Statement on Methylglyoxal

Background

1. As part of its annual horizon scanning discussion in February 2009, the Committee was provided with information on occurrence of methylglyoxal (MG) in food, possibly as an intermediate in the formation of acrylamide, and on the association between endogenously formed MG with a number of diseases. The Committee expressed an interest in a more thorough review of methylglyoxal including, if possible, a comparison between dietary exposure to MG and endogenous production.

Introduction

2. MG is a reactive dicarbonyl compound that is produced endogenously in the body, primarily through anaerobic glycolysis, and has been widely identified in both animal and plant tissues. MG is also known as pyruvaldehyde, 2-ketopropionaldehyde or acetylformaldehyde and the chemical structure can be found below:

3. MG and other products of glycolysis have been shown to produce adducts in both DNA and proteins. Elevated MG levels and associated MG-protein adducts in the kidney, lens and blood have been associated with complications commonly found in patients with diabetes mellitus. Elevated levels of protein adducts have been associated also with aging, renal failure and Alzheimer’s disease and of DNA adducts with cancer. MG also has a role in the formation of reactive oxygen species (ROS) and stable advanced glycation end-products (AGEs).

4. As MG is ubiquitous in living cells, it will be found in food products of both animal and plant origin and therefore exogenous exposure occurs through consumption of all foods. Particularly high levels have been reported in manuka honey and some soft drinks.
5. Reducing saccharides have been found to react with asparagine to form acrylamide. Glucose, on heat treatment, has been shown to degrade into MG and 5-hydroxymethyl-2-furfural (HMF), and these two degradation products have also been found to react with asparagine to form acrylamide \(^8,9\). The conversion of MG and related compounds to acrylamide is likely to be a minor source of acrylamide in the diet when compared to that formed directly from other dietary components \(^10\). There are insufficient data to establish whether efforts to reduce acrylamide levels in food through changes in food processing practices will alter exposure to MG.

Absorption, distribution, metabolism and excretion

6. No data on the absorption of free MG are available, but based on its physical and chemical properties it is expected to be rapidly absorbed from the gastrointestinal tract following oral ingestion.

7. Reported MG plasma concentrations in healthy individuals are in the range 5.8 - 720 μg/L \(^11,12\) although there is some evidence that inadequate control of interferences during processing can lead to overestimation of MG levels by 10-1000 fold \(^11\). Mean blood levels in groups of insulin-dependent diabetics and non-insulin-dependent diabetics were 33.9 μg/L and 20.7 μg/L, respectively compared with a control group with mean blood levels of 5.8 μg/L \(^13\). In individuals found to be compliant with a high protein, low carbohydrate diet, plasma MG levels were found to be 7.1 (standard deviation (SD) 2.5) μg/L at baseline and 15.6 (SD 5.3) μg/L after 14-28 days on the diet. It is not clear from this study whether the diet itself was high in MG or whether it triggered elevated endogenous production of MG \(^14\).

8. One of the most important routes of endogenous MG production is from the triose phosphate intermediates in the glycolytic pathway, which can occur through either spontaneous non-enzymatic elimination of the phosphate group or decomposition of the ene-diol triose intermediate that leaks from the active site of triose phosphate isomerase \(^15\).

9. AGE residues have been shown to have poor bioavailability with less than 10% being absorbed from proteins in ingested foods. They appear in the plasma as AGE-amino acid or AGE-nucleotide complexes or AGE-rich peptides \(^11\).

Metabolism

10. Metabolism of MG primarily occurs through the activity of the zinc-dependent glyoxalase enzymes I and II (GLOI and GLOII) \(^16\). Figure 1 illustrates the main glyoxalase pathway of MG metabolism. D-Lactate hydrogenase transforms the D-lactate into pyruvate. As this system is highly dependent on GSH, conditions of oxidative stress where GSH is depleted can decrease metabolism of MG. In diabetes, cytosolic NADPH is decreased, and as this is required for regeneration of GSH, this may be one route leading to an increase in MG in diabetic patients \(^15\). MG significantly decreased cellular GSH levels in hepatocytes in vitro, although these studies are likely to be of limited relevance to in vivo exposure \(^11,12\). Aldose reductase and betaine aldehyde dehydrogenase are other enzymes involved in the metabolism of MG \(^15\).
Figure 1: The glyoxalase system and metabolism of methylglyoxal to D-lactate.

