Review

Dietary AGEs and ALEs and risk to human health by their interaction with the receptor for advanced glycation endproducts (RAGE) – an introduction

Paul J. Thornalley

Clinical Sciences Research Institute, University of Warwick, University Hospital, Coventry, UK

The receptor for advanced glycation endproducts (RAGE) has a well-substantiated role in cell dysfunction and mechanisms of inflammatory disease. The physiological agonists of RAGE are less certain: S100/calgranulin proteins, high mobility group-1 protein HMGB1 and other proteins are candidate agonists. It increasingly appears unlikely proteins modified by advanced glycation endproducts are effective agonists *in vivo*. In the following debate, Professors Ann Marie Schmidt and Claus Heizmann gave arguments and evidences for and against the motion. Recent evidence suggesting the activation of RAGE impairs the enzymatic defence against glycation provided by glyoxalase 1 (Glo 1) suggests that studies of RAGE will continue to be of importance to our understanding of the physiological significance of protein glycation.

Keywords: Disease / Glycation / Glyoxalase / Receptor / S100 proteins

Received: January 16, 2007; revised: March 17, 2007; accepted: March 20, 2007

This article provides an introduction to "Dietary AGEs and ALEs interact with RAGE".
Pro arguments: http://dx.doi.org/10.1002/mnfr.200700008
Contra arguments: http://dx.doi.org/10.1002/mnfr.200600284

The observation of vascular cell activation and dysfunction following the exposure of cultured cells to proteins highly modified by advanced glycation endproducts (AGEs) led to the hypothesis of cell surface receptors that bind specifically AGE-modified proteins or AGE receptors – reviewed in ref. [1] The receptor for advanced glycation endproducts (RAGE) is one such receptor and has a well-substantiated role in vascular cell dysfunction [2–5]. RAGE was isolated initially from endothelial cells of bovine lung by Stern and coworkers [6]. Human microvascular pericytes, endothelial cells and other cells express splice variants of RAGE: N-terminal truncated, C-terminal truncated and full length forms; only the latter two bound AGE-modified protein [3]. The endogenous secreted C-terminal truncated RAGE, called endogenous secreted RAGE (esRAGE), lacks the cytoplasmic and transmembrane domains of RAGE and is expressed in several human tissues [7]. Recombinant DNA techniques were used to produce a similar C-terminal truncated RAGE called soluble RAGE (sRAGE) [8]. esRAGE and sRAGE act as decoy receptors for RAGE ligands endogenously and experimentally, respectively, and thereby influence RAGE–ligand interactions and signalling [7, 8].

RAGE transgenic and knockout mice have provided functional genomic models to identify the roles of RAGE in several disease states – particularly where an inflammatory mechanism is involved: diabetic complications [9–11], atherosclerosis [12], sepsis [13] and hemorrhagic shock [14]. For example, the RAGE transgenic mice with high expression of full length RAGE were susceptible to the development of diabetic nephropathy [9] and diabetic homozygous RAGE null mice were resistant to the development of diabetic nephropathy [10]. These observations confirm a role for RAGE in the development of diabetic nephropathy. The wealth of evidence linking RAGE to mechanisms of disease was presented in the debate by Dr. Ann Marie Schmidt. The ligand activating RAGE in the development of vascular complications and other disease states

Correspondence: Professor Paul J. Thornalley, Protein Damage and Systems Biology Research Group, Clinical Sciences Research Institute, University of Warwick, University Hospital, Coventry CV2 2DX, UK
E-mail: P.J.Thornalley@warwick.ac.uk
Fax: +44-024-7696-8653

Abbreviations: AGE, advanced glycation endproducts; **ALE,** advanced lipoxidation end products; **RAGE,** receptor for advanced glycation endproducts

is less certain: it may be S100/calgranulin proteins, high mobility group-1 protein HMGB1 (amphoterin) or other proteins. It increasingly appears unlikely to be proteins modified by AGEs.

