IMARS Highlights

Research Commentaries for Members of The International Maillard Reaction Society

A Non-profit Research and Education Organization in Biomedicine and Food Science: http://imars.case.edu

Volume 2, Number 1 **January 15, 2007**

CONTENTS

What's cooking? Notes from the Editor's pot

Greetings and Happy New Year to IMARS members as we begin this second year of IMARS *HIGHLIGHTS*.

We begin the New Year on a very positive note. Below are the names of the winners of the young investigator writing competition, along with the titles of their essays. The essays were judged independently and anonymously. In the Biomedical Sciences category there was also a consensus that an honorable mention should be awarded. The Editorial Board and Executive Council of IMARS congratulate the winners on their efforts. The essays will be published in the March issue of IMARS *HIGHLIGHTS*.

FOOD SCIENCES

Suji George, University of Mumbai

Can non-enzymatic glycation sites in proteins be predicted?

BIOMEDICAL SCIENCES

Ashay Bhatwadekar, Queen's University Belfast

A Puzzle between PPARγ and Advanced Glycation

Honorable Mention

Ina Nemet, Case Western Reserve University, Cleveland, and Rudjer Boskovic Institute, Zagreb

Thiamine and Benfotiamine as potential scavengers of methylglyoxal

Correspondence concerning the topics covered in this or previous issues can be sent to the editor at the address below.

Susan Thorpe
thorpe@mail.chem.sc.edu

Maillard Reaction to reduce food allergy: weak and contrasting evidence up to now.

by Vincenzo Fogliano

Allergic reactions to foods are a widespread and emerging phenomenon. They affect people of all ages, ethnicities and geographic regions. In only a few cases are allergic reactions life-threatening but many subjects suffer chronic disability because of them.

It is easy to imagine that the structural changes caused by the Maillard Reaction (MR) on proteins have the potential to modify the specific IgE-binding ability of many allergens. Particularly when the allergenic peptide contains lysine or arginine residues binding with a carbohydrate moiety could profoundly modify the protein-protein interaction which is the initial event in the allergic cascade.

A recent paper by Nakamura and coworkers (1) published in the Journal of Agricultural and Food Chemistry demonstrates that the Maillard Reaction of squid tropomyosin with ribose reduced the allergenicity of this protein. Similarly, a previous paper by Gruber and coworkers (2) reported that incubation of the major cherry allergen Pru av 1 with glucose and ribose reduce IgE-binding activity and, even more pronounced effects were observed when the protein was reacted with glyceraldehyde and glyoxal. On the other hand, it has been shown that on scallop tropomyosin sugar linked through the MR caused an increase in allergenicity (3) and that the main peanut allergen proteins, namely Ara h1 and Ara h 2, increased their allergenicity both with roasting or with the Maillard reaction with glucose (4).

The findings of these preliminary studies clearly indicates that there is not a direct way to reduce allergenicity by MR. This is not surprising considering that the protein sites undergoing the MR are not specific and many lysine and arginine residues are simultaneously modified. It is also true that the extensive protein cross-linking, occurring in many cases when a protein solution is incubated with very reactive carbohydrates, could mask the allergenic epitopes. However, the aggregation could also generate high molecular weight polymer which can be much more immunogenic than the single polypeptides.

In some proteins preferential sites of reaction with carbohydrates have been identified (5,6), thus suggesting that there is the possibility to control the MR limiting the protein derivatization at these sites. This means that for some allergens, where the epitopes responsible for the IgE-binding are known, it may be possible to address the whole process to specifically modify these sites. In this framework the MR with different carbohydrate could by a potential tool to reduce protein allergenicity.

References

1. Nakamura A, Sasaki F, Watanabe K, Ojima T, Ahn DH, Saeki H. Changes in Allergenicity and Digestibility of Squid Tropomyosin during the Maillard Reaction with Ribose (2006) *J Agric Food Chem.* 54:9529-9534.

2. Gruber P, Vieths S, Wangorsch A, Nerkamp J, Hoffman T (2004) Maillard reaction and enzymatic browning affect the allergenicity of Pru av 1, the major allergen from cherry (*Prunus aVium). J. Agric. Food Chem.* 52:4002-4007.

3. Nakamura A, Watanabe K, Ojima T, Ahn DH,m Saeki H (2005) Effect of Maillard reaction on allergenicity of scallop tropomyosin. *J. Agric. Food Chem.* 53:7559-7564.