**Excretion**

11. Endogenously formed protein glycation adducts are removed from cellular proteins by proteosomal and lysosomal proteolysis. Nucleotide glycation adducts are cleared by nucleotide excision repair and phospholipid AGEs are removed by lipid turnover. Glycation adducts are eliminated as free glycated amino acids or peptides in the urine along with those absorbed from foods.

12. Free MG metabolised to D-lactate will be metabolised initially to pyruvate and then subsequently through the citric acid cycle to form ATP, water and carbon dioxide in the same way as glucose.

**Toxicity studies**

**Mechanisms of toxicity**

13. Three primary mechanisms of MG toxicity have been suggested:
   (i) A direct inhibitory effect of MG on enzymes through the formation of protein adducts leading to a reduction in cellular function.
   (ii) The indirect depletion of GSH and subsequent elevation in ROS which depletes GSH further.
   (iii) Genotoxicity due to the formation of DNA adducts and/or ROS.

**Acute toxicity**

14. The acute oral toxicity of MG was found to vary in Fischer rats, with newborn animals being most sensitive (LD$_{50}$ ~ 600 mg/kg bw), followed by non-pregnant females (LD$_{50}$ ~ 1200 mg/kg bw), then weanling animals (LD$_{50}$ ~ 1800 mg/kg bw), and male adults being the least sensitive to MG (LD$_{50}$ ~ 2400 mg/kg bw). No deaths or adverse health effects were observed in adult Swiss albino mice and Sprague-Dawley rats when MG was administered orally via gastric tube at a dose of 2 g/kg bw.

15. An intraperitoneal dose of 800 mg/kg bw was lethal to mice within 4 hours and a dose of 400 mg/kg bw decreased liver weights and cytosolic GSH, and produced vacuoles in the liver.
Short term toxicity

16. In a non-standard study with no mention of controls, MG was administered orally via gastric tube at a dose of 1g/kg bw/day to Swiss albino mice, Sprague-Dawley rats (6 weeks) and dogs (4 weeks) and at a dose of 0.55 g/kg bw/day to rabbits (6 weeks). There were no overt signs of toxicity and no changes in body weights in any of the species. Biochemical analyses of blood samples were conducted for dogs and rats, and liver, kidney, spleen, duodenum and bone marrow of mice were examined histologically, showing no adverse effects. Rats and mice were allowed to breed; no adverse effects were reported, but no standard reproductive toxicity endpoints were analysed and no statistical analyses were carried out.

17. Wistar-Kyoto rats were given drinking water containing MG at 0.2 % from weeks 0 to 5, 0.4 % from weeks 6 to 10 and 0.8 % from weeks 11 to 18. Approximate mean doses of MG in the treated group were in the region of 270-500 mg/kg bw/day. After 18 weeks, systolic blood pressure, platelet [Ca^{2+}] and kidney aldehyde conjugates were significantly higher and serum nitric oxide levels lower in MG treated rats. Smooth muscle cell hyperplasia in the small arteries of the kidney was also apparent in treated rats. Final body weights were significantly reduced (6%) in the treatment group compared to controls.

18. In a limited study in ddY mice (an outbred strain widely used in some countries), animals were exposed in utero to methylglyoxal (with the dams receiving 1% MG in the drinking water, equivalent to about 1200-2800 mg/kg bw/day) and post weaning (1% in the drinking water), up to 2 months of age. Blood GSH levels and cellular glutathione S-transferase activity were statistically significantly reduced in MG-exposed mice compared with controls. There was also an indication that oral glucose tolerance and erythrocyte resistance to oxidative stress were impaired in treated animals.

Long term toxicity / carcinogenicity

19. No long term or carcinogenicity studies conducted to regulatory standards are available. IARC have determined MG to be not classifiable as a carcinogen (Group III). There have been some studies investigating the tumour-promoting potential of MG but these do not suggest carcinogenic potential. In a study involving subcutaneous injection with 2 mg MG twice a week for 10 weeks, 4 of 18 rats had tumours at the injection site after 20 months. No tumours were observed in the control animals (Nagao et al., 1986). These studies do not provide evidence of systemic carcinogenicity.

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1 For example, no information was provided on how frequently solutions of methylglyoxal were prepared, nor on its stability in these studies. No information was provided on possible impurities in the tap water used in the study. The health checks on the animals prior to study were not to modern standards. Few details are provided on how the animals were housed (cage materials, climate control, bedding). GST was assayed at ambient temperature, which may have varied. The assay used for the determination of GSH is not entirely specific.

