Inhibition of Cytochromes P-450 and Induction of Glutathione S-Transferases by Sulforaphane in Primary Human and Rat Hepatocytes

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Abstract

The isothiocyanate sulforaphane (SF) is thought to be a potential chemoprotective agent. Its effects on Phase I and Phase II enzymes of carcinogen metabolism in primary cultures of rat and human hepatocytes have been investigated. Northern blot analyses of rat hepatocytes showed a dose-dependent induction of mRNA for rat glutathione S-transferases (rGSTs) A1/A2 and P1 but not M1. This was associated with enhanced levels of not only rGSTA1, A2, A4, A5, and P1 but also of rGSTs M1 and M2. On the other hand, the enzyme activities in rat hepatocytes associated with cytochromes P-450 (CYPs) 1A1 and 2B1/2, namely ethoxyresorufin-O-deethylase and pentoxysorufin-O-deethylykase, respectively, were decreased in a dose-dependent manner. In SF-treated human hepatocytes, hGSTA1/2 but not hGSTM1 mRNAs were induced, and the expression of CYP1A2 was unaffected, whereas the expression of CYP3A4, the major CYP in human liver, was markedly decreased at both mRNA and activity levels. These observations demonstrate that in intact human and rat hepatocytes, SF may both induce a number of GSTs and cause enzyme inhibition of some but not all CYPs and, in the case of CYP3A4, inhibit both its enzyme activity and its expression.

Introduction

A number of studies support the view that certain components of plant food derived from members of the family Cruciferae protect against carcinogenesis (1). An important group of compounds that have this property are the glucosinolates that are β-D-thioglucosides of a number of isothiocyanates. These may be released by the stress of tissue damage that activates myrosinase, a glucosinolate-specific glucosidase. One such isothiocyanate is SF (1-isothiocyanate-4-methylsulfinyl butane), which is relatively abundant in certain members of the genus Brassica (2) and is thought to be an anticarcinogen affecting both Phase I and Phase II enzymes of carcinogen metabolism, because it has already proved to be a competitive inhibitor of a CYP associated with the activation of nitrosamines (3) and an inducer of GST activity (2). Thus far, the anticarcinogenic activity of SF has been demonstrated in rodent systems, but in many cases, humans and rodents express different enzymes of detoxication, and to date, the effect of SF on human toxification and detoxication is unknown. AFB₁, metabolism illustrates these differences with respect to those CYPs responsible for the conversion of AFB₁ to its carcinogenic metabolite, AFBO, and the GSTs responsible for its inactivation (4, 5). In the present work, a comparison is made of the effect of SF on the expression of some relevant CYPs and GSTs in primary cultures of rat and human hepatocytes. Results obtained are compared with previous work from our laboratories using 1,2-dithiole-3-thione, another chemoprotective agent from Cruciferae, or its synthetic derivative, OPZ (5, 6). We report that SF, like OPZ, induces all normal rat hepatic GSTs and in addition rGSTP1 and rGSTA5, usually expressed in embryonic and perinatal livers, respectively. The effect of SF on GSTs in human liver is unknown, but with respect to isolated hepatocytes, the present work shows that whereas hGSTA1 and A2 are induced, hGSTM1 is not, and de novo expression of hGSTP1 does not occur. In the case of Phase I enzymes, it is an inhibitor of CYPs 1A and 2B1/2 in rat hepatocytes, whereas in human hepatocytes, CYP3A4 expression is inhibited quite strikingly.

Materials and Methods

Cell Isolation and Culture. Adult rat hepatocytes were obtained from the liver of male Sprague Dawley animals (150–200 g) and human hepatocytes from four adult donors undergoing resection for primary and secondary tumors (7). All experimental procedures complied with French laws and regulations and were approved by the National Ethics Committee. Hepatocytes were seeded in standard medium consisting of 75% MEM and 25% medium 199 supplemented with 10 μg of bovine insulin/mL, 0.2% bovine serum albumin, and 10% fetal calf serum. This medium, supplemented with 5 × 10⁻³m hydrocortisone but lacking serum, was renewed daily. SF was dissolved in DMSO before addition to the culture medium to give final concentrations of 5, 10, or 25 μM (in 0.2% DMSO, v/v). None of the concentrations of SF induced morphological alterations.

Enzyme Assays. Specific activities of GSTs in cell lysates were determined using 1-chloro-2,4-dinitrobenzene as a substrate. EROD, associated with CYP1A1, and PROD, associated mainly with CYP2B1/2, were measured directly on living cells. Reaction rates were determined under linear conditions with respect to incubation time and protein concentration (8).

Oxidation of nifedipine to its pyridine product by CYP3A4 was measured in cells cultured in a medium containing MEM but no phenol red. The concentration of nifedipine was 20 μM. Pyridine was separated and quantitated according to Guengerich et al. (9).