(4) Maleki, SJ, Chung SY, Champagne ET, Raufman JP (2000) The effect of roasting on the allergenic properties of peanut proteins. *J. Allergy Clin. Immunol.* 106:763-768.

(5) Fogliano V, Monti SM, Visconti A, Randazzo G, Facchiano AM, Colonna G, Ritieni A (1998) Identification of a beta-lactoglobulin lactosylation site. *Biochim Biophys Acta* 1388:295-304.

(6) Brock JW, Hinton DJ, Cotham WE, Metz TO, Thorpe SR, Baynes JW, Ames JM (2003) Proteomic analysis of the site specificity of glycation and carboxymethylation of ribonuclease. *J Proteome Res*. 2:506-13.

Sensory neurons respond to AGEs with oxidative stress and apoptosis: a new insight on diabetic neuropathy?

By Alejandro Gugliucci

Diabetic neuropathy (DN) is a main diabetic complication shown by the DCCT and the UKPDS to respond to tight glycemic control. It is a life-threatening condition involving both peripheral and autonomic nerves which results, among other manifestations, in loss of sensation in lower limbs that contribute to foot ulcers, as well as impotence and gastrointestinal dysfunction (1-2). The polyol pathway, enhanced oxidative stress and advanced glycation end products (AGEs) are among the factors implicated in the pathogenesis of diabetic neuropathy (2-5). Their effects are possibly exerted via direct nerve tissue damage or mediated by endothelial injury or vascular dysfunction.

