# Peritoneal, aging during PD: implication of RAGE, the receptor for AGEs

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## SUMMARY

*During last years, the number of patients who have been continuously treated by peritoneal dialysis (PD) for over 5 or 10 years has markedly increased. Sclerosing syndromes and membrane failure are the most common complications that are now currently observed in long-term PD patients. Exposure to conventional PD fluids (PDFs) with poor biocompatibility induces a kind of «chemical peritonitis» in response of bad «biotolerance». The peritoneal fibroblasts, mesothelial cells and especially endothelial cells function as a filtration barrier, but also control intraperitoneal inflammation as well as leukocytes and macrophages.*

*Peritoneal exposure to conventional poorly biocompatible PDFs which combine non-physiological pH, high glucose concentrations, and high levels of glucose degradation products (GDPs), is associated with an accelerated peritoneal aging. Heat sterilization of PDFs induces the formation of GDPs which are involved in the formation of advanced glycation end-products (AGEs). Glucose, GDPs and AGEs participate to the peritoneal membrane failure and aging. AGEs via RAGE (receptor for AGEs) are involved in human peritoneal mesothelial cell (HPMC) activation.*

*In the present work, we summarize our previous* in vitro *works regarding mesothelial RAGE implication in the peritoneal membrane aging. Two periods of aging are distinguished: i) early peritoneal changes related to mesothelial cell activation and loss, ii) late membrane alteration correlated to submesothelial fibrosis and neovascularization.*

Key words: *Peritoneal dialysis. Biocompatibility. Aging. Advanced Glycation End-products (AGEs). RAGE.*

## RESUMEN

**En los últimos años ha aumentado considerablemente el número de pacientes tratados de manera continua con diálisis peritoneal (DP) durante 5 ó 10 años. Los síndromes esclerosantes y el fracaso de la membrana son las complicaciones más frecuentes que se observan actualmente en los pacientes que reciben DP a largo plazo. La exposición a líquidos de (LDP) convencionales con escasa biocompatibilidad induce un tipo de «peritonitis química» en respuesta a una mala «biotolerancia». Los fibroblastos peritoneales, las células mesoteliales y, en especial, las células endoteliales funcionan como una barrera de filtración, pero también controlan la inflamación intraperitoneal y los leucocitos y macrófagos.**

**La exposición peritoneal a LDP convencionales poco biocompatibles, que combinan pH no fisiológico, elevadas concentraciones de glucosa y grandes cantidades de productos de degradación de la glucosa (PDG), acelera el envejecimiento peritoneal. La esterilización por calor de los LDP induce la formación de PDG que están implicados en la formación de productos terminales de glucosilación avanzada (PTGA). La glucosa, los PDG y los PTGA participan en el fracaso y el envejecimiento de la membrana peritoneal. Los PTGA a través de RPTGA (receptor de PTGA) intervienen en la activación de las células mesoteliales peritoneales humanas (CMPH).**

**En el presente trabajo resumimos nuestros estudios anteriores in vitro sobre la implicación del RPTGA mesotelial en el envejecimiento de la membrana peritoneal. Se distinguen dos periodos de envejecimiento: i) alteraciones peritoneales precoces relacionadas con la activación y pérdida de células mesoteliales, ii) alteración tardía de la membrana relacionada con fibrosis y neovascularización submesoteliales.**

*Palabras clave:* Diálisis peritoneal. Biocompatilibidad. Envejecimiento. Productos finales de glicosilación. RAGE.

## INTRODUCTION

When peritoneal dialysis (PD) was extended to the treatment of end-stage renal disease, it could only be used for a short period, since the main complication was the risk of peritoneal infection. Improvement in delivery systems has since reduced the overall rate of infection, and the number of patients who have been continuously treated by PD for more than 5 years has markedly increased.[1,2] Peritoneal membrane aging with sclerotic syndromes or membrane failure is currently observed in long-term PD patients.

