P.43 Metabolism and bioavailability of the benzyl glucosinolate glucotropaeolin in humans investigated by LC-ESI-MS/MS

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The aromatic benzyl glucosinolate glucotropaeolin is the major glucosinolate in nasturtium (Indian cress; Tropaeolum majus L.) and occurs almost exclusively in these Brassicaceae species (1). The potential of this plant can be regarded as being the source of the bioactive breakdown product, benzyl isothiocyanate (BITC) (2). A reliable and sensitive LC-ESI-MS/MS method for the quantification of BITC metabolites in human plasma and urine was developed for the first time. In this study, the following BITC metabolites have been considered: BITC-glutathione, BITC-cysteine-glycine, BITC-cysteine, and BITC-N-acetyl-L-cysteine. The assay development included: (I) synthesis of BITC conjugates as acting reference substances; (II) sample preparation based on protein precipitation and solid-phase extraction; (III) development of a quantitative LC-ESI-MS/MS method working in the multiple-reaction monitoring mode; (IV) validation of the assay; (V) investigation of the stability and the reactivity of BITC conjugates in vitro; (VI) application of the method to samples from a human pilot study (n=4).

The lower limits of quantification were in the range of 2.1-183 nM depending on analyte and matrix, whereas the average recovery rates from spiked plasma and urine were approximately 85% and 75%, respectively. After consumption of nasturtium, containing 1000 μM glucotropaeolin, quantifiable levels of BITC-NAC, BITC-Cys, and BITC-CysGly were found in human urine samples. Maximum levels in urine were determined 4 h after the ingestion of nasturtium. With regard to the human plasma samples, all metabolites were determined at individual distributions. These results will help to understand the bioavailability of BITC in dietary and its effects on human health. Moreover, the assay can be applied for further clinical pharmacological studies of BITC metabolites.

References:


P.44 Anti-carcinogenic effect of glucosinolate degradation products from Brassica local varieties

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The anti-carcinogenic effect of the glucosinolates-hydrolyzed derivatives, isothiocyanates (ITCs), has been extensively proven, essentially for sulforaphane (1). However, just traces of glucoraphanin (the glucosinolate precursor of sulforaphane) are found in most Brassica crops (2). Little is known about the putative anti-carcinogenic effect of ITCs derived from other glucosinolates. Three ITCs (ITC-MBG1, ITC-MBG2, and ITC-MBG3) derived from the most abundant glucosinolates identified in Brassica vegetables grown in Galicia (Northwestern Spain) were assayed to test their in vitro effect on survival and proliferation of cultured PC-3 and DU-145 human prostate cancer cells in the presence or in absence of the anti-cancer drug doxorubicin. The effect of each ITC was evaluated at four doses and three incubation times.

In general terms, the three ITCs showed a remarkable anti-carcinogenic effect, in most cases even higher than that observed with doxorubicin alone. The ITC-MBG1 inhibited cell growth in a dose and time-dependent manner against both PC-3 and DU-145 cells. The effect of this ITC was higher than that observed for doxorubicin, showing both compounds a synergistic effect, suggesting that the ITC-MBG1 could make the cancer cells more susceptible to this chemotherapeutic. ITC-MBG1 induced apoptosis mechanism in both cell types.

The ITC-MBG2 inhibited the cell growth of the PC-3 but not so for the DU-145 cell lines. The growth inhibition of the PC-3 cells was dose and time-dependent but only has a higher effect than doxorubicin after 72 h of incubation. The synergistic effect of these two compounds was partial and data indicate that apoptotic mechanisms are not involved in this inhibition. The performance of the ITC-MBG3 was similar to that observed for the ITC-MBG1. This compound improved the growth of both cell types in a dose-dependent manner. But in this case the maximal effect was achieved at 24 h and does not increase with longer incubation times. The highest concentration (2 and 4 μM) showed higher inhibition than doxorubicin. There is also a synergistic effect when ITC-MBG3 is incubated along with doxorubicin. Similar to ITC-MBG1, apoptotic mechanisms are involved in the inhibitory effect of the ITC-MBG3.

These preliminary results indicate that ITCs from Brassica local varieties could have a remarkable effect inhibiting prostate cancer cells proliferation and survival, and they have the potential to be used as chemotherapeutics in the future. Nevertheless, further investigation is required to understand the mechanism pathways, effects and implications of the adjuvant therapy with these ITCs.

References: