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Editorial comment

Here I am with the March issue of IMARS Highlights. In this issue I have included a special feature by Antonio Ceriello on metabolic memory and glycation. Emile Van Schaftingen and Maria Veiga-da-Cunha de Duve have raised very interesting questions about enzymatic deglycation. I would like to encourage the readers to write to me about their views on these articles. The selected articles by our usual contributing editors - Vincenzo Fogliano, and Alejandro Gugliucci are also included in this month's issue. I am sure readers will enjoy reading them especially Alejandro Gugliucci's article on "the need for a better short-term marker of average glycemia". I have also added an article by Sarah Larkin on "Glyoxalase 1 and multi-drug resistance in cancer chemotherapy" with implications for nucleotide glycation in mechanism of action of anticancer drugs. As always feedback from readers on these and other features in this issue are very welcome.

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“Metabolic Memory and Glycation”

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In the Epidemiology of Diabetes Interventions and Complications (EDIC) trial, a follow-up to the DCCT, patients on the standard treatment regimen during the DCCT still had a higher incidence of complications as compared to their counterparts receiving intensive therapy throughout the trial several years after switching to intensive therapy. Data from the United Kingdom Prospective Diabetes Study (UKPDS) appear to be consistent with this evidence. Specifically, people with lower fasting plasma glucose (FPG) values at the time of diagnosis had fewer vascular complications and fewer adverse clinical outcomes over time as compared to people with higher FPG values, despite similar rates of increasing glycaemia, suggesting that early metabolic control has enduring beneficial effects even in type 2 diabetes. Collectively, these observations support the concept that early glycaemic environment is remembered, and the authors of the DCCT/EDIC have referred to this phenomenon as “metabolic memory”.

Glycation of mitochondrial proteins, oxidative stress and “Metabolic Memory”: Mitochondrial overproduction of $\bullet\text{O}_2^-$ in hyperglycaemia has been suggested as the “*Unifying Hypothesis*” for the development of diabetic complications. Therefore, it is reasonable that mitochondria are also important players in propagating the “metabolic memory”. Chronic hyperglycaemia is thought to alter mitochondrial function through glycation of mitochondrial proteins. Levels of methylglyoxal (MGO), a highly reactive α -dicarbonyl by-product of glycolysis, are increased in diabetes. MGO readily reacts with arginine, lysine and sulfhydryl groups of proteins in addition to nucleic acids, inducing the formation of a variety of structurally identified AGEs, both in target cells and in the plasma. MGO has an inhibitory effect on mitochondrial respiration and MGO-induced modifications are targeted to specific mitochondrial proteins. These premises are important because a recent study, for the first time, has described a direct relationship between the formation of intracellular AGEs on mitochondrial proteins, the decline in mitochondrial function and the excess formation of reactive species. Therefore, mitochondrial respiratory chain proteins which underwent glycation were prone to produce more $\bullet\text{O}_2^-$, independently from the level of hyperglycaemia.

AGE formation is a prolonged phenomenon. In the DCCT, AGE formation was examined in 215 patients who underwent a skin biopsy 1 year before the close of the trial. Compared with conventional treatment, intensive treatment was associated with significantly lower levels of AGEs. Retinopathy, nephropathy and neuropathy outcomes were significantly associated with the levels of AGEs and increased levels of AGEs in the skin has been found to be significantly associated with the outcomes for microvascular complications in the EDIC. Furthermore, it is reasonable that AGEs may also explain the results regarding increased incidence of cardiovascular complications in the EDIC, considering that AGEs have been found associated with CVD even in non diabetic women.

What is really important is the clinical evidence that inclination of proteins, particularly collagen to be glycated is *independent* of the actual ambient glucose level. It has also been proposed that glycation of *extracellular* collagen may be a marker for glycation of *intracellular* proteins and a predictor of end-organ damage. While glycated HbA1c may be partially enzymatically deglycated, such a reaction has been not yet found for AGEs

incorporated into collagen. Therefore, it appears that collagen AGEs formation is an irreversible phenomenon.