AGE-modified proteins are thought to be ligands for RAGE [3, 6]. Many studies of cell activation by RAGE have employed albumin highly modified by AGEs and advanced lipoxidation end products (ALEs) (30 – 40 modified groups *per* protein). Proteins modified so highly by AGEs are rarely found in tissues and body fluids *in vivo* but they are found in thermally processed foods. Proteins of tissues and body fluids contain proteins modified minimally by AGEs and ALEs – they have only one AGE residue *per* protein molecule [15]. The role of AGE-modified proteins as physiological ligands for the RAGE receptor is in doubt, or at least enigmatic. It seems there may be more important ligands for RAGE than AGE or ALE-modified proteins physiologically. There was an agreement that proteins highly modified by AGEs (and hence competent to bind RAGE) are unlikely proteins to exist in physiological systems *in vivo*. In the debate we heard that RAGE binding competence is limited to aggregates of highly glycated proteins from Dr. Claus Heizmann. This may explain why high extents of glycation are required for RAGE binding as only severe glycation damage to proteins leads to multimolecular aggregate formation [16].

The role of endotoxin binding to RAGE in cell activation was raised in the debate and no consensus on the significance of this was reached. As raised in the question and answer session afterwards, contamination of AGE-modified proteins with endotoxin may have compromised many studies of AGE-protein/AGE-receptor involvement in proinflammatory responses.

RAGE binds members of the S100/calgranulin group of proteins: S100A12 (EN-RAGE) and S100b [17]. Dr. Heizmann has longstanding expertise in research of S100 proteins [18 – 20]. S100/calgranulin protein expression was increased in the kidney of diabetic mice with and without overexpression of RAGE [9]. It was also increased in clinical diabetes [21], atherosclerosis [22], arthritis [23] and other inflammatory disorders [24]. S100b induced increased expression of genes linked to inflammatory responses in endothelial cells, whereas endotoxin-free AGE-modified protein did not [5]. Currently, cell activation by S100A12 and S100b appears to be a likely important function of RAGE *in vivo*. The characteristics of molecular recognition of RAGE by S100A12 and the influence of calcium binding of S100A12 on the interaction have been reported recently [25].

It is difficult to establish a consensus of opinion from investigators engaged in RAGE-related research on the importance (or lack of importance) of AGE-modified proteins as agonists of RAGE *in vivo*. There are particular circumstances of extremely high glycation potential where proteins may be highly glycated and competent RAGE ligands. Examples are: (i) end stage renal disease patients receiving peritoneal dialysis therapy with glucose-based dialysis fluids where extracellular matrix proteins of the peritoneal basement membrane are exposed chronically to high concentrations of glucose (up to 593 mM), and (ii) intestinal epithelial cells exposed to high glycated proteins of thermally processing foodstuffs in the gastrointestinal tract epithelium. The latter example is particularly pertinent to the theme of the COST action conference. The importance of RAGE binding of AGE- and ALE-modified proteins at this site is unlikely, however, because of extremely low expression of RAGE in gastrointestinal tract epithelial cells [26].

The association of RAGE with glycation is likely to undergo substantial revision in future research following the work of Bierhaus and coworkers. They found that the engagement of RAGE was associated with decreased expression of Glo1 – an enzyme that protects against protein glycation by dicarbonyls such as methylglyoxal and glyoxal [27]. Induction of diabetes in wild-type mice decreased the expression of Glo1 whereas the induction of diabetes in RAGE (–/–) mice did not [28]. Decreased Glo1 expression leads to increased protein glycation [27]. The mechanistic interpretation of the association of RAGE expression and AGEs in vascular and other tissues [29, 30] may, therefore, require reappraisal. The colocalization of RAGE and AGEs may be due to RAGE activation by S100 proteins (or other non-AGE agonists) decreasing the local expression of Glo1 and thereby increasing the local formation of AGEs. The functional role of decreasing Glo1 activity and increasing protein glycation by RAGE activation is not yet clear. This may be part of an inflammatory response leading to labelling proteins with dicarbonyl-derived hydroimidazolone AGE residues and directing these proteins to the proteasome for destruction. Methylglyoxal modification of proteins is thought to target proteins for proteasomal destruction [31]. If correct, this will link AGE formation to the removal and destruction of proteins, at least for intracellular proteins – a 'twist' or reinterpretation of the role originally attributed to the formation of AGE modified proteins by Cerami [32]. The link of RAGE activation with AGE formation is also a twist to the physiological function of RAGE. As we concluded in the discussion after the debate, continued research on RAGE is likely to remain of importance to our understanding of the physiological significance of protein glycation.

I thank the Wellcome Trust, Juvenile Diabetes Research Foundation, Diabetes UK, Cancer Research UK and European Union 6th Framework Programme for the support for my research and Nestle for support for this debate.

References

[1] Thornalley, P. J., Cell activation by glycated proteins. AGE receptors, receptor recognition factors and functional classification of AGEs, *Cell. Mol. Biol.* 1998, *44*, 1013 – 1023.

