Attempt to Detect Garlic Allyl Sulphides from Saliva after Consumption of Garlic Tablets Using GC-MS

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Abbreviations

DADS: Diallyl disulphide; DAS: Diallyl Sulphide; DATS: Diallyl Trisulphide; DMTS: Dimethyl Trisulphide AM: Allyl Mercaptan; AMDS: Allyl mMethyl Disulphide; AMS: Allyl Methyl Sulphide; AMSO: Allyl Methyl Sulfoxide; AMSO2: Allyl Methyl Sulfone; AMTS: Allyl Methyl Trisulphide; GC-MS: Gas Chromatography Mass Spectrometry

Introduction

Garlic produces volatile sulfur compounds, to which many anti-fungal and antimicrobial properties have been attributed. The authors were interested in the main volatile sulphur compound, allicin, and its potential as a systemic anti-fungal agent. However, the presence of allicin or its metabolites in human fluids has not been reported [1,2].

During the digestive process, allicin quickly metabolizes into other sulphur compounds [3-5]. Thus far, bioavailability has only been measurable by breath studies, which have detected the presence of metabolites AM, AMS and DADS after oral consumption [5,6]. Only traces of allicin were found in blood after being incubated for five minutes [7]. In another in vivo study, DADS was spiked into rat’s blood and identified using GC-MS [8].

Previously in animals, organosulphur compounds from garlic have been detected using GCMS [9,10]. In rats, DADS, AM, AMSO and AMSO2 were found in stomach, liver, plasma and urine after consumption of 200 mg/kg of body weight. Time-dependent tissue concentrations of DADS were highest within 24 hours and not detectable at any time point. In summary, it is possible that these highly volatile compounds may be more readily detectable using a solid phase micro-extraction GC-MS, or headspace analysis methodology, although the less volatile allyl methyl sulfone and allyl methyl sulfoxide may be the main metabolites present.

In this study, an attempt was made to extract and detect allicin transformation products, AMS and DADS, from saliva following ingestion of enteric-coated garlic tablets.

Materials and Methods

Ethics approval was obtained from University of Melbourne Human Research Ethics Committee (ID 1033568.1).

Garlic allyl sulphide standards were purchased from MP Biomedicals Australia for validation and refinement of methods. 25 mg of each of the following standards was purchased: allyl disulphide (purity 91.2%; molecular weight 146.27 g/mol), allyl trisulphide (molecular weight 178.3 g/mol) and allyl methyl sulphide (molecular weight 88.17 g/mol). Standards were diluted in the concentration range of 0.50-50 µg/mL in hexane (w/v) for use as points for a linear standard curve, and to determine limits of detection. Additional testing was performed using steam-distilled garlic oil, diluted in hexan 0.0005% v/v, as a source of a large variety of allyl sulphides.

Using an Agilent 7890a Gas Chromatograph coupled to a 5975C mass selective detector, cryogenic Gas Chromatography (GC) conditions were refined using standards and essential oils. Samples were separated using a 30 m Varian Factor Four VF-5 ms column with a 10m exi-guard column attached. Column dimensions were 250 µm inner diameters with 0.25 µm film thickness. Oven ramp parameters were -20°C held for 2 minutes, 10°C/min to 200°C, 35°C/min to
Results and Discussion

A chromatogram of steam-distilled garlic oil, containing a variety of allyl sulphides, is shown in Figure 2. No allyl sulphides matching known standards were detected in the saliva at any of the time points (Figure 1b).

The method used had a sensitivity of 0.013 umol for AMS or 0.010 umol DADS mL$^{-1}$ saliva. The detection of AMS or DADS would have been possible only if at least 10-50% of the consumed allicin had been metabolized to these compounds. Equal availability in saliva as in the total body water or blood would also have been necessary for detection of these compounds. Organosulphur compounds from garlic have never been detected in human organs or bodily fluids following oral consumption. Detection of garlic compounds in saliva following ingestion of enteric-coated tablets may, with methodological refinement, provide an alternative to breathe studies to prove bioavailability by enabling direct detection of organosulphur compounds in body fluids following consumption. In this small study, standards for allyl sulphides from *Allium sativum* were detected by GC-MS when injected alone and when spiked into saliva (not shown), which enabled full spectral identification of these compounds. However, the sensitivity of the method outlined was not sufficient for detection of physiological levels of allicin metabolites AMS and DADS in saliva after consumption of large amounts of garlic tablets.

The presence of electrolytes in saliva may have affected the solubility of AMS. Measuring peroxidases in saliva [13] for kinetics of oxidation of AMS to AMSO and AMSO2 may have clarified breakdown products present. It is possible that these highly volatile compounds may be more readily detectable using a solid phase micro-extraction GC-MS, or headspace analysis methodology [14-16]. Nevertheless, it appears clear that AMS and DADS are not significantly found in saliva after consuming large amounts of garlic, although the presence of allicin metabolites AMSO and AMSO2 needs yet to be evaluated.

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