The glycation of mitochondrial proteins may be a *contributing* explanation for the phenomenon of the “metabolic memory”. Glycated mitochondria overproducing free radicals, independently from the actual glycaemia, can lead to a catastrophic cycle of mitochondrial DNA (mtDNA) damage as well as functional decline, further oxygen radical generation, and cellular injury, maintaining the activation of the pathways involved in the pathogenesis of diabetic complications. Furthermore, mitochondrial proteins become damaged or posttranslationally modified as a consequence of a major change in a cell's redox status. This, on the other hands, may also affect mitochondrially destined proteins, which imported into the mitochondrial outer membrane, inner membrane or matrix space via specific import machinery transport components. Finally, oxidative stress may alter mitochondrial protein expression and turnover, possibly leading up to a perpetuation of the phenomenon.

In other words, it may be postulated that in the “metabolic memory” the cascade of the events is the same as that proposed by Brownlee - the source of $\bullet\text{O}_2^-$ is still the mitochondria - but that in addition the production of reactive species is unrelated to the presence of hyperglycaemia, depending by the level of glycation of mitochondrial proteins.

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Krebs cycle overflow and protein modification in diabetes: another plausible link to complications

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A key enzyme in glycolysis, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has been made a central player in the pathways that connect hyperglycemia with diabetic complications. In Brownlee's hypothesis, modification of this enzyme and the consequent triose overflow, fit in an explanation for increased glycation, polyol pathway, protein kinase C activation and hexosamine pathway (1). Inhibition of GAPDH activity occurs by excessive activity of poly(ADP-ribose) polymerase caused by mitochondrial ROS. GAPDH function is very dependent on cysteine, which also makes it vulnerable to direct oxidation and or Michael's addition of unsaturated compounds. One such metabolite is fumarate, a classic Krebs cycle intermediate (2). The same block in electron transport that explains oxygen radical generation, results in accumulation of fumarate (as well as other Krebs cycle metabolites). It can easily move out of the mitochondria down a concentration gradient through di-carboxylate transporters.

Recent work by Blatnik et al, (3) has tied together both processes and offered new insights that keep GAPDH on the spotlight while suggesting an even more powerful mechanism to explain its common link role between hyperglycemia and diabetic complications. Previously, work from Thorpe and Baynes's lab had shown that 2-succinyl)cysteine (2SC) is formed by a Michael addition reaction of the Krebs cycle intermediate, fumarate, with cysteine residues in protein (2,4). In this latest work the authors investigated the specific role of fumarate in chemical modification and inhibition of GAPDH, in vitro and in muscle from streptozotocin-induced diabetic rats (3).

Using analysis of tryptic peptides by HPLC- quadrupole/TOF spectrometry the authors determined the sites of 2SC formation. Fumarate and 2SC in muscle of control and streptozotocin-induced diabetic rats were measured by GC/MS or LC/MS, respectively. 2SC was found, both in vitro and in vivo at both active-site Cys-149 and nucleophilic Cys-244. Inactivation of GAPDH by fumarate in vitro correlated with formation of 2SC. In diabetic compared rats, fumarate and 2SC increased approximately fivefold and a 25% decrease in GAPDH specific activity was apparent.

Although muscle is not a site of the major diabetic complications, it offers a convenient, practical model for diabetic metabolism. This work elegantly demonstrates that fumarate contributes to inactivation of GAPDH in diabetes. Adduct formation by Michael's addition of GAPDH and other proteins by fumarate may be a factor in the metabolic changes causing the development of diabetes complications. Fumarate adduct with protein, 2SC, may become a handy biomarker of mitochondrial stress in diabetes.

1. Brownlee M: The pathobiology of diabetic complications: a unifying mechanism. *Diabetes* 54:1615-1625, 2005
2. Alderson NL, Wang Y, Blatnik M, Frizzell N, Walla MD, Lyons TJ, Alt N, Carson JA, Nagai R, Thorpe SR, Baynes JW: S-(2-Succinyl)cysteine: a novel chemical modification of tissue proteins by a Krebs cycle intermediate. *Arch Biochem Biophys* 450:1-8, 2006

3. Blatnik M, Frizzell N, Thorpe SR, Baynes JW: Inactivation of glyceraldehyde-3-phosphate dehydrogenase by fumarate in diabetes: formation of S-(2-succinyl)cysteine, a novel chemical modification of protein and possible biomarker of mitochondrial stress. *Diabetes* 57:41-49, 2008
4. Alt N, Carson JA, Alderson NL, Wang Y, Nagai R, Henle T, Thorpe SR, Baynes JW: Chemical modification of muscle protein in diabetes. *Arch Biochem Biophys* 425:200-206, 2004

Enzymatic Deglycation

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Nature has been confronted with an important dilemma: that of making molecules that are stable, and if extremely stable not reactive at all, or molecules that can be transformed easily, and have therefore some degree of chemical instability. The compromise is somewhere in between: most biomolecules are rather stable, though not completely. If this instability creates a problem (as e.g. DNA instability), enzymatic repair can be a solution.