[2] Schmidt, A.-M., Hori, O., Chen, J. X., Li, J. F., *et al*., Advanced glycation endproducts interacting with their endothelial receptor induce expression of vascular cell adhesion molecule-1 (VCAM-1) in cultured human endothelial cells in mice. A potential mechanism for the accelerated vasculopathy of diabetes, *J. Clin. Invest.* 1995, *96*, 1395 – 1403.

[3] Yonekura, H., Yamamoto, Y., Sakurai, S., Petrova, R. G., *et al*., Novel splice variants of the receptor for advanced glycation end-products expressed in human vascular endothelial cells and pericytes, and their putative roles in diabetes-induced vascular injury, *Biochem. J.* 2003, *370*, 1097 – 1109.

[4] Chavakis, T., Bierhaus, A., Al Fakhri, N., Schneider, D., *et al*., The pattern recognition receptor (RAGE) is a counterreceptor for leukocyte integrins: A novel pathway for inflammatory cell recruitment, *J. Exp. Med.* 2003, *198*, 1507 – 1515.

[5] Valencia, J. V., Mone, M., Zhang, J., Weetall, M., *et al*., Divergent pathways of gene expression are activated by the RAGE ligands S100b and AGE-BSA, *Diabetes* 2004, *53*, 743 – 751.

[6] Neeper, M., Schmidt, A.-M., Brett, J., Yan, S. D., *et al*., Cloning and expression of a cell surface receptor for advanced glycosylation endproducts of proteins, *J. Biol. Chem.* 1992, *267*, 14998 – 15004.

[7] Cheng, C. M., Tsuneyama, K., Kominami, R., Shinohara, H., *et al*., Expression profiling of endogenous secretory receptor for advanced glycation end products in human organs, *Mod. Pathol.* 2005, *18*, 1385 – 1396.

[8] Park, L., Raman, K. G., Lee, K. J., Lu, Y., *et al*., Suppression of accelerated diabetic atherosclerosis by the soluble receptor for advanced glycation endproducts, *Nat. Med.* 1998, *4*, 1025 – 1030.

[9] Yamamoto, Y., Kato, I., Doi, T., Yonekura, H., *et al*., Development and prevention of advanced diabetic nephropathy in RAGE-overexpressing mice, *J. Clin. Invest.* 2001, *108*, 261 – 268.

[10] Wendt, T. M., Tanji, N., Guo, J., Kislinger, T., *et al*., RAGE drives the development of glomerulosclerosis and implicates podocyte activation in the pathogenesis of diabetic nephropathy, *Am. J. Pathol.* 2003, *162*, 1123 – 1137.

[11] Lalla, E., Lamster, I. B., Feit, M., Huang, L., *et al*., Blockade of RAGE suppresses periodontitis-associated bone loss in diabetic mice, *J. Clin. Invest.* 2000, *105*, 1117 – 1124.

[12] Sakaguchi, T., Yan, S. F., Yan, S. D., Belov, D., *et al*., Central role of RAGE-dependent neointimal expansion in arterial restenosis, *J. Clin. Invest.* 2003, *111*, 959 – 972.

[13] Liliensiek, B., Weigand, M. A., Bierhaus, A., Nicklas, W., *et al*., Receptor for advanced glycation end products (RAGE) regulates sepsis but not the adaptive immune response, *J. Clin. Invest.* 2004, *113*, 1641 – 1650.

[14] Raman, K. G., Sappington, P. L., Yang, R., Levy, R. M., *et al*., The role of RAGE in the pathogenesis of intestinal barrier dysfunction after hemorrhagic shock, *Am. J. Physiol. Gastrointest. Liver Physiol.* 2006, *291*, G556 – G565.

[15] Thornalley, P. J., Battah, S., Ahmed, N., Karachalias, N., *et al*., Quantitative screening of advanced glycation endproducts in cellular and extracellular proteins by tandem mass spectrometry, *Biochem. J.* 2003, *375*, 581 – 592.

Dr Paul J. Thornalley is Professor of Systems Biology and Director of the Protein Damage and Systems Biology Research Group, Clinical Sciences Research Institute, University of Warwick, Coventry, U.K. He received his PhD in Biochemistry from the University of Oxford, U.K and has worked for 25 years on protein glycation in diabetes and other diseases, publishing 314 peer-reviewed articles and conference papers. His specialist interests are: LC-MS/MS measurement of protein glycation, oxidation and nitration, the glyoxalase system and thiamine metabolism.

