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Apoptosis: from advances in PD to therapeutic targets in DM

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SUMMARY

A high glucose concentration is shared by peritoneal dialysis (PD) and diabetes mellitus (DM). High glucose leads to tissue injury in diabetes. Peritoneal dialysis research has emphasized the role of glucose degradation products in tissue injury. Apoptosis induction is one of the mechanisms of tissue injury induced both by glucose and glucose degradation products. We now review the role of apoptosis and its regulation by glucose degradation products in antibacterial defense and loss of renal function in diabetes mellitus and peritoneal dialysis. The pathogenic role of the recently identified glucose degradation product 3,4-di-deoxyglucosone-3-ene (3,4-DGE) is detailed. Available therapeutic strategies include the use of peritoneal dialysis solutions containing a low concentration of glucose degradation products. Based on preclinical results, specific targeting of apoptosis regulatory factor should be explored in the clinical setting.

Key words: *Peritoneal dialysis. Diabetes mellitus. Apoptosis.*

RESUMEN

En la diálisis peritoneal (DP) y la diabetes mellitus (DM) altas concentraciones de glucosa se asocian a daño tisular. La apoptosis es uno de los mecanismos de daño tisular. Los productos de degradación de la glucosa (PDGs) se producen a partir de la glucosa tanto *in vivo,* en diabéticos, como durante el procesamiento de las soluciones de DP e inducen apoptosis en distintos tipos celulares. La apoptosis es un modo activo de muerte celular con control molecular, regulada por moléculas intracelulares y extracelulares que dan lugar a distintas vías pro y antiapoptóticas, susceptibles de manipulación terapéutica. Entre estas se encuentran las caspasas, una familia de protein cisteasas que se comportan como moléculas iniciadoras o efectoras de la apoptosis. Entre los PDGs conocidos, la 3,4 dideoxiglucosona (3,4 DGE) es el principal componente letal de las soluciones de DP. La 3,4 DGE induce apoptosis en leucocitos y células tubulares de riñón. La inhibición de la apoptosis de leucocitos mejora la defensa antibacteriana peritoneal. Proponemos que los PDGs pueden estar implicados en el empeoramiento de la defensa antibacteriana y en la pérdida progresiva de la función renal en pacientes diabéticos y en DP. Entre las posibles estrategias terapéuticas destacamos el empleo de soluciones de DP con baja concentración de PDGs, que podrían disminuir la incidencia y gravedad de las peritonitis así como conservar la función renal residual. Otra posible estrategia seria el empleo de fármacos inhibidores de la apoptosis patológica.

Palabras clave: Diálisis peritoneal. Diabetes mellitus. Apoptosis.

APOPTOSIS OVERVIEW

Apoptosis is an active mode of cell death (cell suicide) under molecular control.[1-3] Apoptosis is an essential process to remove unwanted and harmful cells and to maintain homeostasis of cell number (fig. 1). However, excessive, insufficient or untimely apoptosis may result in pathology. Cell number remains stable if cell loss by apoptosis is matched by generation of new cells by mitosis. In the case of migratory cells, chemotaxis plays a role in increasing cell number in a particular tissue. If apoptosis exceeds mitosis or chemotaxis, cell depletion ensues, leading to parenchymal atrophy or leukocyte depletion. If mitosis or chemotaxis exceeds apoptosis, parenchymal hyperplasia or leukocyte accumulation is the result.

Apoptosis is tightly regulated by extracellular and intracellular molecules that provide multiple regulatory and contrarregulatory pathways. Among them, caspases are a family of intracellular cysteine proteases that behave as activators and effectors of apoptosis, and play a central role in the process.[1] These molecules are potential targets for therapeutic intervention. Caspases may be activated by the extrinsic or the intrin-

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sic pathway. The extrinsic pathway consists of lethal cytokine activation of death receptors such as TNFR, Fas or TRAIL receptors, leading to caspase-8 activation. In the intrinsic pathway environmental stressors activate the cytosolic protein Bax which aggregates in mitochondria leading to mitochondrial injury and the release of proapoptotic factors that activate caspase-9. Caspase-8 or -9 activate effector caspases that are responsible for the morphological and functional characteristics of apoptosis. Being an active mode of cell death, apoptosis can be targeted therapeutically.