Peritoneal exposure to conventional poorly biocompatible PD fluids (PDFs) which combine non-physiological pH, high glucose concentrations, and high levels of glucose degradation products (GDPs), is associated with an elevated incidence of peritoneal alterations and accelerated aging.[3] Heat sterilization of PDFs induces the formation of GDPs such as glyoxal, methylglyoxal, 3-deoxyglucosone (3-DG) and 3,4-dideoxyglucosone-3-ene (3,4-DGE) which are involved in the formation of advanced glycation end-products (AGEs) (fig. 1).[3-6] Glucose, GDPs and AGEs participate to the peritoneal membrane failure. AGEs via RAGE (receptor for AGEs) are involved in

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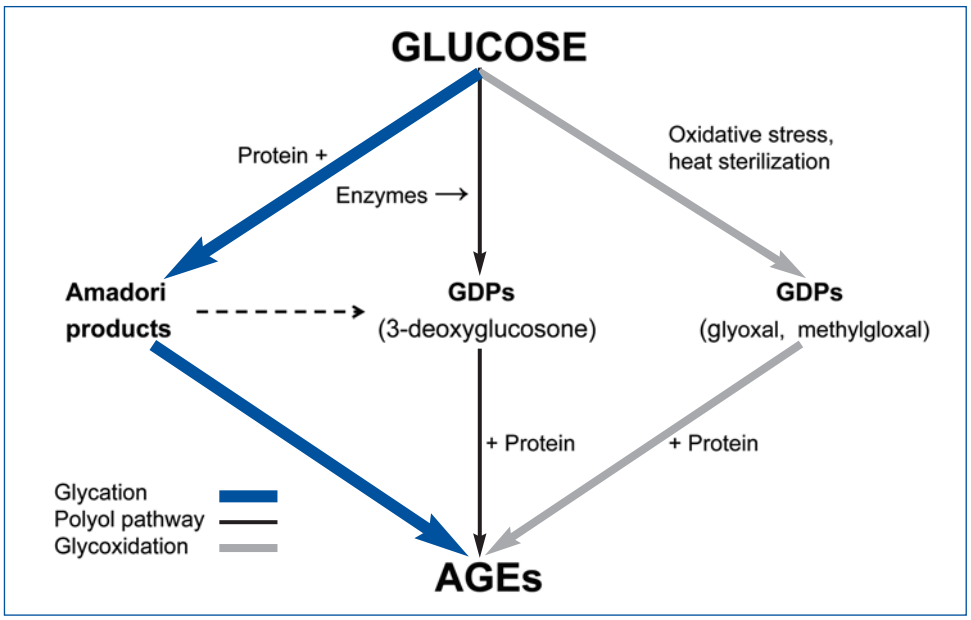


**Figure 1.** Formation of AGEs. Glycation, polyols pathway and glycoxidation. AGEs: advanced glycation end-products; GDPs: glucose degradation products.

human peritoneal mesothelial cell (HPMC) activation. RAGE mRNA and protein were demonstrated to be expressed by cultured HPMCs *in vitro*. The presence of RAGE on HPMCs was confirmed on human peritoneal specimens.[7]

RAGE belongs to the immunoglobulin superfamily of surface membrane molecule. The gene coding for RAGE is located in the major histocompatibility region of chromosome 6.[8] Extracellular domain consists of three immunoglobulin-like regions, one «V»-type followed by two «C»-type and the V-type domain is critical for binding of RAGE ligands. RAGE contains a single transmembrane-spanning domain and a cytosolic tail. RAGE engagement results in a series of intracellular modifications inducing reactive oxygen intermediates (ROIs) formation, and gene transcription. Studies in cell culture show that AGE-RAGE interaction alters cellular properties important in vascular homeostasis. Following engagement of RAGE by AGEs, endothelia increase their expression of vascular cell adhesion molecule-1 (VCAM-1), tissue factor, and interleukin (IL)-6, and their permeability to macromolecules. Two forms of RAGE are described beside the full length RAGE. First, a truncated form of RAGE, which lacks the cytosolic tail, remains firmly embedded in the membrane. Although this form of the receptor is competent to bind the usual complement of RAGE ligands, it acts as a dominant negative (termed «DN-RAGE») receptor, and its expression strikingly suppresses RAGE-mediated signaling. sRAGE (soluble RAGE) is the extracellular domain of the receptor, which binds ligand and blocks interaction with, and activation of, cell-surface RAGE.[9] Blockade of RAGE, employing sRAGE or anti-RAGE antibodies, or in homozygous RAGE null mice, resulted in significantly decreased of AGE deleterious effects.[10,11]

In the present work, we summarize our previous *in vitro* works regarding mesothelial RAGE implication in the peritoneal membrane aging. Two periods of aging are distinguished: i) early peritoneal changes related to mesothelial cell activation and loss, ii) late membrane alteration correlated with submesothelial fibrosis and neovascularization (fig. 2).