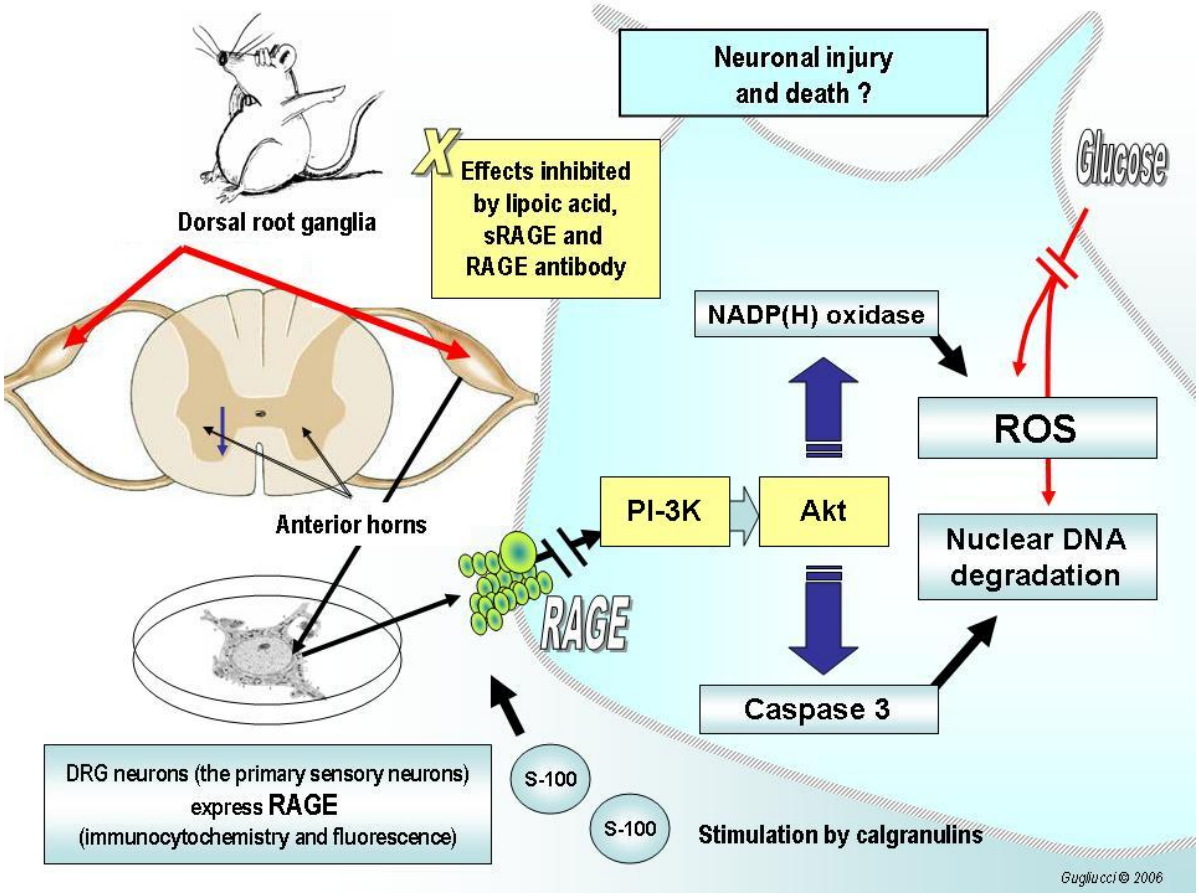

Figure. Sensory neurons (dorsal root ganglia, DRG) express RAGE and its activation leads to oxidative stress and apoptosis, via Akt phosphorylation and increased phosphoinositide 3-kinase activity *(the scheme is this author's interpretation of the paper)*

The dorsal root ganglia (DRG) primary sensory neuron is the key cell in the sensory pathway. However, most of the research in the area had so far concentrated on axonal degeneration, or on the receptor for AGE (RAGE) expressed in endothelial and Schwann cells

(2-5). Interactions between AGEs and RAGE would facilitate endoneural vascular dysfunction, leading to microangiopathy in the peripheral nerve. The attention turned only recently to DRG, it was found that a marked degeneration of these neurons is present in DN, and that their mitochondria are particularly affected. DRG mitochondria are clearly susceptible since they are the site of reactive oxygen species production in the hyperglycemic neuron (6). These findings paved the way for the recent investigation by Andrea Vincent et al, who demonstrate the role or RAGE in DRG apoptosis (7).

The authors used rat DRG neurons in primary cultures. As illustrated in our Figure, they first demonstrate the presence of RAGE in these neurons and its activation by known ligands such as S-100 calgranulins. In a series of elegant experiments, they started dissecting the cell signaling mechanisms that produce apoptosis and oxidative stress as endpoint corollaries of the RAGE activation. As summarized in our Figure, they present evidence suggesting DRG respond to RAGE occupation mainly through Akt phosphorylation and increased phosphoinositide 3-kinase (PI-3K) activity. This cascade ultimately results in NADP(H) oxidase activation and oxidative stress, as well as caspase 3 activation and DNA damage. The serine/threonine protein kinase Akt/Protein kinase B is the cellular homologue of the viral oncogene v-Akt. Several receptors induce the production of second messengers that activate PI-3K). Akt is located downstream of PI-3K. The effects are blocked by sRAGE (soluble fragment of the receptor), RAGE antibodies and the antioxidant alpha lipoic acid. In the same model, glucose also produces oxidative stress and DNA damage, which is inhibited only by the latter, showing therefore at least 2 different damage pathways are functioning in parallel. Judicious use of "extracelular signal-regulated kinase" (ERK) as well as PI-3K inhibitors, allows the authors to strengthen their conclusion.

The molecular mechanisms of AGE-RAGE interactions in the development of diabetic neuropathy still remain speculative, but this article gives a new twist for further exploration by showing, for the first time a direct role of the RAGE axis on sensory neurons leading to oxidative stress and possibly to degeneration. Diabetic neuropathy imposes a considerable burden on a patient's quality of life and the health-care system (1,4). Despite the prevalence and severity of DN there are no effective treatments. This work also points to putative palliative therapeutic alternatives by demonstrating a clear blocking effect for the soluble RAGE and alpha lipoic acid.

References

1. Sullivan KA, Feldman EL (2005) New developments in diabetic neuropathy. *Curr Opin Neurol* 18**:**586-590.

2. Sima AA (2006) Pathological mechanisms involved in diabetic neuropathy: can we slow the process? *Curr Opin Investig Drugs* 7:324-337.

3. Wada R, Yagihashi S (2005), Role of advanced glycation end products and their receptors in development of diabetic neuropathy, *Ann N Y Acad Sci* 1043:598-604.

4. Bierhaus A, Haslbeck KM, Humpert PM, Liliensiek B, Dehmer T, Morcos M, Sayed AA, Andrassy M, Schiekofer S, Schneider JG *et al.*, (2004) Loss of pain perception in diabetes is dependent on a receptor of the immunoglobulin superfamily. *J Clin Invest* 114:1741-1751.

