Estimation of exposure to dietary acrylamide based on mercapturic acid levels in urine (LC-MS)

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Method Article

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Abstract

We determined metabolites of acrylamide and glycidamide concentrations (AAMA and GAMA, respectively) in urine of 93 Polish women within the first days after delivery, using LC-MS/MS. The median AAMA and GAMA levels in urine were 20.9 µg/L (2.3 ÷ 399.0 µg/L) and 8.6 µg/L (1.3 ÷ 85.0 µg/L), respectively. In smokers we found significantly ($p < 0.01$) higher levels of metabolites in comparison with the non-smoking women.

Introduction

Acrylamide (AA) is a chemical compound extensively used in the industry for the production of polyacrylamide polymers. Increased interest in acrylamide has been noted since 2002, when the Swedish National Food Agency in collaboration with scientists from the Stockholm University published for the first time data on the high content of acrylamide in high-carbohydrate products undergoing thermal processing. Acrylamide is rapidly absorbed and, owing to its very good solubility in water, rapidly distributed to various tissues. It is metabolised through two main metabolic pathways: epoxidation to glycidamide and glutathione conjugation to mercapturic acids. The conversion of acrylamide to glycidamide, its main metabolite, is catalysed by an enzyme of cytochrome P450 (isoenzyme CYP2E1). Both acrylamide and glycidamide form adducts with haemoglobin. Acrylamide adducts with N-terminal valine in the haemoglobin molecule (AA-Hg) are considered as biomarkers of long-term exposure to acrylamide. Higher levels of AA-Hg were found in smokers and persons exposed to acrylamide at work. Additionally, glycidamide forms a number of DNA adducts, this is why glycidamide is considered to play a critical role in the carcinogenic action of acrylamide. Both compounds are conjugated with glutathione and further metabolized to form mercapturic acids: acrylamide to N-acetyl-S-(2-carbamoyl-ethyl)-L-cysteine (AAMA) and glycidamide to N-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine (GAMA) and N-acetyl-S-(3-amino-2-hydroxy-3-oxopropyl)-cysteine. Acrylamide and glycidamide metabolites in the form of mercapturic acids are excreted in the urine. Several papers published so far presented the results of studies on urinary levels of mercapturic derivatives of acrylamide and glycidamide in humans. The possibility of use of the mercapturic derivatives levels for the assessment of exposure to acrylamide from food and tobacco smoke was also presented.

Reagents

1. AAMA (N-acetyl-S-(2-carbamoyl-ethyl)-L-cysteine), C/D/N Isotopes Inc. (Quebec, Canada), 2. d4-AAMA (N-acetyl-S-(2-carbamoyl-ethyl-d4)-L-cysteine), C/D/N Isotopes Inc. (Quebec, Canada), 3. GAMA (N-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine dicyclohexylammonium salt), Toronto Research Chemicals Inc. (North York, Canada), 4. d3-GAMA (N-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine-d3 dicyclohexyl-ammonium salt), Toronto Research Chemicals Inc. (North York, Canada), 5. Formic acid 98–100% GR (ACS, Reag.), Merck KGaA (Darmstadt, Germany), 6. Ammonium formate buffer solution (50mmol/L, pH=2.5) prepared from ammonium formate (HPLC, 99.0+%), Fluka (Switzerland), 7. Methanol HPLC (99.9%), Rathburn Chemicals (Walkerburn, Scotland), 8. Deionised water, 9. Diluted