CLINICAL ISSUES IN DIABETES AND PERITONEAL DIALYSIS RELATED TO APOPTOSIS MODULATION

Diabetics have an impaired ability to eradicate certain microorganisms such as *S. aureus.*[4] This is shared by peritoneal dialysis (PD) patients. *S aureus* peritonitis has a mortality of 15%, among the highest for any bacteria.[5] In addition 30% of *S aureus* peritonitis require catheter removal because of failure of antibiotic therapy. Failure to eradicate *S. aureus* complicates patient care and, additionally, favors the development of antimicrobial resistance, thus creating a public health hazard. As an example, vancomycin-resistant *S. aureus* were originally described in diabetic dialysis patients, including PD patients that were unable to control *S. aureus* PD peritonitis, despite long-term antibiogram-directed therapy.[4] Efforts are under way to limit the expansion of these new *S. aureus* strains. However the efficacy of such efforts is limited and the growing problem of antibiotic resistance has recently been highlighted for methicillin-resistant *S. aureus* (MRSA).[6] The burden of invasive MRSA infections in the United States in 2005 was estimated to be a standardized incidence rate of 31.8 per 100,000 and a standardized mortality rate of 6.3 per 100,000. It was preoccupying that community-onset infections accounted for 27% of episodes. According to this estimate, invasive MRSA-related deaths would exceed the total number of deaths attributable to human immunodeficiency virus/AIDS in the United Status.[7] As discussed below, excessive or premature parenchymal cell or leukocyte apoptosis compromises antibacterial responses. Identifying the factors and mechanism responsible for accelerated apoptosis, and designing appropriate therapeutic strategies may prove a more efficient way of boosting antibacterial defenses and limiting the appearance of resistant strains.

In recent yeas new information has become available on the regulation of renal cell apoptosis in the context of diabetic nephropathy and PD. Residual renal function is important for patients undergoing PD. Preserving residual renal function has several advantages, including improved survival, that have been recently reviewed.[8] Preserving renal function is also a high priority in diabetic nephropathy. Apoptosis has been identified as a key mediator of parenchymal cell loss in the progression of chronic kidney disease.[9] Recent transcriptomic analysis of diabetic nephropathy kidney biopsies has identified deregulated apoptosis related genes such as Fas, TRAIL, CD74 and others.[10]

Table I. Low GDP PD fluids reduce the rate of peritonitis and the duration of peritonitis episodes[27]

	Conventional PD fluid	Low GDP PD fluid	P
Peritonitis rate (episodes per patient/year)	0.60	0.34	0.017
Duration of cloudy fluid (h, mean ± SD)	64 ± 29	48 ± 16	0.015

Bicavera®, Fresenius Medical Care.

MODULATION OF APOPTOSIS DURING BACTERIAL INFECTIONS

During infection parenchymal cells and leukocytes are lost by apoptosis. In the case of peritonitis the mesothelium is the most important parenchymal cell target of injury. Cell death through apoptosis of mesothelial cells and leukocytes has been documented in the course of peritoneal injury both in animal models and in clinical PD[11,12] and reviewed in 1). The loss of neutrophils by apoptosis during PD peritonitis is not unexpected, since neutrophils are programmed to die by apoptosis at the site of inflammation.[11,13] In fact, during the evolution of peritonitis, and coinciding with the decrease in total leukocyte number, the percentage of total apoptotic cells increases. The apoptotic demise of leukocytes helps limit the inflammatory response. However, untimely, accelerated leukocyte apoptosis may compromise the peritoneal defense.[11,13] In this regard, maneuvers that decrease neutrophil apoptosis, such as LPS priming, result in accelerated bacterial clearance in mice.[14]

Inflammatory mediators and bacterial products may induce apoptosis.[15] During infection lethal cytokines from the TNF superfamily, including TNF, Fas ligand and TRAIL, are produced that regulate leukocyte and mesothelial cell death.[12]