5. Pop-Busui R, Sima A, Stevens M (2006) Diabetic neuropathy and oxidative stress. *Diabetes Metab Res Rev* 22:257-273.

6. Leinninger GM, Backus, Sastry AM, Yi YB, Wang CW, Feldman EL (2006) Mitochondria in DRG neurons undergo hyperglycemic mediated injury through Bim, Bax and the fission protein Drp1, *Neurobiol Dis* 23:11-22.

7. Vincent AM, Perrone L, Sullivan KA, Backus C, Sastry CM, Lastoskie C, Feldman EL (2006) RAGE Activation Injures Primary Sensory Neurons via Oxidative Stress, *Endocrinology* doi: 10.1210/en.2006-0073

Do green tea polyphenols inhibit protein glycation?

By Teruo Miyazawa

Green tea, derived from the leaves of *Camellia sinensis* L, is one of the most popular drinks in the world. Recent biological studies have succeeded in demonstrating that green tea has numerous positive benefits on human health. These properties of green tea can be ascribed to tea leaf constituents, especially, polyphenolic compounds. The major green tea polyphenols are (-)-epigallocatechin-3-gallate (EGCG), (-)-epicatechin-3-gallate (ECG), (-)-gallocatechin-3-gallate (GCG), (-)-epigallocatechin (EGC), (-)-epicatechin (EC), and (+)-catechin. A typical cup of green tea drink contains between 100-200 mg catechins, of which 40-80 mg is EGCG. Although there is much interest in the bioactivity of green tea polyphenols, the anti-glycative function of green tea polyphenols has been little characterized.

According to a recent paper, Babu et al. (1) have investigated the anti-glycative effect of green tea polyphenols on streptozotocin-induced diabetic rats. After oral supplementation of green tea polyphenol extract (300 mg/kg/day) to diabetic rats for 4 weeks, they found that the extent of protein glycation and the concentrations of lipid peroxides in the heart were decreased.

Vinson and Zhang (2) have investigated the effect of green tea on cataracts in streptozotocin-induced diabetic rats. Plasma and lens lipid thiobarbituric acid-reactive substances and protein glycation were reduced after supplementation of green tea for 3 months.

These reports (1,2) provide in vivo evidence of green tea polyphenols as anti-glycative agents, but the detailed molecular mechanisms are unknown. Further study is needed to evaluate the anti-glycative effect of green tea polyphenols in diabetic condition.

References

1. Babu PV, Sabitha KE, Shyamaladevi CS (2006) Green tea impedes dyslipidemia, lipid peroxidation, protein glycation and ameliorates Ca^{2+}-ATPase and Na^{+}/K^{+}-ATPase activity in the heart of streptozotocin-diabetic rats. *Chem. Biol. Interact.* 25:157-164

2. Vinson JA, Zhang J (2005) Black and green teas equally inhibit diabetic cataracts in a streptozotocin-induced rat model of diabetes. *J. Agric. Food. Chem.* 53:3710-3713

Red wine antioxidants and heterocyclic amine formation: new evidence in the Maillard Reaction-antioxidant relationship

by Vincenzo Fogliano

The presence of mutagenic Heterocyclic Amines (HAs) in foods depends on many factors such as cooking method, time and temperature, the presence of relative amounts of precursors, enhancers and inhibitors, lipids, antioxidants and water content (1). In particular, supplementation with antioxidants is considered to be an effective measure to reduce Has production because of the hypothetical free radical pathways leading to their formation (2). Single antioxidant compounds and complex mixtures of antioxidants have been demonstrated to be effective in suppressing HAs formation. It is likely that this effect is the final result of a variety of actions interfering at various steps of HAs formation. In fact, antioxidants can act as inhibitors along the different pathways of the reaction, preventing mutagen formation, by acting both as free radical quenchers and free radical scavengers.

The experimental work carried out using food-extracts or whole-foods as sources of antioxidants is a potential easy-to-use tool to reduce the HAs dietary intake. Pre-treatment with phenolic antioxidants derived from tea and olive oil results in a marked reduction of HA formation. Therefore cooking meat and fish together with fats, spice or antioxidants containing seasoning may be useful to lessen the levels of carcinogenic HAs produced. In this respect Mediterranean way of cooking can be regarded as particularly suitable, as it is based on the use of sauces and spices rich in antioxidants.