The formation of Amadori products from glucose is the perfect illustration of the reactivity of free sugars. It became only recently clear that the formation of Amadori products on proteins can be enzymatically reversed. This repair reaction requires energy (ATP), is partial and takes place only inside cells.

As other known protein repair mechanisms, enzymatic “deglycation” was serendipitously found. By running NMR spectra on tissue extracts, Ben Szwegold and coworkers identified almost 20 years ago an unexpected phosphate ester, fructose 3-phosphate, in the lenses and in the erythrocytes of different species [1,2]. The discovery of fructose 3-phosphate was surprising as there was no indication that it belonged to any known biochemical pathway. However, further studies by Ben Szwegold’s group on erythrocytes suggested that there was an enzyme that was able to phosphorylate fructose on its third carbon, yet this enzyme had an extremely low affinity for fructose [3]: its K_m was about 40-fold higher than the fructose concentration found in blood after a fructose-rich meal. Furthermore the V_{max} of “fructose 3-kinase” was orders of magnitude lower than the V_{max} of fructokinase, the enzyme metabolizing fructose in liver. Finally, fructose 3-phosphate was spontaneously degraded to 3-deoxyglucosone (a compound with bad reputation) and inorganic phosphate. It was obvious that this enzyme had some other role to play than to phosphorylate fructose to a potentially toxic product.

Our own lab has long worked on phosphate esters of fructose (fructose 2,6-bisphosphate, fructose 1-phosphate), which explains how our interest in this problem arose. By 1997, we started working on the idea that the real substrates for “fructose 3-kinase” were fructosamines, without knowing that Ben Szwegold was formulating the same idea. The only commercially available fructosamine was deoxymorpholinofructose, which luckily proved to be an excellent substrate for “fructose 3-kinase”. Consequently, “fructose 3-kinase” was since rechristened “fructosamine 3-kinase” or “FN3K”[4]. The availability of a good substrate facilitated the purification of FN3K from human erythrocytes. The purification and characterization of FN3K and the cloning of its cDNA could soon be reported [5], quickly followed by similar findings made by Szwegold and coworkers [6]. Of interest was the finding that FN3K was as good at phosphorylating *protein-bound* as well as low-molecular-weight fructosamines (such as fructose-?-lysine).

As for the physiological function of FN3K, the obvious possibility was that the phosphorylation of fructosamines on their third carbon could initiate a deglycation process. We actively searched for an enzyme that could metabolize fructosamine 3-phosphates but failed to find one. Szwegold and coworkers showed that fructosamine 3-phosphates are unstable, decomposing to deoxyglucosone, inorganic phosphate and a free amine, with a half-life of 8 hours [6]. *Ex vivo* experiments on human erythrocytes incubated with 200 mM

glucose showed that the accumulation of glycated hemoglobin was faster in the presence of deoxymorpholinofructose (used to inhibit FN3K competitively) than in its absence [7], indicating that FN3K partially antagonizes glycation. FN3K is not able to prevent glycation completely because it is unable to phosphorylate *all* fructosamines present on a protein: the fructosamines on the N-terminal valines of the β -chain of hemoglobin and those on some less accessible lysine residues are virtually not phosphorylated by FN3K, whereas other more exposed lysine-bound fructosamines are excellent substrates [8]. The old finding that glycation of hemoglobin *in vitro* leads to the formation of fructosamines on lysines that are apparently not glycated *in vivo* [9] is explained by deglycation taking place in the latter situation.