## EARLY PERITONEAL MEMBRANE CHANGES

### Proinflammatory cell response: overexpression of VCAM-1 after mesothelial RAGE activation[7]

Increased levels have been found in the peritoneal fluids of several mediators which are involved in the inflammatory reaction such as prostaglandins, leukotrienes, cytokines IL-1α, IL-1β, IL-6, IL-8, and tumor necrosis factor-α (TNF-α).[3,12-16] Other molecules which modulate mesothelial cell, fibroblast, and macrophage function can participate in protein synthesis and also in protein degradation: transforming growth factor-β1 (TGF-β1), and matrix metalloproteinase 9 (MMP-9).[14,17] In addition, the activation of coagulation by tissue factor synthesis, or the reduction of plasminogen activator inhibitor-1 (PAI-1) may contribute to the inflammatory reaction[18].

In a previous work, we have demonstrated that VCAM-1 expression by HPMC was potentiated after stimulation by carboxymethyllysine (CML)-albumin, the well-defined AGE ligand.[7] Blockade of RAGE, employing sRAGE or anti-RAGE antibodies, both inhibited the stimulation of VCAM-1 induced by AGEs. This showed that, in addition to other inflammatory cytokines, AGEs can modulate VCAM-1 expression by HPMC. Contrary to VCAM-1, intercellular adhesion molecule-1 (ICAM-1) expression on HPMC was not affected by addition of CML-albumin. This different effect regarding adhesion molecule expression was similar to the results observed with endothelial cells (HUVECs, human umbilical vein endothelial cells) and suggested that the transduction pathways and gene transcription regulation differ after cytokine or AGE stimulation, respectively. VCAM-1 expression after RAGE activation was accompanied by enhanced leukocyte adhesion, indicating that this mechanism may participate in the inflammatory process and could have an influence on HPMCs or monocyte function, especially as regards peritoneal macrophages that can be activated by AGEs and produce TNFα.[19] Adherent leukocytes may infiltrate and migrate through the mesothelial cells; in addition, they can produce inflammatory mediators.[20,21]

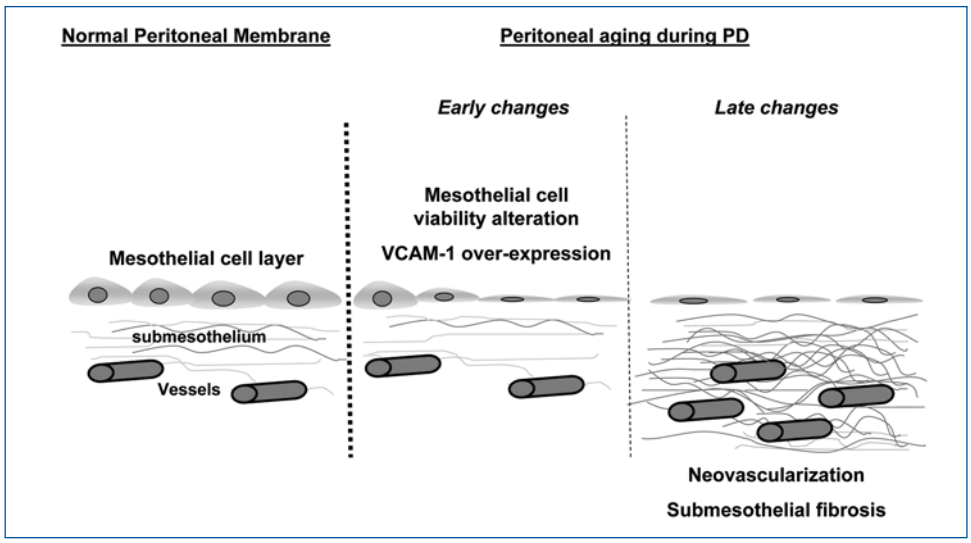


**Figure 2.** Peritoneal membrane aging during PD. Two periods of aging are distinguished: i) early peritoneal changes related to mesothelial cell activation and loss, ii) late membrane alteration correlated with submesothelial fibrosis and neovascularization.

