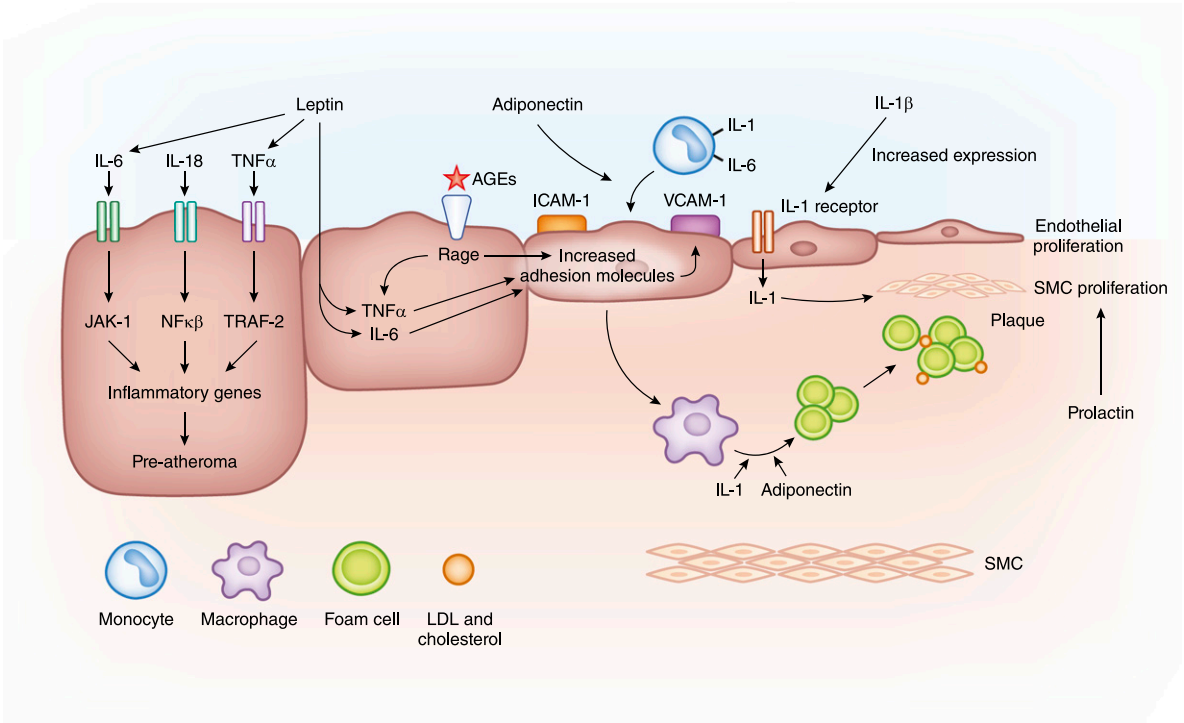

Figure 2. | **Large middle molecules are pathologically involved in atherosclerosis.** Large middle molecules and atherosclerosis. The cytokines IL-6, IL-18, and TNF-α result in increased expression of inflammatory genes, which are proatheroma formation. Advanced glycosylation end products (AGEs), TNF-α, IL-6, and adiponectin all result in increased expression of adhesion molecules on the vascular endothelium. Elevated levels of IL-1b result in increased expression of IL-1 receptors, which in turn, results in monocyte recruitment, macrophage activation, and the proliferation of smooth muscle cells (SMCs) and endothelium. In addition, prolactin stimulates SMC proliferation.

