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LETTER TO THE EDITOR

Biomarkers of exposure to endogenous oxidative and aldehyde stress

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Abstract
We observed an unexpectedly strong association of three different endogenous aldehydes and noted that the association could be explained by multiple reactions in which oxidative stress increased the formation of endogenous aldehydes and endogenous aldehydes increased oxidative stress. These interactions make it reasonable to assess multiple exposures to endogenous oxidative and aldehyde stress with less specific measures such as advanced glycation end-products or protein carbonyls.

Keywords: Colorectal cancer, oxidative stress, fructose, endogenous aldehydes, advanced glycation end-products (AGEs), protein carbonyls

We recently observed an unexpectedly strong association of plasma levels of glyoxal, methylglyoxal and 4-hydroxynonenal in patients at possible risk for colorectal cancer (CRC). The plasma samples had been obtained in a pilot study of biomarkers of exposure designed to assess our hypothesis, that risk of CRC was associated with exposure to the toxic products formed from the interaction of oxidative stress with aldehydes resulting from excess energy substrates (Lee et al. 2009; McKeown-Eyssen et al., 2010). Herein, we describe the pilot study briefly, suggest mechanisms that could explain the strong associations, and identify more robust biomarkers of exposure to endogenous oxidative and aldehyde stress that could be used to assess such risks in the origin of CRC and other chronic diseases.

The pilot study was based on plasma samples collected from patients at the Gastroenterology Endoscopy Clinic, St. Michael's Hospital and University of Toronto (protocol approved by the institution's Research Ethics Board). Briefly, fasting plasma samples were collected from sequential consenting non-diabetic patients on their normal diets, with and without colonic polyps ($n = 21$ and 22, respectively). They were analyzed for insulin, triglyceride, lipopolysaccharide, lipopolysaccharide binding protein, soluble CD14, folate, homocysteine, phosphatidylcholine, phosphatidylethanolamine, plasma alanine aminotransferase and γ-glutamyltransferase, the advanced glycation end-product (AGE), carboxymethylysine, and plasma glyceraldehyde, glyoxal, methylglyoxal and 4-hydroxynonenal. For the plasma aldehydes, samples were analyzed by a modification of a previously described method (Luo et al. 1995; Mak et al. 2000) in which the PFB-oxime-TMS derivatives were analyzed by gas chromatography-electron ionization mass spectrometry (GC-EI-MS) to give control values for glyceraldehyde of 187 nmol/L (105–259 (95% CI)), glyoxal of 287 (236–351), methylglyoxal of 271 (218–320) and 4-hydroxynonenal of 10.5 (0.0–17.6).

The results of all the assays for the two groups were compared by the Wilcoxon Rank Sum Test and the t-test, after transformation as necessary. As anticipated because of the limited power of the pilot study, none of the differences between polyp and non-polyp patients' results reached statistical significance. Correlations of

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age, weight, height, BMI and all laboratory data were then determined after transformations (reciprocal, reciprocal and logarithmic) to correct for skewness in these data. They demonstrated a very strong association between concentration of glyoxal and methylglyoxal ($r = 0.920$, $p < 0.00001$), a high correlation between glyoxal and 4-hydroxynonenal ($r = 0.516$, $p < 0.00056$) and a strong association between methylglyoxal and 4-hydroxynonenal ($r = 0.468$, $p = 0.0021$). The correlations were evident in both the case and the control series. Glyoxal, methylglyoxal and 4-hydroxynonenal were not correlated with glyceraldehyde, none exceeding a correlation of greater than 0.11.

The mechanisms responsible for the close association of glyoxal, methylglyoxal and 4-hydroxynonenal was surprising as these compounds are thought to be formed in cells in quite different ways: glyoxal is derived mostly from the autoxidative degradation of nucleic acids and monosaccharides (Murata-Kamiya et al., 1995; Thornalley et al., 1999); methylglyoxal generated spontaneously from dihydroxyacetone and enzymatically as a product of the triose phosphate isomerase reaction (Richard, 1991; Phillips & Thornalley, 1993; Thornalley, 1993); 4-hydroxynonenal is formed as an oxidation product of polyunsaturated fatty acids such as arachidonic acid (Esterbauer & Zollner, 1989). However, oxidative stress can increase the formation of endogenous aldehydes, and endogenous aldehydes can increase oxidative stress. In the former case, oxidative stress resulting from reactive oxygen species formation can increase the formation of endogenous aldehydes: (i) by lipid peroxidation (Esterbauer & Zollner, 1989); (ii) by the oxidative inactivation of glyceraldehyde-3-phosphate dehydrogenase with the resulting increase of glyceraldehyde-3-phosphate and glyceraldehyde (Eaton et al., 2002); and (iii) by the one-electron oxidation and activation of short chain sugars and their metabolic and oxidation products (Benov & Beema, 2003; O'Brien et al., 2010). In the latter case, endogenous aldehydes such as methylglyoxal, glyoxal and 4-hydroxynonenal can increase oxidative stress: (i) by depleting NADPH (Vander Jagt, 2008; Chaplen et al., 1998; Vander Jagt et al., 1992; Vander Jagt et al., 1995; O'connor et al., 1999), when they are detoxified by aldose reductase (AKR1B1) or by aldehyde reductase (AKR1A1); (ii) by inactivating glutathione reductase, as in the case of methylglyoxal and 4-hydroxynonenal, (Vander Jagt et al., 1997); (iii) by reacting with the α-NH_2 group of the glutamate residue of glutathione (rather than the SH group), as in the case of glyoxal, thus depleting glutathione and thereby decreasing the detoxication efficacy of glutathione peroxidase and glutathione S-transferase (Nomi et al., 2009); (iv) by decreasing the level of glutathione and as a result limiting the detoxification of methylglyoxal through glyoxylase I and II (Vander Jagt, 2008; Shinohara et al., 1998; Vander Jagt, 1989); and (v) by activating NADPH oxidase, with the formation of AGEs and their interaction with the AGE receptors (RAGEs) (Yan et al., 2003; Tan et al., 2007; Wautier et al., 2001). We suggest that these feedback cycles led to a parallel, correlated increase in the endogenous aldehyde products, glyoxal, methylglyoxal and 4-hydroxy-nonenal concentrations.