The creation of a mouse knockout model of FN3K [10] brought the final proof that FN3K is a deglycating enzyme, but it also opened new questions on the role of this type of protein repair. Deglycation deficient mice have about 1.5 to 2.5-fold more glycated cytosolic proteins than control mice. Since there is no difference in their blood glucose level and in their concentration of extracellular fructosamines, this proves that indeed FN3K serves to remove fructosamines. The proportion of glycated proteins can rise to at least 30% for a given protein in FN3K deficient diabetic mice. Yet this high level of glycation is not accompanied by an obvious increase in mortality or morbidity, at least in our experimental conditions. Does this mean that deglycation has no physiological significance? Probably not: FN3K is a conserved enzyme in mammalian and avian species, which suggests that having FN3K must offer a selective advantage. Would protein lose activity if they are not deglycated? Probably only minimally, since the glycation extent of proteins in normoglycemic FN3K deficient mice amounts to only \sim 5-10 % and this amount is distributed on several lysines. Would fructosamines interfere with the function of a protein, e.g. by allowing them to be recognized by intracellular lectins? Would “non-deglycated” proteins be more rapidly degraded? Does anything happen to intracellular fructosamines? At this stage, we simply don’t know.

Besides FN3K, there is a second enzyme, sharing 65 % sequence identity with FN3K, and called FN3K-related protein. This enzyme does not act on fructosamines, but it does phosphorylate other Amadori products: ribulosamines and erythrulosamines [11]. Intriguingly, the tissue distribution of this enzyme is wider than that of FN3K, it is more active than the latter and the homologues of FN3K/FN3K-RP that are found in fishes, plants and bacteria also act on ribulosamines and erythrulosamines but *never* on fructosamines [12-14]. This could imply that phosphorylation of ribulosamines and/or erythrulosamines is a more ancient function than phosphorylation of fructosamines. Ribulosamines and erythrulosamines are most probably formed from ribose 5-phosphate and erythrose 4-phosphate, which are extremely potent glycating agents. Accordingly, we recently described that low-molecular-weight “tyrosine-phosphatase” is better at converting ribulosamine 5-phosphates to ribulosamines than at dephosphorylating tyrosine-phosphates! [15] Similarly, another phosphatase (Magnesium dependent phosphatase-1, MDP-1) is extremely good at dephosphorylating fructosamine 6-phosphates [16]. These findings raise additional questions on the function of deglycation. Is it directed against protein-bound or low-molecular-weight ketoamines? Is the formation of ribulosamines and/or erythrulosamines a spontaneous or an enzyme-catalyzed process? Is there any other unknown molecule, resembling ketoamines, which would be the ‘true’ substrate for the deglycating enzymes?

REFERENCES

- [1] Szwergold *et al.* Science (1990) 247:451-4.
- [2] Petersen *et al.* J Biol Chem (1990) 265:17424-7.

- [3] Petersen *et al.* Biochem J (1992) 284:363-6.
- [4] Delpierre *et al.* Biochem J (2000) 352:835-9.
- [5] Delpierre *et al.* Diabetes (2000) 49:1627-34.
- [6] Szwergold *et al.* Diabetes (2001) 50:2139-47.
- [7] Delpierre *et al.* (2002) Biochem J 365:801-8.
- [8] Delpierre *et al.* (2004) J Biol Chem. 279:27613-20.
- [9] Shapiro R *et al.*(1980) J Biol Chem 255:3120-7.
- [10] Veiga-da-Cunha *et al.* (2006) Biochem J 399:257-64.
- [11] Collard *et al.* (2003) Diabetes 52:2888-95.
- [12] Delplanque *et al.* (2004) J Biol Chem 279:46606-13.
- [13] Fortpied *et al.* (2005) Biochem J. 388:795-802.
- [14] Gemayel *et al.* (2007) FEBS J 274:4360-74.
- [15] Fortpied *et al.* (2007) Biochem J 406:139-45.
- [16] Fortpied *et al.* (2006) J Biol Chem 281:18378-85.

The ACCORD discord: only a media misinformation campaign or the need for a better short-term marker of average glycemia?

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Maintaining glycated hemoglobin (HbA1c) levels at less than 6%, means different things for people with normal glycemic excursions, than for high-risk type 2 diabetic patients. Given the long time of response of this marker, HbA1c levels of less than 6% in diabetic patients may hide too frequent borderline or overt hypoglycemias. Although this is still not clear, it might have been one of the underlying problems in the recently publicized NIH-sponsored ACCORD study (1-2).

It is well documented that patients with type 2 diabetes mellitus die of cardiovascular disease (CVD) at rates 2-4 times higher than patients without diabetes but with similar demographic characteristics (2). In the 90's the DCCT (for type 1 diabetic patients) and the UKPDS (for type 2 diabetic patients) have shown that tight glycemic control reduces dramatically the incidence or progression of microangiopathy. There is an independent progressive epidemiologic relation between glycemia and CVD events; however, whether lowering glucose levels with currently available therapies can reduce CVD events remains unknown. The Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial was designed (in one of its arms) to answer this question in high-risk patients with type 2 diabetes mellitus (1-2). More than 10,000 patients with type 2 diabetes and other CVD risk factors or CVD were randomly allocated to intensive glycemic control, targeting a glycosylated hemoglobin (HbA1c) level <6%, or standard glycemic control, targeting an HbA1c level of 7.0%-7.9%.

Most patients with type 2 diabetes mellitus develop CVD, with significant loss of life expectancy. And the impact of the microvascular complications and its relationship with glycemic control is an established fact. But we have all witnessed the media frenzy when an unexpected increase in deaths in the intense treatment arm precipitated a stop of this part of the study and threatened to jeopardize the efforts that have been made in the past 15 years to educate the patient population and the public in general. Although similar results did not come out of the ADVANCE study (3), an explanation for the ACCORD findings has to be found, and of course it would not be simplistic.

One possible inroad to delve in is the treatment goal and the marker employed. The glycemia trial was testing the hypothesis that a therapeutic strategy that targets a HbA1c level of <6.0% will reduce the rate of CVD events more than a strategy that targets an HbA1c level of 7.0%-7.9%. The primary outcome measure for this (and the other 2) research questions is the first occurrence of a major CVD event, specifically nonfatal myocardial infarction, nonfatal stroke, or cardiovascular death (1-2).

All participants in the intensive glycemic control group were started on up to 2 classes of agents. Doses were intensified or a new medication class was added every month if HbA1c levels were > or = 6% or if >50% of pre or postprandial capillary glycemia >5.6 mmol/L or >7.8 mmol/L, respectively (1-2). Although ACCORD focused on the levels of glycemia achieved using a variety of strategies, the main endpoints were a couple of glycemia (pre and post prandial) and the target was the HbA1c value. It is plausible to think that a shorter term marker between glycemia and HbA1c could have provided valuable additional information and a better metabolic control.

One such marker is glycated albumin, which had fallen out of favor, due mainly to a bad reputation when it was measured essentially by the fructosamine test (4). The half-life of albumin being approximately 3 weeks, a sensitive and specific assay for it should, a priori, provide glycemic averages estimation in the period of 10-30 days. In fact, several new investigations are evaluating new tests for glycated albumin and are re-examining the clinical performance of this marker in different patient populations (5-9). The issue has not been resolved; however, this represents a good trend toward revalorization of an old marker with new technology that could give us also a new tool for much needed clinical trials.

1. Buse JB, Bigger JT, Byington RP, Cooper LS, Cushman WC, Friedewald WT, Genuth S, Gerstein HC, Ginsberg HN, Goff DC, Jr., Grimm RH, Jr., Margolis KL, Probstfield JL, Simons-Morton DG, Sullivan MD: Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial: design and methods. *Am J Cardiol* 99:21i-33i, 2007
2. Goff DC, Jr., Gerstein HC, Ginsberg HN, Cushman WC, Margolis KL, Byington RP, Buse JB, Genuth S, Probstfield JL, Simons-Morton DG: Prevention of cardiovascular disease in persons with type 2 diabetes mellitus: current knowledge and rationale for the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial. *Am J Cardiol* 99:4i-20i, 2007
3. Bloch MJ, Basile J: Advance trial supports current recommendations for blood pressure goals and not the use of any specific combination of antihypertensive agents in patients with type 2 diabetes. *J Clin Hypertens (Greenwich)* 10:81-84, 2008
4. Armbruster DA: Fructosamine: structure, analysis, and clinical usefulness. *Clin Chem* 33:2153-2163, 1987
5. Takahashi S, Uchino H, Shimizu T, Kanazawa A, Tamura Y, Sakai K, Watada H, Hirose T, Kawamori R, Tanaka Y: Comparison of glycated albumin (GA) and glycated hemoglobin (HbA1c) in type 2 diabetic patients: usefulness of GA for evaluation of short-term changes in glycemic control. *Endocr J* 54:139-144, 2007
6. Inaba M, Okuno S, Kumeda Y, Yamada S, Imanishi Y, Tabata T, Okamura M, Okada S, Yamakawa T, Ishimura E, Nishizawa Y: Glycated albumin is a better glycemic indicator than glycated hemoglobin values in hemodialysis patients with diabetes: effect of anemia and erythropoietin injection. *J Am Soc Nephrol* 18:896-903, 2007
7. Paroni R, Ceriotti F, Galanello R, Battista Leoni G, Panico A, Scurati E, Paleari R, Chemello L, Quaino V, Scaldaferrì L, Lapolla A, Mosca A: Performance characteristics and clinical utility of an enzymatic method for the measurement of glycated albumin in plasma. *Clin Biochem* 40:1398-1405, 2007
8. Ahmed N, Dobler D, Dean M, Thornalley PJ: Peptide mapping identifies hotspot site of modification in human serum albumin by methylglyoxal involved in ligand binding and esterase activity. *J Biol Chem* 280:5724-5732, 2005
9. Klonoff DC: Serum fructosamine as a screening test for type 2 diabetes. *Diabetes Technol Ther* 2:537-539, 2000