Table 3. Involvement of large middle molecules with cardiovascular disease

Middle Molecule	Association	Possible Mechanisms
IL-18	Cardiovascular mortality; aortic pulse wave velocity; unstable coronary plaque; coronary and thoracic aortic calcification	Promotion of atherosclerotic plaque instability, induction of IFN-γ, promotion of collagen and lipid deposition
IL-6	Left ventricular hypertrophy, systolic dysfunction; cardiovascular mortality	Coordination of local inflammatory cell influx and lymphocyte proliferation; promotion of coagulation
IL-1β	Left ventricular hypertrophy	Promotion of local inflammatory response within plaque
TNF-α	Left ventricular hypertrophy	Promotion of cardiac inflammatory response to stress
Pentraxin-3	Unstable coronary plaque	Infiltration of neutrophils into atherosclerotic plaque, prothrombotic effects, impairment of NO production
β-Trace protein	Atherosclerotic plaque; cardiovascular mortality	Possible functions acting against platelet aggregation *via* catalyzation of PGD2 production
Prolactin	Cardiovascular mortality	Proliferation of vascular smooth muscle cells, promotion of vasoconstriction
AGEs	Cardiovascular mortality	Deposition within vessel wall; induction of oxidative stress, inflammation, and endothelial dysfunction
Visfatin	Unstable atherosclerotic plaque	Induction of inflammatory macrophages within atherosclerotic plaque
Adiponectin	Atherosclerotic plaque	Expression of adhesion molecules; foam cell formation
Leptin	Atherosclerotic plaque	Expression of adhesion molecules; production of MCP-1, IL-6, and TNF-α
FGF-2	Cardiac hypertrophy	Induction of cardiomyocyte hypertrophic response
FGF-23	Cardiac hypertrophy	Induction of cardiomyocyte hypertrophic response

NO, nitric oxide; AGE, advanced glycosylation end products FGF, fibroblast growth factor.

Influence on Secondary Immune Deficiency

ESKD is associated with significant immune dysfunction. Despite high cytokine levels, immune cell function is impaired, and patients on dialysis experience high rates of infection-related morbidity and mortality (31). Several large middle molecules have been shown to directly cause impairment of immune cell function in experimental models (Table 4). Ig free light chains were shown to reduce glucose uptake by polymorphonuclear leukocytes *in vitro* and reduce chemotaxis (32). Additionally, serum free light chain levels were an independent risk factor for death by infectious cause in a CKD population (33).

RBP4 also inhibits the chemotactic movement of polymorphonuclear leukocytes in a concentration-dependent fashion, and it inhibits oxidative metabolism and apoptosis (34). FGF-23 has also been shown to exert inhibitory effects on leukocytes in a mouse CKD model in a dose-dependent fashion, which was reversible with a neutralizing antibody toward FGF-23 (35).

Additionally, α1-acid glycoprotein inhibits the migration of neutrophils to infectious foci (36) and is associated with the susceptibility to infections in individuals with diabetes (36).

Protein-Energy Wasting in CKD

There is evidence linking IL-6, TNF-α, and IL-1β to anorexia and protein-energy wasting in cancer, AIDS, and geriatric cachexia, and this is supported by interventional animal models. Evidence specific to patients with CKD and patients on dialysis is also growing stronger. Elevated levels of the adipokine leptin can contribute to protein-energy wasting by inhibiting food intake and increasing energy expenditure.

Of the cytokines, IL-6 has a clear inverse relationship with albumin levels in patients on dialysis and has been found to negatively correlate with muscle mass (37). TNF-α levels were higher in patients on dialysis with poor appetite or evidence of anorexia, nausea, or vomiting compared with those without. Higher TNF-α levels are also associated with lower prealbumin and body mass index (38). Higher IL-1β levels were associated with lower physical activity scores and faster declines in a bioimpedence-derived measure of body

Table 4. Involvement of large middle molecules with secondary immunodeficiency

Middle Molecule	Associations	Possible Mechanisms
Ig light chains	Impaired PMNL function; infectious mortality	Interference with caspase-3 activity; interference with normal PMNL apoptosis
Retinol binding protein 4	Impaired PMNL function	Interference with upstream complement receptor signaling within PMNLs
FGF-23	Leukocyte inhibition	Interference with chemokine signaling
α1-Acid glycoprotein	Impaired PMNL function	Neutrophil migration

PMNL, polymorphonuclear leukocyte; FGF, fibroblast growth factor.

cell mass when patients on hemodialysis were followed longitudinally (39).