2 In an apparent miscalculation, the authors cited the dose as 1.7µmol/mouse, i.e. about 5mg/kg bw/day. A 1% solution consumed by 25g mice, assuming consumption of 3-7 ml water per day, gives a dose of 1200-2800 mg/kg bw/day.
Genotoxicity

20. Positive results for MG have been obtained in reverse mutation assays in the presence and absence of metabolic activation using S. typhimurium strains TA97, TA98, TA100, TA102 and TA104 at concentrations of 12 – 470 μg/plate. Negative results were obtained using strains TA1535 and TA1538. MG produced mutations in S. cerevisiae (strain D7) at concentrations of 0.5-1.5 mg/ml and the diphtheria toxin resistance mutation in Chinese hamster lung cells at concentrations of 24-66 μg/ml. It induced micronuclei in human lymphocytes without, but not with, a microsomal enzyme preparation (S9). DNA cross linking was apparent after 90 minutes incubation of Chinese hamster ovary cells with 1.5 mM MG solution without S9. Cross-linking was significantly reduced in the presence of S9.

21. MG has been shown to form DNA adducts in vitro, primarily derived from deoxyguanosine. Both endogenous and concentration-related induced MG DNA adducts have been demonstrated in cultured human buccal epithelial cells.

22. In a non-standard study, oral administration of 600 mg/kg bw MG to mice resulted in an increased incidence of sister chromatid exchange but not chromosomal aberrations in duodenal cells. No effects were seen in the ileum or at 400 mg/kg bw MG (Migliore et al., 1990). No other in vivo genotoxicity tests have been identified.

Human Studies

23. MG was administered orally to 86 patients with various types of tumours and at varying stages of the disease at doses of 25-30 mg/kg bw/day for approximately 10 weeks and then 14-16 mg/kg bw/day for 15 weeks. Individuals were also given multi-vitamin supplements daily. In a second group of patients, dosing was similarly timed but levels varied depending on the patient’s response. No apparent treatment-related effects were observed. These studies did not appear to follow established criteria for medical trials, they were not controlled, and no biochemical parameters were assessed.

24. In a four-week study, 26 non-diabetic renal failure patients on maintenance peritoneal dialysis were randomized to a high or low AGE diet obtained through using different cooking methods. Three-day dietary records, fasting blood, 24 hour urine and dialysate fluid collections were obtained at baseline and at the end of the study. AGE levels were determined using N-carboxymethyl-lysine (CML) as a biomarker. Compared to baseline, patients on the low AGE diet showed decreased serum CML and MG, and high dietary AGE intake appeared to increase these parameters compared to baseline values.

Exposure

Exogenous

Table 1 shows the MG content of a number of foodstuffs as reported in the literature.
<table>
<thead>
<tr>
<th>Foodstuff</th>
<th>Level of MG</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuka Honey</td>
<td>38 – 829 mg/kg</td>
<td>[39, 39, 40, 40]</td>
</tr>
<tr>
<td>Other honey</td>
<td>0 – 135 mg/kg</td>
<td></td>
</tr>
<tr>
<td>Soya sauce</td>
<td>8.7 mg/L</td>
<td>[30]</td>
</tr>
<tr>
<td>Bread</td>
<td>0.79 mg/kg</td>
<td></td>
</tr>
<tr>
<td>Alcoholic beverages</td>
<td>0.26 - 1.5 mg/L</td>
<td></td>
</tr>
<tr>
<td>Brewed coffee</td>
<td>7 mg/L</td>
<td></td>
</tr>
<tr>
<td>Cod liver oil*</td>
<td>2.03 (SD 0.13) mg/L</td>
<td>[41]</td>
</tr>
<tr>
<td>Tuna oil*</td>
<td>2.89 (SD 0.11) mg/L</td>
<td></td>
</tr>
<tr>
<td>Olive oil*</td>
<td>0.61 (SD 0.03) mg/L</td>
<td></td>
</tr>
<tr>
<td>Rice, millet, mustard</td>
<td>2.2 - 5.4 mg/kg</td>
<td>[15]</td>
</tr>
<tr>
<td>Soft drinks containing high fructose corn syrup</td>
<td>23.5 - 267 mg/L</td>
<td>[42, 43, 44]</td>
</tr>
<tr>
<td>Diet soft drinks</td>
<td>7.1 - 31.5 mg/L</td>
<td></td>
</tr>
<tr>
<td>Sweetened tea beverages</td>
<td>32.1 - 98.1 mg/L</td>
<td></td>
</tr>
<tr>
<td>Diet tea beverages</td>
<td>26.2 - 42.5 mg/L</td>
<td></td>
</tr>
<tr>
<td>Cheddar cheese</td>
<td>10.89 mg/kg</td>
<td>[44]</td>
</tr>
<tr>
<td>Swiss cheese</td>
<td>1.98 mg/kg</td>
<td></td>
</tr>
<tr>
<td>Mozzarella</td>
<td>4.06 mg/kg</td>
<td></td>
</tr>
<tr>
<td>Beer</td>
<td>0.072 mg/L</td>
<td>[45]</td>
</tr>
<tr>
<td>Wine</td>
<td>0.65 - 2.88 mg/L</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Foodstuffs and corresponding MG levels from the literature. * indicates oils analysed following accelerated conditions of use (60°C for 3-7 days and 200°C for 1 hour).