## Glucose, 3-DG and AGEs have distinct deleterious effects on HPMC viability[22]

Glucose represents the major osmotic agent contained in most PDFs. However, it has deleterious effects on PDF biocompatibility and could be a source of potentially harmful derivative products. During heat sterilization GDPs are formed, and these are precursors of AGE generation. These two glucose derivatives have independent and synergistic deleterious effects when combined with glucose on HPMC viability (fig. 3).

We previously described that heat-sterilized dextrose-lactate PDF and a high glucose concentration are predominant factors in HPMC proliferation inhibition.[22] Compared to filtered dextrose-lactate-based PDF (no inhibition), we demonstrated a significant inhibition in HPMC proliferation when cell were exposed to heat-sterilized dextrose-lactate-based PDF (high inhibition). This indicated that agents such as GDPs formed during heat sterilization may participate in the mechanism of cell proliferation inhibition. A direct effect of 3-DG on cell culture was consistent with this hypothesis, as well as with its potentiation of the deleterious effect of high glucose concentration. Two other GDPs, i.e. 3,4-dideoxyglucosone-3-ene (3,4-DGE) and methylglyoxal, are of particular interest. 3,4-DGE has been quantified as a novel reactive GDP, the concentrations of which increase after heat sterilization of PDF.[5] Compared to heat sterilization, less peritoneal injury developed in rats receiving a daily intraperitoneal infusion of lactate sterilized by filtration. This result indicates that besides pH level and glucose concentration, GDPs can also be involved in the genesis of mesothelial damage.

Cell death may occur in a disorganized and chaotic manner, associated with swelling of the cell, a process that is known as necrosis or more specifically, oncosis.[23] In contrast, apoptosis is a highly regulated form of programmed cell death. Morp-

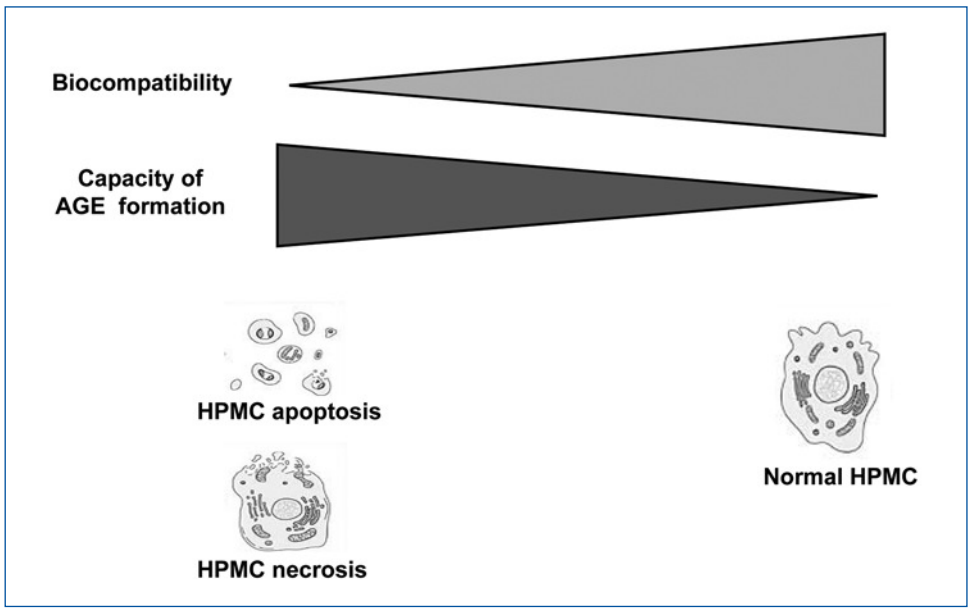


**Figure 3.** PD fluids: Biocompatibility and capacity to form AGEs. HPMCs: human peritoneal mesothelial cells; AGEs: advanced glycation end-products.

