Oxidative Conversion of Isothiocyanates to Isocyanates by Rat Liver

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This report describes the oxidative metabolism of isothiocyanates to isocyanates catalyzed by rat liver microsomes. Incubation of 2-naphthylisothiocyanate, microsomes, and NADPH yielded either N,N'-di-2-naphthylurea or, on inclusion of 2-aminofluorene in the incubations, N-2-naphthyl-N'-2-fluorenylurea. These ureas were formed by the production of the known genotoxicant, 2-naphthylisocyanate, which reacted with its hydrolysis product, 2-aminonaphthalene, to yield the symmetrical urea, or with 2-aminofluorene to form the mixed urea. Formation of N,N'-di-2-naphthylthiourea was also observed because 2-aminonaphthalene reacted with the substrate. Urea formation was dependent on the microsomes, NADPH, and oxygen. Use of microsomes from rats previously treated with Aroclor 1254 increased urea formation greater than 10-fold. The enzyme activity was inhibited by α-naphthoflavone, flavone, or CO, and slightly inhibited by metyrapone, 7-ethoxycoumarin, or SKF-525A. It was not inhibited by methimazole or paraxoxon, suggesting that neither flavin-containing monoxygenase nor hydrolytic enzyme was involved. These data are consistent with a cytochrome P450-dependent, oxidative desulfuration of 2-naphthylisothiocyanate to yield 2-naphthylisocyanate. Further studies with the isomeric 1-naphthylisothiocyanate and the dietary benzylisothiocyanate showed that they can also be metabolized to their isocyanates, as evidenced by the trapping of isocyanates with 2-aminofluorene to form the mixed ureas. — Environ Health Perspect 102(Suppl 6):115-118 (1994)

Key words: isothiocyanates, isocyanates, Aroclor-induced rat liver microsomes, cytochrome P450, NADPH, 2-naphthylisothiocyanate, 1-naphthylisothiocyanate, benzylisothiocyanate.

Introduction

Organic isothiocyanates (R=N=C=S) are used extensively in the production of pesticides, and polyurethanes or polyureas for the manufacture of varnishes, paints, foams, and plastics (1). Chemotherapeutic dialkyl- nitrosoureas (2) and alkylformamides that are used as industrial solvents (3) can produce isocyanates. Chemically, isocyanates are capable of modifying proteins (4) and nucleic acids (5,6); biologically, they can cause chromosome aberrations and sister chromatid exchanges (7-9), mutations (6,10), and cancer (11,12).

A related class of compounds, the isothiocyanates (R=N=C=O), possess a wide range of biologic activities (13).

Moreover, some isothiocyanates can be generated from a variety of vegetables by enzymatic hydrolysis of their glucosinolates (14). Several synthetic and dietary isothiocyanates can inhibit the metabolic activation of certain carcinogens in vitro (15-17) and tumorigenesis in experimental animals (15,18-21). Although relatively little attention has been focused on the possible adverse long-term effects exerted by isothiocyanates, a number of isothiocyanates have been found to be mutagenic for Salmonella typhimurium TA100; among them, dietary allyl isothiocyanate is the most potent (22,23). Furthermore, allyl isothiocyanate has been found to cause bladder tumors in F344 rats (24).

Organic isothiocyanates are considerably less reactive than their isocyanate counterparts. Although examples of oxidative removal of sulfur such as the conversions of parathion to paraoxon (25) and α-naaphthylthiourea to α-naaphthylurea (26) through metabolism are known, the analogous metabolic conversion of 2-naphthylisothiocyanate (2-NITC), an antitumor agent (20), to the more reactive known genotoxicant 2-naphthylisocyanate (2-NIC) (6), has only recently been reported (27). Further studies showed that 1-naphthylisothiocyanate (1-NITC), a chemopreventive agent (20,21) and benzyl- isothiocyanate (BITC), a component of cruciferous vegetables (14) and an antitumor agent (18,19), can also be converted to their isocyanates.

Materials and Methods

Materials, synthetic and commercial, and the methods required to characterize the metabolic activation of 2-NITC were described previously (27). N-1-Naphthyl- N'-2-fluorenylurea (1-NFU) was prepared by reacting equimolar amounts of 2-aminofluorene (2-AP) and 1-naphthylisocyanтate (1-NIC) in dry acetone. N-Benzyl-N'-2-fluorenylurea (BFU) was prepared by reacting equimolar amounts of 2-AF and benzylisocyanate in dry ether. The instrumental procedures for the analyses of the products from the metabolism of 1-NITC and BITC are similar to those for analyses of products from 2-NITC (27) but use different HPLC solvent systems. For analyses of the mixed urea formation from 1-NITC, the effluents were monitored at 284 nm at a flow rate of 1 ml/min using solvent system A: stepwise linear gradient from 30% methanol/H2O to 65% methanol in 5 min, to 75% methanol in 30 min, then to 100% methanol in 5 min. Rf (min) are: 1-NITC, 33.2 and 1-NFU, 31.1. For analyses of the mixed urea from BITC, the effluents were monitored at 286 nm at a flow rate of 1 ml/min using solvent system B: linear gradient from 30% acetonitrile/H2O to 60% acetonitrile in 10 min, at 60% acetonitrile for 15 min, then to 100% acetonitrile linearly in 5 min. Rf (min) are BITC, 18.6 and BFU, 19.4.
Results and Discussion