Some years ago it was shown that burgers fried in extra virgin olive oils having high concentrations of phenolic compounds have between 20 and 50% less HAs; the effect is lost when refined olive oil is used and strongly reduced after one year of storage as a consequence of phenol hydrolysis during extra virgin olive oil storage [3]. In a recent paper from the Skog group (4) red wine marinade, which is a typical ingredient in Mediterranean recipes for cooking meat, was assayed to verify the effect on HA formation during frying. Red wine marinade reduced the formation of some of the HAs during chicken breast frying. PhIP (2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine), with a reduction of up to 88%, was the most minimized amine, but the formation of Harman (1-methyl-9H-pyrido[3,4-b]indole) was enhanced.

The possibility of relating the nature and the concentration of the antioxidants used in the different experiments to the effect on HA formation represents a stimulating challenge for research in this field. The practical objective of reducing dietary HA exposure is combined with the need to understand the role of antioxidants in the development of the Maillard Reaction which is still a “grey box” for Maillard chemists. It is clear that to achieve this goal the complete chemical characterization of the system studied is a mandatory prerequisite. Unfortunately in this particular case the three red wines used have very similar total phenol concentration while the flavonoid and anthocyanin composition was not reported. Nonetheless, the study emphasizes the complex effects of the food antioxidants on the Maillard Reaction leading both to the formation or inhibition of HAs during cooking.

References

1. Skog K, Solyakov A, Arvidsson P, Jagerstad M (1998) Analysis of nonpolar heterocyclic amines in cooked foods and meat extracts using gas chromatography-mass spectrometry. *J Chromatogr A* 803:227-33.

2. Oguri A, Suda M, Totsuka Y, Sugimura T, Wakabayashi K (1998) Inhibitory effects of antioxidants on formation of heterocyclic amines. *Mutat. Res.* 402: 237-45.

3. Persson E, Graziani G, Ferracane R, Fogliano V, Skog K (2003) Influence of antioxidants in virgin olive oil on the formation of heterocyclic amines in fried beefburgers. *Food Chem Toxicol* 41:1587-97.

4. Busquets R, Puignou L, Galceran MT, Skog K (2006). Effect of Red Wine Marinades on the Formation of Heterocyclic Amines in Fried Chicken Breast *J. Agric. Food Chem* 54, 8376-8384

Effective blocking of dietary Maillard products absorption: a therapeutic novelty for renal failure?

By Alejandro Gugliucci

Circulating Maillard products are extremely high in end stage renal disease (ESRD) patients, since the kidney plays a key role in their elimination by filtration, reabsorption and metabolism (1-3). They have been linked to the accelerated atherogenesis that characterizes this phase of renal failure (3-5). Due to their impaired excretion, retention of absorbed food Maillard products is particularly striking in ESRD patients (1,3,6). Avoiding foods with browning products has been advocated as one palliative measure in these patients (6). A less stringent or more pleasant approach would be to be able to enjoy the organoleptic properties of Maillard delicacies while avoiding their absorption. In a recent article, Seiji Ueda *et al.* report the results of a clinical trial that show the benefit of AST-120 in curtailing the deleterious effects of absorbed AGEs in ESRD patients (7).