The Effects of Maillard Reaction Products on Virulence Bacteria

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The antibacterial effects of MRPs is an old story, but there was a renewed interest on the possibility to use as antibacterial agents compounds which are naturally formed during thermal treatment of various foods. During the eighties the pioneering works of Einarsson and coworkers (1) demonstrated that different mixtures of monosaccharides and amino acids are able to inhibit bacterial growth on fish products. The following investigations showed that the inhibition is dependent by the type of MRPs and by the bacteria strain suggesting that the inhibitory effect is somehow specific (2).

A paper by Sheikh-Zeinoddin et al (3) demonstrated that heat treatment of bacterial media generating browning in the solution is accompanied by the suppression of a virulence gene expression in *Listeria monocytogenes*. Although quite indirectly, this finding suggested that it is possible to establish a one to one interaction between a specific MRP and single virulence gene. However, Ermolaeva and others (4) did not find an effect of MRPs on the amount of PrfA protein in *L. monocytogenes*, which regulates the levels of listerial virulence gene expression.

In a recent paper in J. Food Science by Kundinger (5) the influence on the virulence of *Salmonella* Typhimurium EE658 of three MR model systems made up with glucose reacted with histidine, asparagine and lysine respectively was tested. The interesting aspect of this paper was that the influence of the various MRPs was evaluated both on bacteria growth and on the expression of *hila* gene. *hila* is a transcriptional activator and it is a main regulator of the bacterial pathogenicity, its expression was evaluated both by real time PCR and by beta-galactosidase gene fusion assay.

Data shows that, while the addition of MRP did not affect the growth of the food borne pathogen, the histidine and arginine containing mixture dramatically increase *hila* expression when measured by beta-galactosidase gene fusion assay. Results were confirmed also by real time PCR where, due to a higher sensitivity of the assay, also lysine-containing MRPs showed a moderate induction of *hila* expression.

This preliminary evidence sheds some light on the potential of MRP serving as a bacterial virulence modulator. Beside these promising results new studies using single MRPs or at least partially purified fraction from MR mixture are necessary to obtain more significant and reproducible results.

References

Einarsson H, Snygg BG, Eriksson C. 1983. Inhibition of bacterial growth by Maillard reaction products. J Agric Food Chem 31:1043-7.

Marounek M, Březina P. 1993. Heat-induced formation of soluble Maillard reaction products and its influence on utilization of glucose by rumen bacteria. Arch Anim Nutr 43:45-51.

Sheikh-Zeinoddin M, Pehinec TM, Hill SE, Rees CED. 2000. Maillard reaction causes suppression of virulence gene expression in *Listeria monocytogenes*. Int J Food Microbiol 61:41-9.

Ermolaeva S, Novella S, Vega Y, Ripio M-T, Scotti M, Vázquez-Boland JA. 2004. Negative control of *Listeria monocytogenes* virulence genes by a diffusible autorepressor. Mol Microbiol 52:601-11.

Kundinger MM, Zabala-Díaz IB, Chalova VI, Ricke SC. Effects of Maillard reaction products on *hila* expression in *Salmonella typhimurium*. J Food Sci. 2008 73: 32-5.