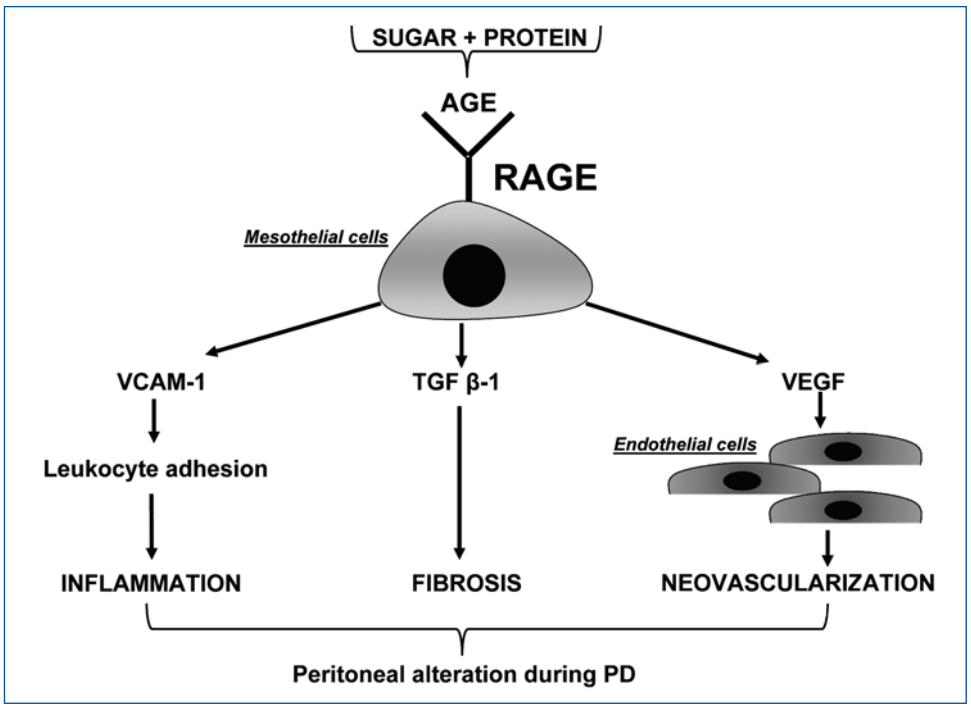


**Figure 4.** HPMC response after RAGE activation. AGEs: advanced glycation end-products; RAGE: receptor of AGEs; VCAM-1: vascular cell adhesion molecule-1; TGF-β-1: transforming growth factor-β1; VEGF, vascular endothelial growth factor.

hological alterations such as shrinkage and blebbing are observed. Apoptotic cells retain an intact cellular membrane and undergo removal by macrophages. We have demonstrated that 3-DG and CML-albumin have distinct deleterious effects on HPMC viability.[22] CML-albumin potentiated the proapoptotic effect of glucose at intermediate glucose concentrations (25 g/L), but this effect was not detectable at a higher glucose concentration (45 g/L). Blockade of AGE-RAGE interaction partially prevented the PDF-induced apoptosis, indicating that besides AGEs other glucose-derived compounds such as 3-DG can also alter HPMC functions via a RAGE-independent pathway.

## LATE PERITONEAL MEMBRANE CHANGES

### Peritoneal fibrosis: production of TGFβ-1 after mesothelial RAGE activation[24]

It has been demonstrated that conventional PDF-induced TGF-β1 production by HPMCs was slightly increased compared to low-GDP-PDF at 15 g/L glucose.[24] At a 42.5 g/L glucose concentration, PDF-induced TGF-β1 secretion was significantly higher after exposure to conventional PDFs than after exposure to two other PDFs offering physiological pH (bicarbonate or lacatate-based-PDF, pH 7.4 to 7, respectively) which showed identical results. This over production of TGF-β1 by HPMCs exposed to high GDP content PDFs could be partially prevented by PDF preincubation with sRAGE. This result suggested that, beside pH, AGE engagement to RAGE is implicated, at least in part, in TGF-β1 overproduction.

In diabetic rats, blockade of RAGE by anti-RAGE antibodies prevented the upregulation of TGFβ-1 and the development of submesothelial fibrosis, but did not affect the upregulation of endothelial nitric oxide synthase expression.[25] These results are consistent with the partial inhibition of the deleterious effect of PDFs containing AGE precursors.

### Peritoneal neovascularization: production of Vascular Endothelial Growth Factor (VEGF) after mesothelial RAGE activation[26]