25. Using the occurrence data in Table 1 and food consumption data from the National Diet and Nutrition Surveys (NDNS) [46, 47, 48], dietary exposure to MG has been estimated as 1.3 mg/kg bw/day and 3.9 mg/kg bw/day for mean and high level (97.5th percentile) adult consumers, respectively. In toddlers (the group with the highest predicted exposures relative to bodyweight) estimates were 7.7 mg/kg bw/day and 22.8 mg/kg bw/day, for mean and high level (97.5th percentile) consumers respectively. The highest reported levels in each food group were used to calculate the above exposure estimates. However, since data are not available for foods not mentioned in Table 1, these could be underestimates of actual dietary exposure. The Committee noted that the estimates of dietary exposure in children may be out of date and could be updated when sufficient data to generate robust estimates of food intake for these age-groups are available from the new NDNS rolling programme.

26. Exposure to MG may also occur through catabolism of glycated proteins, from both endogenous and exogenous sources. The rate of MG production at the cellular level in normal healthy individuals is largely unknown and the relative significance of the different sources of MG is unclear. Furthermore, it is not possible to determine whether MG levels in blood reflect total body burden of MG. Thus a comparison between endogenous and exogenous exposure to MG is currently not possible.

Conclusions

27. MG can be present as a free molecule in the diet and can be found bound to biological material, such as proteins, as AGEs, which are poorly absorbed.

28. MG is primarily metabolised through the activity of enzymes that are dependent on cellular GSH.
29. The database on toxicity of MG is poor, and inadequate for characterisation of dose-response relationships. MG is of low acute toxicity, although one report indicates that new-born rats may be more sensitive than adults.

30. MG can bind to cellular macromolecules and can form DNA adducts. There is in vitro evidence that MG is genotoxic, particularly in the absence of an exogenous metabolising system, but the in vivo relevance is unclear. The limited data available do not suggest that MG is systemically carcinogenic.

31. No overt signs of toxicity were seen in short-term studies in adult mice, rats and dogs dosed orally by gavage with MG at 1000 mg/kg bw/day. An 18-week study in rats indicated effects on blood pressure and clinical chemistry at doses in the region of 500 mg/kg bw/day.

32. Dietary exposure to MG has been estimated at 1.3 mg/kg bw/day and 3.9 mg/kg bw/day for mean and high level adult consumers, respectively. In toddlers (the group with the highest predicted exposures relative to bodyweight) estimates were 7.7 mg/kg bw/day and 22.8 mg/kg bw/day, for mean and high level consumers respectively. Based on the margins of 25-500 between these exposures and the dose reported to cause biological changes in rats, the Committee concluded that short term dietary intakes of MG were unlikely to be of concern.

33. MG exposure can also result from dietary AGEs and from endogenous production. The Committee concluded that it is currently not possible to assess the relative importance of dietary sources of MG or endogenous production, or their expected biological effects.

34. In order to support an evaluation of the risks associated with MG, the Committee suggested that a study to investigate the kinetics of MG would be the first priority, followed by a 90-day study to assess the toxicity of MG and an in vivo genotoxicity study.

COT statement 2009/04
November 2009
References