[16] Valencia, J. V., Weldon, S. C., Quinn, D., Kiers, G. H., *et al*., Advanced glycation end product ligands for the receptor for advanced glycation end products: Biochemical characterization and formation kinetics, *Anal. Biochem.* 2004, *324*, 68 – 78.

[17] Hofmann, M. A., Drury, S., Fu, C. F., Qu, W., *et al*., RAGE mediates a novel proinflammatory axis: A central cell surface receptor for S100/calgranulin polypeptides, *Cell* 1999, *97*, 889901.

[18] Schafer, B. W., Heizmann, C. W., The S100 family of EF-hand calcium-binding proteins: Functions and pathology, *Trends Biochem. Sci.* 1996, *21*, 134 – 140.

[19] Hsieh, H. L., Schafer, B. W., Weigle, B., Heizmann, C. W., S100 protein translocation in response to extracellular S100 is mediated by receptor for advanced glycation endproducts in human endothelial cells, *Biochem. Biophys. Res. Commun.* 2004, *316*, 949 – 959.

[20] Marenholz, I., Heizmann, C. W., Fritz, G., S100 proteins in mouse and man: From evolution to function and pathology (including an update of the nomenclature), *Biochem. Biophys. Res. Commun.* 2004, *322*, 1111 – 1122.

[21] Kosaki, A., Hasegawa, T., Kimura, T., Iida, K., *et al*., Increased plasma S100A12 (EN-RAGE) levels in patients with type 2 diabetes, *J. Clin. Endocrinol. Metab.* 2004, *89*, 5423 – 5428.

[22] Kosaki, A., Mori, Y., Kimura, T., Nishikawa, M., *et al*., S100A12: A potential new marker for accelerated atherosclerosis in end stage renal disease patients with diabetes mellitus, *Diabetes* 2005, *54*, A189 – A189.

[23] Foell, D., Kane, D., Bresnihan, B., Vogl, T., *et al*., Expression of the pro-inflammatory protein S100A12 (EN-RAGE) in rheumatoid and psoriatic arthritis, *Rheumatology* 2003, *42*, 1383 – 1389.

[24] Foell, D., Seeliger, S., Vogl, T., Koch, H. G., *et al*., Expression of S100A12 (EN-RAGE) in cystic fibrosis, *Thorax* 2003, *58*, 613 – 617.

[25] Xie, J., Burz, D. S., He, W., Bronstein, I. B., *et al*., Hexameric calgranulin C (S100A12) binds to the receptor for advanced glycated end products (RAGE) using symmetric hydrophobic target-binding patches, *J. Biol. Chem.* 2007, *282*, 4218 – 4231.

[26] Brett, J., Schmidt, A.-M., Yan, S. D., Zou, S., *et al*., Survey of the distribution of a newly characterized receptor for advanced glycation end-products in tissues, *Am. J. Pathol.* 1993, *143*, 1699 – 1712.

[27] Thornalley, P. J., Glyoxalase I – structure, function and a critical role in the enzymatic defence against glycation, *Biochem. Soc. Trans.* 2003, *31*, 1343 – 1348.

[28] Bierhaus, A., Stoyanov, S., Haag, G. M., Konrade, I., *et al*., RAGE-deficiency reduces diabetes-associated impairment of glyoxalase-1 in neuronal cells, *Diabetes* 2006, *55*, A511.

[29] Soulis, T., Thallas, V., Toussef, S., Gilbert, R. E., *et al*., Advanced glycation end products and their receptors co-localise in rat organs susceptible to diabetic vascular injury, *Diabetologia* 1997, *40*, 619 – 628.

[30] Hammes, H. P., Hoerauf, H., Alt, A., Schleicher, E., *et al*., Ne(Carboxymethyl)lysine and the AGE receptor RAGE colocalize in age-rRelated macular degeneration, *Invest. Ophthalmol. Vis. Sci.* 1999, *40*, 1855 – 1859.

[31] Du, J., Zeng, J., Ou, X., Ren, X., *et al*., Methylglyoxal down-regulates Raf-1 protein through a ubiquitination-mediated mechanism, *Int. J. Biochem. Cell Biol.* 2006, *38*, 1084 – 1091.

[32] Cerami, A., Aging of proteins and nucleic acids: What is the role of glucose? *TIBS* 1986, *11*, 311 – 314.