In a recent work, we have investigated whether the effect of glucose and AGEs on HPMCs might alter the release of VEGF and subsequently the formation of capillary tubes by endothelial cells. HUVECs have the capacity to spontaneously form capillary tubes in Matrigel basement membrane matrix (3 dimensions). This property was used to assess the effect of glucose and AGEs as a model for angiogenesis. To mimic the peritoneal membrane exposed to PDF, we have developed a co-culture system without direct contact between HPMCs and HUVECs. HMPCs were cultured on a polyester membrane in an intercup chamber and HUVECs were co-cultured in well bottom (of 24 well-plates) in Matrigel. The effect of capillary tube formation was studied after HPMC stimulation by AGEs. During monoculture, capillary tube formation by HUVECs was inhibited by high glucose concentrations and AGEs.[26] In the co-culture system, the capillary tube formation by HUVECs was potentiated when HPMCs were prestimulated by AGEs and co-cultured with HUVECs. The capillary tube formation promoted by the co-culture of HUVECs with stimulated HPMCs was mediated by mesothelial RAGE, since RAGE blockade by anti-RAGE antibody significantly limited capillary tube development. The addition of anti-VEGF antibody to HPMCs significantly reduced capillary tube formation by HUVECs indica-

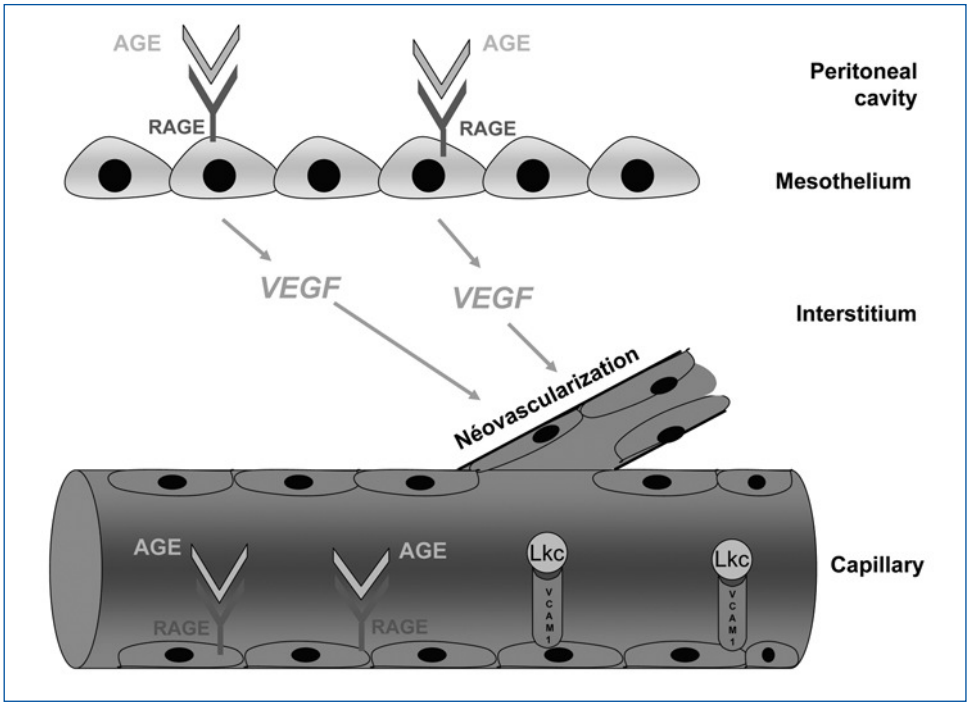


**Figure 5.** Cross talk between mesothelial and endothelial cells. VEGF production is stimulated by AGEs through mesothelial RAGE activation, and may well be responsible for a large proportion of the peritoneal neovascularization. Endothelial cells are also stimulated after RAGE activation by AGE formed through uremic carbonyl stress. AGEs: advanced glycation end-products; RAGE: receptor of AGEs; VEGF, vascular endothelial growth factor; VCAM-1: vascular cell adhesion molecule-1; Lkc: leukocytes.

ting that VEGF production is stimulated by AGEs through mesothelial RAGE activation, and may well be responsible for a large proportion of the increase in capillary tube formation.

In animal study, it has been demonstrated that RAGE-deficient mice failed to develop peritoneal neoangiogenesis and increased VEGF expression after exposure to high GDP-containing PDFs compared to control mice.[27]

## CONCLUSION

HPMCs, which constitute the innermost layer of the peritoneum, are a source of a large number of cytokines and growth factors whose production is regulated by various factors including AGEs. AGEs binding mesothelial RAGE are clearly implicated in peritoneum aging (fig. 4).