Identification of Products from the Metabolism of 2-NITC

2-NITC on incubation with NADPH and liver microsomes from an Aroclor 1254-treated rat yielded the HPLC profile shown in Figure 1A (27). The two products were identified as di-2-naphthylthiourea (D-2-NT) and di-2-naphthylurea (D-2-NU) based on comparisons of HPLC retention times, UV spectra, and, in the case of D-2-NT, mass spectra, with those of authentic compounds. When 2-AF was included in the incubation to provide a higher and more constant level of amine, ethyl acetate extracts of the incubation mixture gave the typical HPLC profile shown in Figure 1B (27). In addition to the two peaks identified as D-2-NT and D-2-NU, the peak at 25.8 min was identified as N-2-naphthyl-N'-2-fluorenylurea (2-NFU) by comparing the retention time, UV, and mass spectra with those of the authentic compound.

Figure 1C (27) shows the dependency on microsomes of this reaction in the presence of 2-AF. In the absence of microsomes, 2-AF reacted with the substrate to give N-2-naphthyl-N'-2-fluorenylthiourea (2-NFT). When NADPH was omitted from the incubation in the presence of 2-AF, an HPLC profile similar to Figure 1C was obtained, indicating the dependency of the reaction on NADPH as well. These results indicated that 2-NITC was enzymatically converted to 2-NIC, which hydrolyzed readily to 2-aminonaphthalene (2-AN). Reaction of 2-AN with 2-NITC yielded D-2-NT and, with 2-NIC, formed D-2-NU. In the presence of 2-AF, 2-NFU was also formed, confirming the formation of 2-NIC as a reactive metabolite.

In order to determine whether the di-substituted urea products D-2-NU and 2-NFU might be generated enzymatically from the initial products D-2-NT and 2-NFT, these compounds were subjected to the same incubation conditions in the presence of 2-AF. The results disclosed that the thioureas were stable under these conditions and that no ureas were formed. These results further confirmed that reactive 2-NIC was formed in the enzymatic incubation mixture. The metabolic conversion of 2-NITC to 2-NIC and further reactions are outlined in Reaction Scheme 1 (27).

Inducibility of the Enzyme(s) Responsible for the Metabolic Activation

In order to determine whether the enzyme(s) responsible for this reaction is(are) inducible, incubations were carried out with induced or uninduced liver microsomes. Data presented in Table 1 (27) clearly indicate that the transformation of 2-NITC to 2-NIC is mediated by enzyme(s) inducible with Aroclor 1254, and that the Aroclor induction increased the formation of D-2-NT and 2-NFU by approximately 3- and 10-fold, respectively.

**Effects of Argon and Paraoxon on the Formation of D-2-NT and 2-NFU from the Metabolism of 2-NITC in the Presence of 2-AF**

Argon was used to exclude oxygen from the incubation mixture to determine if formation of both products is oxygen dependent. D-2-NT is produced from the reaction of 2-NITC with 2-AN, which could be derived either through reduction or hydrolysis of 2-NITC. Since 2-NIC may also be derived from a hydrolytic reaction of 2-NITC, paraoxon, an inhibitor of hydrolytic enzymes, was used. The results (27) showed that argon reduced the formation of 2-NFU by 49% and D-2-NT by 25%. Thus, these two products were derived at least in part from an oxidative metabolism. Paryonon did not inhibit the production of these compounds, thereby supporting the conclusion that these products did not result from a hydrolytic process.

