

## **Profiling of potential anti-cancer metabolites of glucosinolate-enhanced broccoli (super broccoli) in human volunteers**

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Isothiocyanates (ITCs) are biologically active, and have been implicated in the anti-carcinogenic activity of cruciferous vegetables, demonstrated both from epidemiological studies and the use of animal models. ITCs such as sulforaphane (4-methylsulphinyl-butylisothiocyanate) and iberin (3-methylsulphinylpropyl isothiocyanate) are potent inducers of phase II enzymes and can induce cell cycle arrest and apoptosis. Glucosinolates (GSLs), sulphur-containing glycosides from which ITCs are derived, are found in all cruciferous crops, and those with methylsulfinylalkyl side chains occur in broccoli (*Brassica oleracea var italica*). To enhance their levels a breeding programme has developed GSL-enriched broccoli via introgression of genomic segments from a wild broccoli relative, *B. villosa*, into an agronomically superior broccoli genetic background. The known mercapturic acid pathway metabolites of sulforaphane and iberin have been synthesized and characterized to use as authentic standards in this study by using the method of Kassahun et al. (1997). The stability of sulforaphane and iberin metabolites has been determined at different pH and temperature in buffered aqueous solution and human plasma. Stability results have been employed to optimize the conditions to collect blood and urine from human volunteers and sample preparation before analysis. Acidic media were found to substantially reduce the rate of decomposition of sulforaphane and iberin conjugates. Knowledge of the stability of sulforaphane and iberin metabolites in human plasma at different temperatures gave credence to results obtained by the analytical method. This study reports a novel LC-MS/MS analytical method for the simultaneous determination of sulforaphane and iberin and their glutathione, cysteine, cysteine-glycine, *N*-acetylcysteine conjugates in human plasma and urine. The analytes were monitored by the positive ion-electrospray-ionization mass spectrometry (ESI-MS-MS). Quantitation of sulforaphane, iberin and their metabolites in human plasma and urine was carried out in multiple reaction monitoring (MRM) mode. This method had a linear calibration range of 0.03-300  $\mu\text{mole/L}$  and 0.025-15  $\mu\text{mole/L}$  with a correlation coefficient of  $\geq 0.96$  for sulforaphane, iberin and their metabolites in human urine and plasma respectively. The lower limit of quantification (LLOQ) for sulforaphane in human plasma was 15 nmole/L. It was demonstrated that the method had high recovery and accuracy (85-99% and 99-104%), as well as excellent intra- and inter-assay precision, thus meeting the international standards in bioanalytical validation required for a human volunteer trial. A human intervention study was undertaken to assess plasma concentrations of ITC and ITC-derived metabolites following consumption of either standard or GSL-enriched broccoli. For the first time, using this method it was possible to report the individual quantitative measurement of each of the conjugates, unconjugated sulforaphane and iberin in both plasma and urine in human volunteers. Full biokinetic information of the time course of disposition of the ITCs and their metabolites was also determined. The  $\text{AUC}_{0-24\text{h}}$  and  $\text{C}_{\text{max}}$  values for sulforaphane and their metabolites in human plasma after consumption of GSL-enriched broccoli was about 3-4 fold the  $\text{AUC}_{0-24\text{h}}$  of the normal broccoli (37.74  $\mu\text{M/hr}$ , compared to 11.17  $\mu\text{M/hr}$ ). This result was consistent with the total amounts of sulforaphane and its metabolites excreted in urine. Glutathione S-transferase M1 (GSTM1) positive individuals excreted less sulforaphane than GSTM1 nulls. There were no significant differences in the plasma kinetics of sulforaphane between the two GSTM1 genotype. GSTM1 nulls excreted 100% of ingested sulforaphane from standard broccoli, but only 69% from super broccoli. These results provide very good evidence to understand the contradiction between the epidemiological studies in US and epidemiological studies in Singapore and Shanghai on the effect of GSTM1 on the risk of cancer.