During PD, at least two factors may lead to AGE formation: high glucose concentrations in PD solutions and renal failure. Uremia is associated with carbonyl and oxidative stress, which results in the enhanced formation of glycation and oxidation products respectively. We have demonstrated that PD is associated with an increase in the levels of blood glycation end-products particularly in diabetic patients, but also with a decrease in oxidative products such as advanced oxidative protein products (AOPP) especially in non-diabetic subjects.[28] We have to keep in mind that AGEs have been described as inducing VCAM-1 expression on endothelial cells, and have also been implicated in human atherosclerotic lesions and diabetic vascular disorders (fig. 5).[29]

Animal and in vitro studies have evidenced that AGE/RAGE interaction blockade by soluble RAGE or anti-RAGE antibodies could reduce the cellular and organic alterations secondary to RAGE activation, but these molecules have not been used in human therapy.

### Conflict of interest statement

*Eric Boulanger and Maïté Daroux: have no conflict of interest.*

## REFERENCES

1. Davies SJ, Phillips L, Griffiths AM, Russell LH, Naish PF, Russell GL. What really happens to people on long-term peritoneal dialysis? *Kidney Int* 1998; 54: 2207-2217.
2. Topley N. Membrane longevity in peritoneal dialysis: impact of infection and bio-incompatible solutions. *Adv Ren Replace Ther* 1998; 5: 179-184.
3. Witowski J, Korybalska K, Wisniewska J, Breborowicz A, Gahl GM, Frei U, Passlick-Deetjen J, Jorres A. Effect of glucose degradation products on human peritoneal mesothelial cell function. *J Am Soc Nephrol* 2000; 11: 729-739.
4. Miyata T, Horie K, Ueda Y, Fujita Y, Izuhara Y, Hirano H, Uchida K, Saito A, Van Ypersele de Strihou C, Kurokawa K. Advanced glycation and lipidoxidation of the peritoneal membrane: respective roles of serum and peritoneal fluid reactive carbonyl compounds. *Kidney Int* 2000; 58: 425-435.
5. Linden T, Cohen A, Deppisch R, Kjellstrand P, Wieslander A. 3,4-Dideoxyglucosone-3-ene (3,4-DGE): a cytotoxic glucose degradation product in fluids for peritoneal dialysis. *Kidney Int* 2002; 62: 697-703.
6. Linden T, Forsback G, Deppisch R, Henle T, Wieslander A. 3-Deoxyglucosone, a promoter of advanced glycation end products in fluids for peritoneal dialysis. *Perit Dial Int* 1998; 18: 290-293.
7. Boulanger E, Wautier MP, Wautier JL, Boval B, Panis Y, Wernert N, Danze P, Dequiedt P. AGEs bind to mesothelial cells via RAGE and stimulate VCAM-1 expression. *Kidney Int* 2002; 61: 148-156.
8. Neeper M, Schmidt AM, Brett J, Yan SD, Wang F, Pan YC, Elliston K, Stern D, Shaw A. Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins. *J Biol Chem* 1992; 267: 14998-15004.
9. Schmidt AM, Yan SD, Yan SF, Stern DM. The multiligand receptor RAGE as a progression factor amplifying immune and inflammatory responses. *J Clin Invest* 2001; 108: 949-955.
10. Sakaguchi T, Yan SF, Yan SD, Belov D, Rong LL, Sousa M, Andrassy M, Marso SP, Duda S, Arnold B, Liliensiek B, Nawroth PP, Stern DM, Schmidt AM, Naka Y. Central role of RAGE-dependent neointimal expansion in arterial restenosis. *J Clin Invest* 2003; 111: 959-972.