Contributions to Chronic Inflammation

Chronic inflammation in patients on dialysis is multifactorial due, in part, to the retention of inflammatory cytokines, proteins, and other factors that induce inflammation. IL-6 release from both leukocytes and peripheral tissues is upregulated in uremia (40,41), and both IL-1β and its circulating receptor antagonist IL-1Ra are increased (42). Although IL-1Ra is an antagonist, a large excess of IL-1Ra is required to block effects of IL-1β, and it is, therefore, likely that elevated IL-1β and IL-1Ra as seen in patients on dialysis act predominantly as an agonist to the IL-1 receptor (43). Both TNF-α and the soluble TNF receptors 1 and 2 (38) are increased in CKD. The increased soluble TNF receptors levels increase the circulating $t_{1/2}$ of TNF-α, contributing to the chronic inflammation of ESKD.

In addition to the retention of these proinflammatory middle molecules, other molecules not directly involved in the inflammatory cascades can still stimulate inflammation. For example, AGE levels correlated independently with CRP (44), and there is suspicion that tissue accumulation of AGEs induces chronic inflammation.

Clinical Evaluation of MCO Dialyzers

Large middle molecules would seem to be a clinically relevant group of uremic toxins to be removed from patients with ESKD. However, a number of steps now need to occur to determine if patient outcomes are genuinely improved with this new dialysis membrane. Safety is paramount. With an increasingly porous membrane, such as those in the MCO dialyzers, there are two principle safety concerns: albumin loss and back filtration of endotoxins. Schepers *et al.* (45) assessed the back filtration of endotoxins *in vitro* and identified no increased back filtration with MCO dialyzers compared with high-flux membranes.

Hemodialysis with the MCO membranes is associated with albumin loss of approximately 3 g per session (6). In an initial assessment of MCO dialyzers, Belmouaz *et al.* (46) monitored ten patients converted from online HDF to MCO dialysis and did not identify a significant change in serum albumin concentrations, suggesting that this level of albumin loss is tolerable. The REMOVAL-HD Study in Australia and New Zealand is a single-arm, multicenter study designed to specifically address the safety of MCO dialysis (ANZCTRN 12616000804482). Powered to detect a 5% change in predialysis serum albumin concentrations over 6 months, the study is now fully recruited and will report in 2018.

In addition to safety, the efficacy of MCO dialysis for the removal of large middle molecules should also be assessed. Kirsch *et al.* (9) showed that MCO dialysis provides increased clearance rates of Complement factor D (24 kD), α1-microglobulin (33 kD), and YKL-40 (40 kD) in comparison with high-flux dialysis sessions. The ability of these increased clearance rates to provide sustained reductions in predialysis concentrations of large middle molecules is being assessed in the REMOVAL-HD Study, which is measuring λ-free light chain, one of the largest middle molecules (45 kD).

After the reporting of these assessments of safety and efficacy of MCO dialysis, robust clinical trials will then be required to determine patient benefit. This review of the literature suggests that increased removal of large middle molecules is most likely to provide patient benefit by the reduction in cardiovascular disease. Evidence of potential benefit is growing rapidly. In small studies to date, dialysis with MCO membranes has been associated with a reduction in the activation of the renin-angiotensin system (47), reduced serum concentrations and expression of proinflammatory cytokines (TNF-α and IL-6) (48), and reduced induction of vascular calcification (49).

It would, therefore, be logical that future clinical trials could potentially have major cardiovascular events as a primary outcome when comparing MCO dialysis with current standards of care; this would fit with the recommendations of the SONG initiative, which identified MACE as one of the four principal outcomes for dialysis studies (50).

Conclusions

Individuals with ESKD requiring dialysis support continue to have high morbidity and mortality. When the causes of this disease burden are analyzed, chronic inflammation, protein-energy wasting, cardiovascular disease, and secondary immunodeficiency are key culprits. In this review, we have assessed whether the large middle molecules that are inadequately removed by current dialysis strategies could be contributing to this disease burden. Of the 27 large middle molecules identified, many have biologic pathways through which they can contribute to chronic inflammation, protein-energy wasting, cardiovascular disease, and secondary immunodeficiency. Robust clinical trials are now required to determine if increasing their removal by dialysis can translate to improved clinical outcomes.

Disclosures

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