Effects of ICZ on the Expression of GST Isoenzymes in Hepa-1 Cells

Since ICZ can enhance total GST activities in Hepa-1 cells, its effects on the GST isoenzyme proteins were examined to understand whether the induced GST activity was due to increased expression of the GST isoenzymes, GST-α, GST-μ, and GST-π. Parallel to the enzyme activity, levels of GST-α protein increased with increasing concentrations of ICZ after 24 h of treatment, but the quantity was less than that of the BNF-treated group (Fig. 5). However, under the same conditions, no dose effect of ICZ on GST-μ and GST-π proteins was observed, and it seems that ICZ treatment tended to decrease the expression of these 2 proteins.

DISCUSSION

Induction of phase II enzymes including GST plays an important role in the detoxification of chemical carcinogens. Many animal experiments have indicated that hepatic GST activity can be increased after oral administration of glucosinolates or I3C from Brussels sprouts or other cruciferous vegetables. Indole glucosinolates are 1 group of glucosinolates present in large amounts in cruciferous vegetables. Glucosinolates can be hydrolyzed by the enzyme, myrosinase, which exists in plant tissues, to produce other compounds including I3C. Oral administration of I3C has been shown to increase hepatic xenobiotic-metabolizing enzymes, such as CYP1A, quinone reductase, and GST, in animals.[9,10,14,20] whereas when I3C was given i.p., no such induction was observed.[9] Therefore, the inducing activity of I3C may be due to other compounds formed in the gastrointestinal tract. We demonstrate that ICZ, one of the acid products of I3C, enhances GST enzyme activity in Hepa-1 cells. This suggests that the ICZ formed in the stomach contributes, at least partially, to the GST-induction activity evoked by the ingestion of cruciferous vegetables or I3C. However, other components in cruciferous vegetables and other I3C derivatives, such as isothiocyanate sulforaphane and crambene, have also been shown to increase GST in rats and in hepatocytes.[15,21]

In the present study, we observed differential expression of GST isoenzymes, in which GST-α is inducible by treatment with ICZ. On the contrary, ICZ seemed to suppress the constitutively expressed GST-μ and GST-π proteins. On the other hand, it is interesting to observe that BNF showed no pronounced effects on GST-μ protein and suppressed effects on GST-π protein. BNF has been shown to enhance total GST activity and to enhance gene expression of the rat GST Ya gene which corresponds to mouse GST-π.[22,23] Therefore, the regulation of GST isoenzymes by various xenobiotics may occur through different mechanisms. Likewise, because Hayes and Pulford indicated that GST isoenzymes are differentially expressed in different species and in different tissues,[5] our results suggest that the expression of GST isoenzymes might also be regulated in different ways. Overexpression of GST, especially GST-π, has been reported to be associated with drug resistance in cancer cell lines.[24] Therefore, suppression of GST-π by ICZ may play a role in chemotherapy.

The results demonstrate that the induction of the GST-α isoenzyme might be responsible for ICZ-induced GST activity. This is comparable to the studies done by Nijhoff et al. and Bogaards et al. in which they showed that plasma GST-α and rectal GST-α and GST-π were enhanced after human subjects consumed 300 g Brussels sprouts for 7 days.[25-27] Additionally, Mahéo et al. reported that isothiocyanate sulforaphane may induce mRNAs for rat GSTA1/A2 and GSTP1 but not for GSTM1 in rat hepatocytes. In addition, other isoenzymes, such as GST-θ, may also play a role in the induction of total GST activity by ICZ. However, we were unable to detect increased expression of GST activity by ICZ in HepG2 cells. This result is consistent to that of a previous study done by Castro et al. who reported that HepG2 cells do not express the GST-π enzyme.[28] Because HepG2 cells normally do not express GST-π, the most abundant enzyme in most tumor cells, the murine hepatoma cell line, Hepa-1, seems to be a better model to study the effect of ICZ on the induction of GST.

It has been shown that ICZ possesses CYP1A1 enzyme-inducing activity both in rats and in cultured cells,[10,11] and the induction occurs through the binding of ICZ to the Ah receptor. The induction of CYP1A1