11. Yamamoto Y, Kato I, Doi T, Yonekura H, Ohashi S, Takeuchi M, Watanabe T, Yamagishi S, Sakurai S, Takasawa S, Okamoto H, Yamamoto H. Development and prevention of advanced diabetic nephropathy in RAGE-overexpressing mice. *J Clin Invest* 2001; 108: 261-268.
12. Douvdevani A, Rapoport J, Konforty A, Argov S, Ovnat A, Chaimovitz C. Human peritoneal mesothelial cells synthesize IL-1 alpha and beta. *Kidney Int* 1994; 46: 993-1001.
13. Topley N, Jorres A, Luttmann W, Petersen MM, Lang MJ, Thierauch KH, Muller C, Coles GA, Davies M, Williams JD. Human peritoneal mesothelial cells synthesize interleukin-6: induction by IL-1 beta and TNF alpha. *Kidney Int* 1993; 43: 226-233.
14. Offner FA, Feichtinger H, Stadlmann S, Obrist P, Marth C, Klingler P, Grage B, Schmahl M, Knabbe C. Transforming growth factor-beta synthesis by human peritoneal mesothelial cells. Induction by interleukin-1. *Am J Pathol* 1996; 148: 1679-1688.
15. Cannistra SA, Ottensmeier C, Tidy J, DeFranzo B. Vascular cell adhesion molecule-1 expressed by peritoneal mesothelium partly mediates the binding of activated human T lymphocytes. *Exp Hematol* 1994; 22: 996-1002.
16. Topley N, Kaur D, Petersen MM, Jorres A, Williams JD, Faict D, Holmes CJ. *In vitro* effects of bicarbonate and bicarbonate-lactate buffered peritoneal dialysis solutions on mesothelial and neutrophil function. *J Am Soc Nephrol* 1996; 7: 218-224.
17. Rougier JP, Moullier P, Piedagnel R, Ronco PM. Hyperosmolality suppresses but TGF beta 1 increases MMP9 in human peritoneal mesothelial cells. *Kidney Int* 1997; 51: 337-347.
18. Rougier JP, Guia S, Hagege J, Nguyen G, Ronco PM. PAI-1 secretion and matrix deposition in human peritoneal mesothelial cell cultures: transcriptional regulation by TGF-beta 1. *Kidney Int* 1998; 54: 87-98.
19. Rogachev B, Hausmann MJ, Yulzari R, Weiler M, Holmes C, Faict D, Chaimovitz C, Douvdevani A. Effect of bicarbonate-based dialysis solutions on intracellular pH (pHi) and TNFalpha production by peritoneal macrophages. *Perit Dial Int* 1997; 17: 546-553.
20. Li FK, Davenport A, Robson RL, Loetscher P, Rothlein R, Williams JD, Topley N. Leukocyte migration across human peritoneal mesothelial cells is dependent on directed chemokine secretion and ICAM-1 expression. *Kidney Int* 1998; 54: 2170-2183.
21. Liang Y, Sasaki K. Expression of adhesion molecules relevant to leukocyte migration on the microvilli of liver peritoneal mesothelial cells. *Anat Rec* 2000; 258: 39-46.
22. Boulanger E, Wautier MP, Gane P, Mariette C, Devuyst O, Wautier JL. The triggering of human peritoneal mesothelial cell apoptosis and oncosis by glucose and glycoxydation products. *Nephrol Dial Transplant* 2004; 19: 2208-2216.
23. Majno G, Joris I. Apoptosis, oncosis, and necrosis. An overview of cell death. *Am J Pathol* 1995; 146: 3-15.
24. Grossin N, Wautier MP, Wautier JL, Gane P, Taamma R, Boulanger E. Improved *in vitro* biocompatibility of bicarbonate-buffered peritoneal dialysis fluid. *Perit Dial Int* 2006; 26: 664-670.
25. De Vriese AS, Flyvbjerg A, Mortier S, Tilton RG, Lameire NH. Inhibition of the Interaction of AGE-RAGE Prevents Hyperglycemia-Induced Fibrosis of the Peritoneal Membrane. *J Am Soc Nephrol* 2003; 14: 2109-2118.
26. Boulanger E, Grossin N, Wautier MP, Taamma R, Wautier JL. Mesothelial RAGE activation by AGEs enhances VEGF release and potentiates capillary tube formation. *Kidney Int* 2007; 71: 126-133.
27. Schwenger V, Morath C, Salava A, Amann K, Seregin Y, Deppisch R, Ritz E, Bierhaus A, Nawroth P, Zeier M. Damage to the peritoneal membrane by glucose degradation products is mediated by the receptor for advanced glycation end-products. *J Am Soc Nephrol* 2006; 17: 199-207.
28. Boulanger E, Moranne O, Wautier MP, Witko-Sarsat V, Descamps-Latscha B, Kandoussi A, Grossin N, Wautier JL. Changes in glycation and oxidation markers in patients starting peritoneal dialysis: a pilot study. *Perit Dial Int* 2006; 26: 207-212.
29. Vlassara H, Fuh H, Donnelly T, Cybulsky M. Advanced glycation endproducts promote adhesion molecule (VCAM-1, ICAM-1) expression and atheroma formation in normal rabbits. *Mol Med* 1995; 1: 447-456.