In diabetes and PD high glucose and glucose degradation products (GDPs) concentrations may also play a role in apoptosis induction.[1,16,17] Heat-sterilized glucose-containing medical fluids contain GDPs.[18] These are created through thermally driven non-enzymatic processes during the heat sterilization as well as during the subsequent storage. GDPs are a heterogeneous group of compounds that may have heterogeneous biological roles. In addition, the concentration of several GDPs is increased in diabetic individuals, particularly in the presence of uncontrolled hyperglycemia.[19]

GDPs accelerate neutrophil and peripheral blood mononuclear cell apoptosis in a caspase-dependent manner.[13,20] Rescue from apoptosis by caspase inhibition restores the defensive function of neutrophils.[20] Glucose promotes apoptosis in several cell types, including vascular endothelium, renal tubular cells and the blastocyst.[21,22] However, the leukocyte toxicity of conventional, high-glucose PD solutions is not related to lactate, glucose or pH.[20] Newer, low GDP-containing solutions do not accelerate neutrophil apoptosis[20] and a GDPs, 3,4-dideoxyglucosone-3-ene (3,4-DGE), was identified as the compound responsible for induction of leukocyte apoptosis.[13] 3,4-DGE was recently isolated from PD fluid.[23] Studies to date suggest that it is the most cytotoxic GDP and that the mechanism of cytotoxicity is induction of apoptosis[13] which may underlie its immunosuppressive properties *in vivo.*[24,25] Apoptosis

Table II. Low GDP PD fluids may better preserve residual renal function

	Conventional PD fluid	Low GDP PD fluid	Ref.	Study design
Initial Final (30 ± 13 months) Mean ± SD	7.1 ± 1.5 2.3 ± 2.2*	7.5 ± 1.8 4.1 ± 3.4	(27)	Single center, open, non-randomized, prospective, observational[1]
Initial Final (3 months) Median (25-75%)	4.5 (2.9–7.2) 3.5 (2.4–6.5)**	4.9 (2.9–6.5) 5.2 (4.4-8.9)	(30)	Multicenter, open, randomized, prospective with a crossover design and parallel arms[2]

*p = 0.004 *vs* final low GDP PD fluid; ** P = 0.007 *vs* initial conventional PD fluid.
Residual renal function = C urea + C creatinine)/2, expressed as ml/min/1.73 m².
[1]BicaVera®, [2]Balance®, Fresenius Medical Care. Similar results reported for other glucose containing low GDP PD solutions in abstract form (Haag-Weber et al. ISPD 2006. A470).

induced by 3,4-DGE was caspase-dependent and could be prevented by the broad-spectrum caspase inhibitor zVAD-fmk.[13] The serum or tissue levels of 3,4-DGE in diabetics are not known. However, it is conceivable that 3,4-DGE is generated from increased concentrations of its precursor 3-DG.[19] A higher temperature favors the conversion from 3,4-DGE to 3-DG.[26] Infection-triggered fever may contribute to the generation of 3,4-DGE and, thus to an accelerated untimely loss of leukocytes through apoptosis. The resulting impaired antibacterial defense may preclude eradication of the microorganism.

Most GDPs are not cytotoxic for human leukocytes.[13] 3-deoxyglucosone (3-DG) promotes apoptosis in a variety of cell types. However, only a not statistically significant trend toward increased apoptosis was noted in human neutrophils exposed to 3-DG.[13] No cytotoxicity was observed following the addition of methylglyoxal, acetaldehyde, or formaldehyde at concentrations found in PD solutions.[13]

CLINICAL CONSEQUENCES FOR INFECTION

How can leukocyte apoptosis induced by GDPs present in conventional PD solutions be avoided? GDP-containing PD solutions can be substituted for newer more biocompatible solutions that may decrease the incidence and severity of peritonitis[27,28] (table I).