RNA Isolation and Northern Blot Analysis. Total RNA was extracted by the method of Chomczynski and Sacchi (10). Ten μg of total RNA was used for electrophoresis in a denaturing 6% (v/v) formaldehyde-1.2% (w/v) agarose gel and transferred onto Hybond-N nylon filters (Amersham, Arlington Heights, IL). After prehybridization, the filters were hybridized with 32P-labeled cDNA probes, washed, dried, and autoradiographed at −80°C. Specific cDNA probes for rGSTA1/2 and rGSTM1 were prepared by PCR (11). The other probes used were as follows: rGSTP1 (12), hGSTA1/2 (13), hGSTM1 (14), CYP3A4 (15), and CYP1A2 (16). An oligonucleotide specific for the 18S rRNA was 32P-labeled and used as a control. Relative amounts of mRNA were determined by densitometry and integration of unsaturated signals (Densyslab; Microvision instruments, Evry, France).
GST Subunit Separation. Cytosolic protein was obtained from washed cells by sonication and sedimentation of cell debris. GST subunits were separated on a 5-μm particle size C-18 HPLC column (218TP54 protein and peptide column; Vydac, Theale, United Kingdom) according to Östlund-Farrants et al. (17). In most separations, an acetonitrile gradient from 30—65% was attained in 60 min with a flow rate of 1 ml/min, but when a separation of GSTA3 and A5 was required, the gradient between 43 and 49% was reduced by extending its attainment to 40 min.

Statistical Analysis. Numerical values are expressed as the mean ± SD, and significance, using Student’s t test, was set at a limit of ≤5%.

Results

Effects of SF on GSTs. After exposure of rat hepatocytes to 10 μM SF for 48 and 72 h, GST-1-chloro-2,4-dinitrobenzene transferase activity was increased 2- and 3-fold, respectively, compared with that of controls. The effect of SF on the expression of GST mRNAs, as determined by Northern blots, varied with the GST. After 24 h of treatment with 5, 10, or 25 μM SF, mRNAs hybridizing with rGST A1/A2 and P1 cDNAs increased in a dose-dependent manner, but mRNA hybridizing with cDNA for rGSTM1 was little affected. For example, exposure to 25 μM SF for 24 h increased the expression of combined mRNAs for rGSTA1 and A2 8-fold and increased the cDNA for rGSTP1 11-fold (Fig. 1). Induction did not persist and was no longer apparent 48 h after the above-mentioned exposure to SF (data not shown). HPLC analysis of GST subunits after 72 h of treatment with 10 μM SF showed induction of all subunits present in the normal adult rat liver, namely GSTs A1, A2, A3, A4, GSTM1, and GSTM2, together with GSTP1, a characteristic of embryonic liver, and GSTA5, usually restricted to the perinatal period (Fig. 2).

In human hepatocytes treated with SF for 72 h, hGSTA1/2 mRNAs gave a dose-dependent response such that 5, 10, and 25 μM SF resulted in 5-, 9-, and 11-fold increases in hGSTA1/2 mRNA, respectively (Fig. 1). One of the above schedules of SF treatment affected hGSTM1 expression in the one available cell population that was hGSTM1 positive. hGSTP1 was not detected in either control or SF-treated human hepatocyte cultures.

Effects of SF on CYPs. Both EROD and PROD activities were measured in control and SF-treated rat hepatocytes. A time course analysis determined from 6—72 h after the addition of either 5, 10, or 25 μM SF to the culture medium showed a dose-dependent inhibition of EROD activity beginning with 6 h of treatment with 5 μM SF and reaching more than 80% after 24 h of treatment with 25 μM SF. Effects on PROD were less pronounced. Thus, after a 6-h exposure, 25 μM SF was required to give an observable decrease in activity. After 24 h, a dose-dependent inhibition was obtained, but maximum inhibition with 25 μM at 24 h did not exceed 60%. If incubation with SF was not continued after the first 24-h treatment period, inhibition of EROD and PROD was no longer detectable after 48 and 72 h, respectively (Fig. 3). In human hepatocytes, SF was without effect on the expression of CYP1A2 mRNA, whatever the concentration. In contrast, the expression of CYP3A4 mRNA was decreased by SF. After a 72-h treatment with 10 or 25 μM SF, the decrease was 2.5- and 1.5-fold, respectively (Fig. 1). The oxidation of nifedipine (a CYP3A4-specific substrate) in human hepatocytes was also inhibited in the presence of SF. Indeed, treatment with 10 and 25 μM SF for 48 h resulted in a decrease in nifedipine oxidation of 30 and 45%, respectively (Fig. 4).