Fasting glyceraldehyde concentrations appeared as an exception to this scheme. Glyceraldehyde is produced in large amounts in the first steps of fructose metabolism by glycolysis and is usually metabolized rapidly, initially phosphorylated (catalyzed by triokinase) and then oxidized (catalyzed by glyceraldehyde-3-phosphate dehydrogenase). High, intermittent concentrations of glyceraldehyde could temporarily overwhelm the ability of triokinase to phosphorylate it for the further steps in glycolysis. Therefore, high intermittent or average concentrations of glyceraldehyde may be more relevant than low-fasting concentrations for the further metabolism and oxidation of "free" glyceraldehyde to further reactive short-chain sugars (Benov & Beema, 2003; O'Brien et al., 2010). The concentration of many aldehydes may similarly vary through the day reflecting the changing concentrations of fructose and glucose which may also vary from tissue to tissue.

Long-term exposure to reactive endogenously-formed aldehydes could be assessed by measuring the concentrations of AGEs and advanced lipid oxidation end-products (ALEs). Aldehydes form protein adducts and rearrange to form these stable end products that can be detected in plasma and tissues by immunologic, mass-spectroscopic or by fluorescence techniques (Takeuchi et al., 2001; Tessier et al., 2003; Usui et al., 2008). A possible limitation to their use is the large number of different AGEs that can result from the multiple reactive species and multiple sites for adduct formation. Long-term exposure to reactive aldehydes could also be assessed with protein carbonyls detected by colorimetric, ELISA or fluorometric assays (Reznick & Packer, 1994; Buss et al., 1997; Mohanty et al., 2010). A possible limitation is the single measure it provides for carbonyls formed both from reactive aldehydes (Mehta et al., 2009) and from the oxidative modification of amino acids (Berlett & Stadtman, 1997). Protein carbonyls have also not been found to be sensitive measures of oxidative stress in some animal and clinical models (Kadiiska et al., 2005; Il'yasova et al., 2009). However, the animal studies used only diets with slowly digested complex carbohydates and the clinical study did not provide any information concerning the consumption of fructose or sucrose during the chemotherapy exposure. The close association we have observed between our measures of oxidative and aldehyde stress suggests that single measures such as glyceraldehyde- or methylglyoxal-derived AGEs, or protein carbonyls could provide a suitable initial biomarker of exposure to these reactive species for an assessment of their role in the origin of chronic disease.

Indeed, both AGEs and protein carbonyls have been found to be associated with many chronic diseases observed in developed countries (Hyogo et al., 2007; Takeuchi et al., 1999; Kilhovd et al., 2009; Ni et al., 2009; Ahmed et al., 2003; Dalle-Donne et al., 2003) including CRC (Chang et al., 2008; Yeh et al., 2010). While these

studies do not rule out the possibility that the observed association is the result of the disease influencing the levels of the markers, we consider more plausible that the association is the result of an exposure of tissues and cells to cyto- and genotoxic-reactive species that may well have been involved in the development of the disease. This relationship has been appreciated in age-related eye diseases and diabetes-related complications, but has not been noted or generally appreciated for other chronic disease processes. Cell populations in the body appear to be exposed to a wide range of oxidative and aldehyde species which could have resulted from exposures to lifestyle and dietary factors typical of developed countries. Disease processes could be better defined with studies directed toward identifying the relations between the following: lifestyle and dietary factors; biomarkers of exposure to oxidative stress, as well as to oxidative stress combined with aldehyde stress; and evidence of disease. The apparent close association of products of oxidative and aldehyde stress, and with AGEs and protein carbonylation should facilitate such studies.

Our small study of the correlation of reactive carbonyls is only a step in the eventual definition of the relation between lifestyle factors and chronic disease. Short-term clinical studies are needed in which diet and time of collection are controlled and the samples assessed rapidly by methods optimized for mono-carbonyls and the more reactive dicarbonyls (Mitchel & Birnboim, 1977; Thornalley et al., 1999; Mak et al., 2000), AGEs and protein carbonyls in plasma and serum. Other studies are needed to assess the association of the biomarkers in plasma with those in the tissues at risk. Finally, intervention studies will be needed to demonstrate feasible approaches to decrease the biomarkers of aldehyde exposure, approaches that hopefully will also decrease diseases.

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Declaration of interest

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