Glyoxalase I in cancer chemotherapy and multidrug resistance

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Current available therapies are able to cure just over half of cancer patients, with surgery and radiotherapy being the most effective treatments. The success rate for chemotherapy is much lower. The statistics can be misleading, however, because chemotherapy is often used as a last resort after other therapies are unsuccessful. Much of the failure to cure cancer patients with chemotherapy can be attributed to multidrug resistance (MDR). Improving the efficacy of existing chemotherapeutic drugs by targeting mechanisms of MDR will lead to a better prognosis for patients who develop resistance and improve the survival rates for many of the cancers of highest mortality.

A much-overlooked target in the fight against drug resistance is the glyoxalase system. Several research groups have found a positive correlation between resistance of tumours to chemotherapy and both transcription and activity of glyoxalase I (Glo1) (1-4). In a study by Sakamoto and colleagues (5) Glo1 was quantified and its activity measured in 38 human solid tumour cell lines including cells from lung, colon, stomach, breast, ovarian, brain, renal and prostate cancers. Higher Glo1 activity was seen in all of the cells tested compared to normal tissue samples. The most marked difference was seen in NCI-H522 lung cancer cells, where Glo1 activity was 8.47 ± 0.10 units/mg protein compared to less than 0.5 units/mg protein seen in non-malignant lung epithelial cell controls. Furthermore, a positive correlation was observed between Glo1 activity and sensitivity to the cell-permeable Glo1 inhibitor S-p-bromobenzylcyclopentyl diester. In addition to this work, the same group performed a cDNA subtractive hybridisation to compare levels of mRNA in human monocytic leukaemia U937 cells and the variant UK711, which is resistant to apoptosis caused by antitumour agents (6). They found that Glo1 was selectively overexpressed in the UK711 drug-resistant cells. Glo1 expression was elevated in UK110 and K562/ADM (doxorubicin resistant) leukaemia cells. Furthermore, overexpression of Glo1 in Jurkat cells conferred resistance to apoptosis induced by both doxorubicin and etoposide. This evidence suggests a correlation between drug resistance and Glo1 activity across a broad range of tumours. This resistance can also be induced by overexpressing Glo1. Since tumours exhibit elevated Glo1 activity, Glo1 inhibitors could be used as cytotoxic agents that selectively target cancer cells.

As well as having their own inherent anticancer activity, dicarbonyls have been investigated as possible mediators of the anticancer activity of other chemotherapy drugs. Godbout *et al* showed that methylglyoxal (MG) potentiated the cytotoxic activity of cisplatin, an effect mediated by PKCd. When cells were treated with 3 $\mu\text{g/ml}$ cisplatin in conjunction with 5 μM MG, the percentage of apoptotic cells increased from $18 \pm 4\%$ for cisplatin treatment alone, to $35 \pm 7\%$ for cisplatin plus methylglyoxal (7).

The efficacy of Glo1 inhibitors as antitumour agents *in vivo* was investigated by Sharkey *et al*. They showed that a mechanism-based competitive inhibitor of Glo1 could inhibit the growth of solid tumours in mice with efficacy comparable to that of the commonly used anticancer drugs doxorubicin (murine B16 melanomas), cisplatin (human prostate PC3 tumours) and vincristine (human colon HT-29 tumours). The inhibitor appeared rapidly in tissues, including the tumours, with no visible adverse side effects (8).

These findings all suggest that Glo1 inhibitors have an important role to play in the treatment of cancer, both as anticancer agents in their own right and as potentiators of the antitumour activity of existing anticancer drugs. Used in conjunction with conventional treatments, Glo1 inhibitors have the potential to alleviate resistance and increase the therapeutic potential of a wide spectrum of anticancer drugs.