A second approach would be to use pharmacological inhibitors of apoptosis for limited time periods. PD peritonitis is usually a mild complication characterized by a rapid response to i.p. antibiotics. However, agents such as *S. aureus* or gram negative bacteria may cause severe and protracted peritonitis even when antibiotic treatment is optimized. Protracted infection increases morbidity and may become a public health hazard.[4,7] A pan-caspase inhibitor increased the number of viable neutrophils and accelerated clearance of *S. aureus* in a murine model of peritonitis in the presence of PD solutions.[11] These results are consistent with those reported in experimental models of septic polymicrobial peritonitis in the absence of PD solutions.[29]

Once safe caspase inhibitors are available for their use in humans, trials should be designed to test whether their administration together with antibiotics in the first 24-48 h can accelerate the recovery of the more severe cases of peritonitis. Similar strategies may be applied to the early stages of severe infection in diabetics.

CLINICAL CONSEQUENCES FOR RESIDUAL RENAL FUNCTION

There is evidence that GDPs from PD fluid enter the systemic circulation, where they increase plasma advanced glycation end-products (AGEs) and may influence appetite.[30,31] In addition, PD solutions with a low GDP content better preserve residual renal function in PD patients than traditional, high-GDP PD solutions[27,30] (table II). As already commented, serum and intracellular concentrations of GDPs are increased in diabetics and have been associated to diabetic nephropathy.[19] It is interesting to note that acute renal failure is more frequent in diabetic patients with bacteriemia (and fever) than in non-diabetic individuals. Fever may tip the 3-DG/3,4-DGE balance toward the generation of 3,4-DGE.

Glucose and glucose degradation products induce apoptosis in renal cells.[10,32,33] In particular 3,4-DGE promotes apoptosis of cultured tubular cells by a Bax- and caspase-dependent mechanism.[34] Broad-spectrum caspase inhibition prevented apoptosis

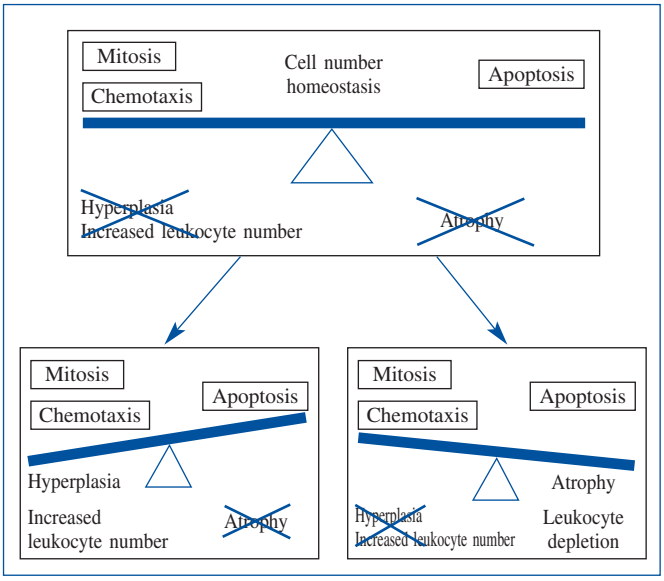

Figure 1. Cell number remains stable if cell loss by apoptosis is matched by generation of new cells by mitosis. In the case of migratory cells, chemotaxis plays a role in increasing cell number in a particular tissue. If apoptosis exceeds mitosis or chemotaxis, cell depletion ensues, leading to parenchymal atrophy or leukocyte depletion. If mitosis or chemotaxis exceeds apoptosis, parenchymal hyperplasia or leukocyte accumulation is the result.

but could not prevent eventual tubular cell death.[34] This is in contrast to cells with a shorter half-life, such as neutrophils, where prolongation of life by caspase inhibition is enough to allow them to carry their defensive function. As opposed to the unsatisfactory effect of caspase inhibition in tubular cells, antagonism of Bax prevented both apoptosis and non apoptotic tubular cell death.[34] Bax expression is increased in diabetic nephropathy[10,33] and Bax involvement is critical for apoptosis in the presence of hyperglycemia.[22] We hypothesize that the nephrotoxic potential of 3,4-DGE may have contributed to the preservation of renal function observed in clinical studies of low GDP PD solutions[30] (table II). Bax is a therapeutic target in preservation of renal function shared by diabetics and PD patients.

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Conflicto de intereses

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