**Effects of Oxidative Enzyme Inhibitors on the Formation of 2-NFU from Metabolism of 2-NITC in the Presence of 2-AF**

In order to determine whether either cytochrome P450 (P450) or flavin-containing monoxygenase (FMO) was involved...
Table 1. Effect of Aroclor treatment on metabolism of 2-NITC.

| Microsomes | Incubation components | D-2-NITC, nmol/mg protein/min | 2-NFU, nmol/mg protein/min |
|------------|-----------------------|-------------------------------|----------------------------|
| Induced    | Complete system       | 0.97 ± 0.35 (3)              | 1.01 ± 0.15 (3)            |
|            | - NADPH              | 0.12 ± 0.10 (3)              | 0.00 (3)                  |
| Uninduced  | Complete system       | 0.30, 0.13 (3)               | 0.11, 0.22 (3)            |
|            | - NADPH              | 0.17, 0.06 (3)               | 0.02, 0.14 (3)            |

*incubations were carried out with 2-NITC, 2-AF, NADPH, and microsomes (0.3 or 0.4 mg) (complete system), or complete system without NADPH, or complete system without microsomes at 37°C for 30 min. Experimental details are as described (27). Values represent the means ± SD obtained with microsomes from three animals. 

The mean value of nmol per incubation from three rats was 27 times as high as that of the complete system without microsomes. 

The mean value of nmol per incubation from three rats was 40% more than that of the complete system without microsomes. 

Individual values from the microsomes of two rats are shown. The mean value of nmol per incubation from two rats was the same as that of the complete system without microsomes.

The mean value of nmol per incubation from two rats was 7% more than that of the complete system without microsomes.

Table 3. Comparisons of the formation of mixed ureas (2-naphthyl-N'-2-fluorenylurea, 1-naphthyl-N'-2-fluorenylurea, benzyl-N'-2-fluorenylurea, respectively) in the metabolism of three isothiocyanates in the presence of 2-AF.

| Microsomes | Substrate | Incubation components | Mixed urea, nmol/mg protein/min |
|------------|-----------|-----------------------|--------------------------------|
| Aroclor-induced | 2-NITC | Complete system | 11.60 ± 2.73 |
|             | 2-AF     | Complete system | 11.65 ± 0.36 |
| Uninduced   | 2-NITC   | Complete system | < 0.1 |
|             | 2-AF     | Complete system | < 0.1 |
| Aroclor-induced | 1-NITC | Complete system | 1.17 ± 1.15 |
|             | 2-AF     | Complete system | 1.12 ± 0.36 |
| Uninduced   | 1-NITC   | Complete system | 1.17 ± 1.15 |
|             | 2-AF     | Complete system | 1.12 ± 0.36 |
| Aroclor-induced | BITC   | Complete system | 1.95 ± 0.12 |
|             | BITC     | Complete system | 2.10, 2.14 |
| Uninduced   | BITC     | Complete system | 1.95 ± 0.12 |
|             | BITC     | Complete system | 2.10, 2.14 |

*incubations were carried out as described in Table 1 using 0.4 mg of microsomes from Aroclor-treated or untreated rats. Products from 2-NITC metabolism were analyzed using HPLC system II described previously (27); analyses of products from 1-NITC and BITC were carried out using solvent system A and B, respectively, as described in "Materials and Methods." Data are either mean ± SD obtained from microsomes from three rats or individual numbers from microsomes from two rats. Omission of microsomes from the complete system decreased product formation by approximately 50%.

Comparisons of the Metabolism of 2-NITC, 1-NITC, or BITC with Induced or Uninduced Rat Liver Microsomes in the Presence of 2-AF

Incubations were carried out as described (27). Identification of products from 1-NITC and BITC was based on comparison of HPLC retention times and UV spectra with the authentic compounds. As shown in Table 3, the reactivity of 1-NITC is comparable to that of 2-NITC and they may be catalyzed by the same Aroclor-inducible P450 system. In contrast, dietary BITC has a lower reactivity than that of the other two compounds, and the use of Aroclor 1254-induced microsomes did not increase the activity. These results suggest that the P450 that activates BITC may be different from that responsible for the activation of the other two compounds. This is the first demonstration of this type of metabolic activation of a dietary isothiocyanate. Further studies are necessary to gain insight into the systems responsible for the activation of dietary isothiocyanates.

Conclusions

The metabolic transformation reported here has only recently been described (27). Although El-Hawari and Plaa (28) had shown previously that the NADPH-dependent protein binding of isothiocyanates 1-NITC required the oxidative conversion of the compound to a metabolite in rat liver microsomes by a P450 system, they did not succeed in identifying the reactive binding species, presumably 1-naphthylisothiocyanate by analogy to the observations described herein. The recent study showed that 2-NITC can be converted oxidatively to 2-NIC (27), which has been found to be mutagenic (6). 2-NITC also may be converted to its hydrolysis product, 2-AN, which is a carcinogenic aromatic amine (29). Thus, this type of conversion has the potential to cause genetic effects. Since the dietary isothiocyanates that can be generated from a variety of vegetables encompass diverse structural features (14), it seems prudent to clarify their potential for inducing genetic damage before their use as anticarcinogens is emphasized.
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