References

1. Davidson SD, Cherry J P, Choudhury M S, Tazaki H, Mallouh C and Konno S (1999) Glyoxalase I Activity in Human Prostate Cancer: a Potential Marker and Importance in Chemotherapy. *J Urol* **161**: pp 690-691.
2. Mearini E, Romani R, Mearini L, Antognelli C, Zucchi A, Baroni M, Porena M and Talesa V (2002) Differing Expression of Enzymes of the Glyoxalase System in Superficial and Invasive Bladder Carcinomas. *Eur J Cancer* **38**: pp 1964-1950.
3. Rulli A, Carli L, Romani R, Baroni T, Giovannini E, Rosi G and Talesa V (2001) Expression of Glyoxalase I and II in Normal and Breast Cancer Tissues. *Breast Cancer Res Treatment* **66**: pp 72.
4. Samadi AA, Fullerton S A, Tortorelis D G, Johnson G B, Davidson S D, Choudhury M S, Mallouh C, Tazaki H and Konno S (2001) Glyoxalase I Phenotype As a Potential Risk Factor for Prostate Carcinoma. *Urology* **57**: pp 183-187.
5. Sakamoto H, Mashima T, Sato S, Hashimoto Y, Yamori T and Tsuruo T (2001) Selective Activation of Apoptosis Program by S-p-Bromobenzylglutathione Cyclopentyl Diester in Glyoxalase I-Overexpressing Human Lung Cancer Cells. *Clin Cancer Res* **7**: pp 2513-2518.
6. Sakamoto H, Mashima T, Kizaki A, Dan S, Hashimoto Y, Naito M and Tsuruo T (2000) Glyoxalase I is involved in resistance of human leukemia cells to antitumor agent-induced apoptosis. *Blood* **95** (10) pp3214-3218.
7. Godbout J, Pesavento J, Hartman M, Manson S and Freund G (2002) *J. Biol. Chem.* **227** (4) pp 2554-2561.
8. Sharkey E, O'Neill H, Kavarana M, Wang H, Creighton D, Sentz D and Eiseman J (2000) Pharmacokinetics and antitumour properties in tumour-bearing mice of an enediol analogue inhibitor of glyoxalase I. *Cancer Chemother Pharmacol* **46** pp 156-166.

Highlights of the glycation literature January– February 2008

Breyer, V., M.Frischmann, C.Bidmon, A.Schemm, K.Schiebel, and M.Pischetsrieder.2008. Analysis and biological relevance of advanced glycation end-products of DNA in eukaryotic cells. *Eur J Biochem* 275:914-925.

Chetyrkin, S.V., W.Zhang, B.G.Hudson, A.S.Serianni, and P.A.Voziyan. 2008. Pyridoxamine Protects Proteins from Functional Damage by 3-Deoxyglucosone: Mechanism of Action of Pyridoxamine. *Biochemistry* 47:997-1006.

Peng, X., Z.Zheng, K.W.Cheng, F.Shan, G.X.Ren, F.Chen, and M.Wang. 2008. Inhibitory effect of mung bean extract and its constituents vitexin and isovitexin on the formation of advanced glycation endproducts. *Food Chemistry* 106:475-481.

Waanders, F., E.van den Berg, R.Nagai, I.van Veen, G.Navis, and H.van Goor. 2008. Renoprotective effects of the AGE-inhibitor pyridoxamine in experimental chronic allograft nephropathy in rats. *Nephrology Dialysis Transplantation* 23:518-524.

Yuyuan Li, Menashi A. Cohenford, Udayan Dutta, Joel A. Dain. 2008. The structural modification of DNA nucleosides by nonenzymatic glycation: an in vitro study based on the reactions of glyoxal and methylglyoxal with 2'-deoxyguanosine. *Anal Bioanal Chem* (2008) 390:679–688

Jakas, A; Horvat,S. 2008. Reactivity and oxidative potential of fructose and glucose in enkephalin-sugar model systems, *Amino Acids* (2008) 34: 329–332

PHD STUDENTSHIP OPPORTUNITY

A PhD studentship funded by the charity Diabetes UK is available in the Protein Damage and Systems Biology Research Group, Clinical Sciences Research Institute, University of Warwick, University Hospital, Coventry, U.K. It is available from October 2008 for 3 years. Citizens of the UK and European Union are eligible. The project is “Mechanism of increased renal clearance of thiamine in hyperglycaemia associated with diabetes”. The project will be supervised jointly by Dr Naila Rabbani and Professor Paul J Thornalley, with systems modelling assistance from Dr Hugo van den Berg (Systems Biology Centre, University of Warwick). The project involves characterisation of thiamine and thiamine metabolites and their transport in physiological tissues to construct a physiological-based pharmacokinetic model of thiamine metabolism. The disturbance of thiamine handling in diabetes and effect of high dose thiamine/Benfotiamine supplements will then be characterised and modelled. The project will provide training in translational medicine and will involve assay of thiamine metabolites and related enzyme and transporter activities and expression. For further details and application form, please contact Paul Thornalley, email: P.J.Thornalley@warwick.ac.uk












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