Discussion

The present work is a study of the chemoprotective potential of the isothiocyanate SF. A number of other isothiocyanates have been associated with chemoprotection against a variety of chemical carcinogens (2), and SF has already been shown to prevent the development of mammary tumors in Sprague Dawley rats treated with 9,10-dimethyl-1,2-benzanthracene (18). It has been shown to induce GST activity and to behave as a competitive inhibitor of CYP2E1 (an enzyme involved in nitrosamine activation), but its potential for chemoprotection against hepatocarcinogenesis has yet to be investigated.

Recently, we have shown that OPZ, a well-known chemoprotective agent with respect to AFBl carcinogenesis in the rat, induces GSTs including rGSTA5 with high activity toward AFBO, the carcinogenic metabolite of AFBl. These conclusions result from experiments with the whole animal (6, 19) and isolated rat hepatocytes in primary culture (6). As a result, it was concluded that hepatocytes in primary culture were a good surrogate for the intact liver, and for this reason, they should enable the acquisition of otherwise unavailable information concerning mechanisms of chemoprotection from hepatocarcino-
genesis in man (5). It was shown that although induction of hGSTs occurred, it did not involve enzymes with a high capacity to detoxify AFBO (5), and it was also noted that the hGSTs with the highest activities, namely hGSTM1 and T1, were subject to frequent genetic polymorphism, resulting in 50 and 20% of the population with null phenotypes, respectively, and 10% lacking both phenotypes (20, 21).

By using cultured rat hepatocytes, we have shown that like OPZ, SF induces all hepatic GSTs, including rGSTA5, and inhibits CYP1A and CYP2B1/2 activities in a reversible fashion. These observations are persuasive evidence that both induction of Phase II enzymes and inhibition of Phase I enzyme activities together may play a major role in chemoprotection by SF.

In human hepatocytes, SF behaves in a manner similar to that of OPZ with respect to hGSTs, inducing hGSTA1/2; therefore, like OPZ, it can only have a small effect on AFB1 detoxication via GSTs, because enzymes with good activity toward AFBO (like rGSTA5) are neither present in the normal human liver nor induced de novo. However, with respect to CYP1A2 and CYP3A4, there was an interesting difference. SF inhibits CYP1A2 activity more effectively than OPZ in the only case studied (data not shown). SF also has a negative effect on the expression

![Graph A](image1.png)

Fig. 2. GST subunit composition in 48-h control and SF-treated hepatocytes. GST subunits from untreated (C) or SF-treated (SF) hepatocytes were purified by a glutathione-affinity column and separated by HPLC as described in “Materials and Methods.” Inset, separation of rGSTA3 and rGSTA5.

![Graph B](image2.png)

Fig. 3. Effects of SF on EROD and PROD activities in rat hepatocytes in primary culture. Rat hepatocytes were cultured for 0—48 h in the absence or presence of 5 (○), 10 (■), and 25 (△) μM SF; and EROD (A) and PROD (B) activities were measured at different time intervals. Rat hepatocytes were cultured for 24 h in the presence of 25 μM SF, and EROD and PROD activities were measured 48 h after a unique treatment of 25 μM SF during 24 h (○). Both activities are expressed as the mean percentage of control ± SD of three independent experiments.
hepatocytes are a priority in our future research. Carcinogenesis in rat and man has yet to be clarified. Pharmacokinetic expressed as nanomoles of metabolites (dihydroxypyridine nifedipine) formed per minute per milligram of protein. Three experiments were performed in triplicate on three human cases. Significant differences between treatment and control groups, as determined by Student’s t test (P ≤ 5%), are denoted by an asterisk.

of CYP3A4. By comparing CYP inhibition performed on rat hepatocytes in culture after OPZ treatment (4) or SF treatment, we can conclude that CYP1A1 and CYP2B1 inhibitions are much more persistent with SF. Indeed, after OPZ treatment, the inhibition appeared after 40 min but is not detectable 24 h after the beginning of the treatment. By contrast, 6 h of treatment with SF are needed to detect CYP inhibition, which is still detectable until at least 48 h. These observations suggest that SF could produce more prolonged inhibition of AFB1 activation than OPZ. On this basis, we could assume that this compound is at least as effective as OPZ in chemoprotection against AFB1 hepatocarcinogenesis in the rat.

To summarize, our results demonstrate for the first time that SF can inhibit CYPs and induce GSTs in intact liver cells from both rat and man. Like other chemoprotectors, the effectiveness of SF depends on whether or not it affects enzymes specific for the activation and inactivation of the carcinogen in question. For example, from the present results, SF (like OPZ) would seem to give better protection against AFB1 carcinogenesis in the rat than in man. The principal reason for this is that unlike the situation in the rat, neither of these substances induces human enzyme sufficiently powerfully to detoxify AFB1. The role of CYP inhibition in chemoprotection of AFB1 carcinogenesis in rat and man has yet to be clarified. Pharmacokinetic studies of AFB1 metabolism in both control and SF-treated human hepatocytes are a priority in our future research.

References

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