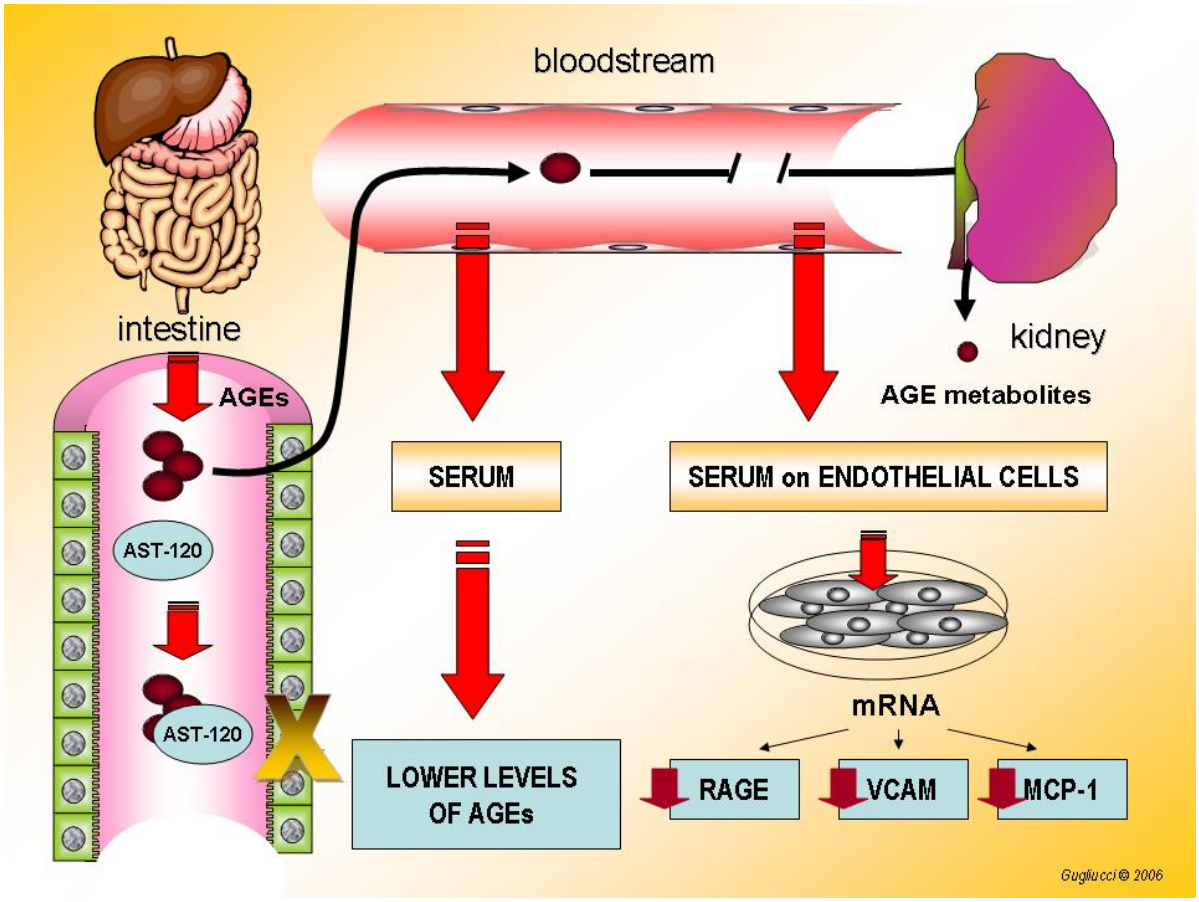

Figure. AST-120 blocks AGEs absorption and decreases circulating AGEs in renal failure patients *(the scheme is this author's interpretation of the paper)*

AST-120 has been used in Japan for several years as an alternative to charcoal-based compounds, to decrease absorption of indols and other molecules that are considered uremic toxins (8). The investigators hypothesized that AST-120 would also block AGEs absorption and

not only decrease circulating AGEs but their atherogenic effects as well. They conducted a clinical trial on non-diabetic patients in ESRD (n=10) and measured several parameters before and after 3 months of treatment (compared to a control subset of age and renal function-matched patients). As we illustrate in the Figure, AST-120 treatment resulted in a significant decrease (25-40%) in circulating AGEs (glucose-derived AGEs, glyceraldehyde-derived AGEs and CML). To demonstrate an antiatherogenc effect, the authors went on to incubate human umbilical vein endothelial cells in the presence of patient sera obtained before and after the treatment (see Figure). They measured the expression of three atherosclerosis-related genes: monocyte chemoattractant protein 1 (MCP-1); vascular adhesion molecule-1 (VCAM) and the receptor for AGE (RAGE). All three markers were decreased after treatment. Finally the authors showed in vitro direct proof of absorption of CML (an AGE) by AST-120.

Two limitations of the study are the low number of patients involved, and the use of immunological techniques to measure the AGEs. As previously reported in the Highlights by Miyata, there are inherent problems in AGE detection with antibodies. However, the comparison here is done with the patients as their own control, therefore the results are valid. Although more research needs to be done to definitely establish the former as an effective treatment to retard atherogenesis in ESRD patients, this article paves the way for that by showing, for the first time, a clear beneficial effect for AST-120 therapy on Maillard products and their effects. This adds a new, potentially important property to a drug that is already in use and may offer a tool for research on the AGE-hypothesis for the pathogenesis or accelerated atherosclerosis in renal failure.

References

1. Monnier VM, Sell DR, Nagaraj RH, Miyata S, Grandhee S, Odetti P, Ibrahim SA (1992) Maillard reaction-mediated molecular damage to extracellular matrix and other tissue proteins in diabetes, aging, and uremia. *Diabetes* 41 Suppl 2: 36-41.