formic acid (pH = 2.5) prepared from formic acid 98–100% GR (ACS, Reag.), Merck KGaA (Darmstadt, Germany), 10. 10% (v/v) methanol (HPLC) in diluted formic acid (pH = 2.5), 11. 1% (v/v) formic acid (ACS, Reag) in methanol (HPLC), 12. Formic acid LC-MS (98%), Fluka (Germany), 13. 0.1% formic acid (LC-MS) in deionised water, 14. Methanol LC-MS (99.8+), Avantor Performance Materials B.V. (Deventer, Netherlands), 15. Acetonitrile LC-MS (99.9+), Avantor Performance Materials B.V. (Deventer, Netherlands), 16. Mobile phase A: deionised water with 0.2% formic acid (LC-MS) and 2mmol/L ammonium formate, 17. Mobile phase B: acetonitrile (LC-MS) with 0.2% formic acid (LC-MS) and 2mmol/L ammonium formate, and 18. ISOLUTE ENV+ (100 mg; 10 ml) columns, Biotage AB (Uppsala, Sweden). CALIBRATION STANDARDS 1. AAMA and GAMA (1 µg/L); d4-AAMA and d3-GAMA (1 µg/L) 2. AAMA and GAMA (2.5 µg/L); d4-AAMA and d3-GAMA (1 µg/L) 3. AAMA and GAMA (5 µg/L); d4-AAMA and d3-GAMA (1 µg/L) 4. AAMA and GAMA (10 µg/L); d4-AAMA and d3-GAMA (1 µg/L) 5. AAMA and GAMA (25 µg/L); d4-AAMA and d3-GAMA (1 µg/L) 6. AAMA and GAMA (50 µg/L); d4-AAMA and d3-GAMA (1 µg/L) 7. AAMA and GAMA (100 µg/L); d4-AAMA and d3-GAMA (1 µg/L) 8. AAMA and GAMA (250 µg/L); d4-AAMA and d3-GAMA (1 µg/L) 9. AAMA and GAMA (500 µg/L); d4-AAMA and d3-GAMA (1 µg/L)

Equipment

1. Water purification Systems (Direct_Q, Millipore) 2. Shaker PROMAX 1020 (Heidolph) 3. High Speed Brushless Centrifuge MPW-350 R 4. SPE clean-up 5. Evaporation system (N₂) with heating (400°C) 6. Microcentrifuge MPW-55 7. Kinetex 2.6u XB-C18 column (Phenomenex) 8. LC-MS/MS system: UltiMate 3000 (Dionex) with mass spectrometer 3200 QTrap (ABSciex)

Procedure

The samples were prepared according to Boettcher and Angerer (2). We describe here how to prepare urine samples to LC-MS/MS. 1. Thaw urine samples to room temperature and then mix them (257 rpm, 5 min). 2. 4 ml of urine withdrawn from each sample and add 30 µl of the mixture of d3- and d4-internal standard solutions (10 mg/L). 3. The samples were vortex-mixed and centrifuged (3000 rpm, 10 min). 4. Purifying on SPE columns: ☐ Conditioning the column with methanol (4 ml), water (2 ml) and diluted formic acid (2 ml). ☐ Add 7.5 ml of supernatant of the centrifugated sample solution. ☐ Wash the column with diluted formic acid (2 ml) and 10% methanol in formic acid (0.8 ml). ☐ Elute with 1% formic acid in methanol (1.7 ml). 5. Evaporate to dryness. 6. Residue reconstitute in 0.5 ml 0.1 formic acid. 7. 10 microlitres of this solution inject into LC-MS/MS system. LC conditions: ☐ flow rate – 1 mL/min, ☐ mobile phase (A : B – 96 : 4) ☐ column temperature – 40°C, ☐ runtime – 1.5 min. MS/MS conditions: ☐ MRM mode, ☐ curtain gas – nitrogen (CUR = 30), ☐ ion source temperature – 6000°C, ☐ electrospray capillary voltage (IS) – - 4.500 V, ☐ dwell-time – 50 ms, ☐ collision energy (CE): a) CE = - 22 for m/z 233/ 104 (AAMA) and for m/z 236.9 / 108 (d4-AAMA), b) CE = - 54 for m/z 233 / 58 (AAMA), c) CE = - 24 for m/z 248.9 / 120.1 (GAMA) and for m/z 252/ 119.9 (d3-GAMA), d) CE = - 18 for m/z 248.9/ 127.9 (GAMA). ☐ Monitored ions: a) for the purposes of quantitative determination of AAMA and GAMA levels in the

urine: m/z 233 104 \(\text{AAMA}\), m/z 236.9 / 108 \(\text{d4-AAMA}\), m/z 248.9 / 120.1 \(\text{GAMA}\) and m/z 252/ 119.9 \(\text{d3-GAMA}\), b) for the purposes of verification of the results obtained: while m/z 233/ 58 \(\text{AAMA}\) and m/z 248.9 / 127.9 \(\text{GAMA}\) \(\text{(fig. 1)}\).

Anticipated Results

For representative urinary levels of AAMA and GAMA results, please refer to the full article where the results are presented and discussed in details.

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