2. Gugliucci A, Bendayan M (1996) Renal fate of circulating advanced glycated end products (AGE): evidence for reabsorption and catabolism of AGE-peptides by renal proximal tubular cells. *Diabetologia* 39:149-160.

3. Thornalley PJ (2005) Glycation free adduct accumulation in renal disease: the new AGE. *Pediatr Nephrol* 20:1515-1522.

4. Fort J (2005) Chronic renal failure: a cardiovascular risk factor, *Kidney Intl.* S25-29.

5. Makita Z, Yanagisawa K, Kuwajima S, Bucala R, Vlassara H, Koike T (1996) The role of advanced glycosylation end-products in the pathogenesis of atherosclerosis. *Nephrol Dial Transplant* 11 Suppl 5: 31-33.

6. Peppa M, Uribarri J, Cai W, Lu M, Vlassara H (2004) Glycoxidation and inflammation in renal failure patients. *Am J Kidney Dis* 43: 690-695.

7. Ueda S, Yamagishi S, Takeuchi M, Kohno K, Shibata R, Matsumoto Y, Kaneyuki U, Fujimura T, Hayashida A, Okuda S (2006) Oral adsorbent AST-120 decreases serum levels of AGEs in patients with chronic renal failure. *Mol Med* 12:180-184.

8. Niwa T, Nomura T, Sugiyama S, Miyazaki T, Tsukushi S, Tsutsui S (1997) The protein metabolite hypothesis, a model for the progression of renal failure: an oral adsorbent lowers indoxyl sulfate levels in undialyzed uremic patients. *Kidney Int Suppl* 62: S23-28.

Suppressed colonization of *Helicobacter pylori* by food protein-derived melanoidins

By Teruo Miyazawa

Helicobacter pylori which is the major cause of peptic ulcer and gastric cancer, infects half of the world population (1). The adhesion of *H. pylori* to the gastric mucosa is a critical first step in host colonization by *H. pylori*. Previously, it has been reported that acidic polysaccharides inhibit the adhesion of *H. pylori* urease to mucin (2). Hence, certain acidic polymers may have a similar effect in inhibiting the adhesion of *H. pylori* to gastric mucin.

Because melanoidins derived from the Maillard reaction are acidic polymers, Hiramoto et al. (3) recently studied the inhibitory activity of melanoidins on the adherence of *H. pylori* urease to gastric mucin. These authors found that food protein-derived melanoidins inhibited the urease-gastric mucin adhesion. In particular, melanoidin I prepared from casein and lactose suppressed colonization of *H. pylori* in mice.

Melanoidins are not only a source of the flavor and browning of heat-treated foods but also have a variety of physiological activities, such as anti-mutagenic and anti-oxidant functions. Generally, *H. pylori* eradication therapy involves administration of proton pump inhibitors along with antimicrobials. However, this reports show us the new possibility of melanoidins and the foods that contain them as therapeutic agents.

References

1. Taylor DN, Blaser MJ (1991) The epidemiology of *Helicobacter pylori* infection. *Epidemiol. Rev.* 13:42-59

2. Icatlo FC, Goshima H, Kimura N, Kodama Y (2000) Acid-dependent adherence of *Helicobacter pylori* urease to diverse polysaccharides. *Gastroenterol.* 119:358-367

3. Hiramoto S, Itoh K, Shizuuchi S, Kawachi Y, Morishita Y, Nagase M, Suzuki Y, Nobuta Y, Sudou Y, Nakamura O, Kagaya I, Goshima H, Kodama Y, Icatro FC, Koizumi W, Saigenji K, Miura S, Sugiyama T, Kimura N (2004) Melanoidin, a food protein-derived advanced maillard reaction product, suppresses *Helicobacter pylori* in vitro and in vivo. *Helicobacter* 9:429-435

INTERNATIONAL MAILLARD REACTION SOCIETY

Charting the future of carbonyl research in food and medicine

THANKS TO OUR GENEROUS SPONSORS

Nestlé

Good Food, Good Life

RenaScience

Bristol-Myers Squibb

PRESCRIPTIVES A DIVISION OF ESTÉE LAUDER COMPANIES

Firmenich

Kowa Kowa Company, Ltd.

L'ORÉAL