Relative Importance of Bacterial and Mammalian Nitroreductases for Niridazole Mutagenesis

Jeffrey L. Blumer, Allen Friedman, LeRoy W. Meyer, Edward Fairchild, Leslie T. Webster, Jr., and William T. Speck

Divisions of Pediatric Pharmacology and Infectious Disease, Department of Pediatrics, Rainbow Babies and Children's Hospital; [J. L. B., A. F., L. W. M., W. T. S.]; and the Genetics Center [J. L. B.] and Department of Pharmacology [J. L. B., E. F., L. T. W.], Case Western Reserve University School of Medicine, Cleveland, Ohio 44106

ABSTRACT

Niridazole is a nitrothiazole anthelmintic agent used to treat schistosomiasis. Its antibacterial activity was found to require the presence of the nitro group; a synthetic desnitro analog was completely inactive. Niridazole was mutagenic for Salmonella tester strains TA1538, TA98, and TA100, suggesting that it was both a frame-shift and a base substitution-type mutagen. It was effective under both aerobic and anaerobic conditions, while similar testing of the desnitro niridazole produced consistently negative results. Addition of rat liver S-9 fraction under either aerobic or anaerobic conditions did not enhance mutagenicity. However, since bacterial killing limited the dose of niridazole to 0.33 µg/plate in standard tester strains (1/20 K m for the mammalian liver enzymes), further studies were performed using niridazole-resistant, histidine-dependent mutants derived from strains TA98 and TA100. These mutants were found to be nitroreductase deficient and to resist the mutagenic effects of niridazole, in the presence or absence of S-9, up to concentrations of 10 µg/plate. In addition, even at niridazole concentrations of up to 100 µg/plate, rat liver S-9 was ineffective in enhancing the mutagenicity of niridazole. These results suggest that the mutagenicity of niridazole is dependent on its aromatic nitro group and a specific bacterial nitroreductase.

INTRODUCTION

During the past 50 years, a variety of structurally diverse nitroheterocycles have been synthesized and used as food additives (26), antibacterial agents (17), and antiparasitic agents (17, 18). A majority of these compounds are nitrofuran derivatives, among which nitrofurazone has been most extensively studied. The antimicrobial activity of these nitrofuran derivatives has been demonstrated for anaerobic and facultative aerobic bacteria. Studies on the mechanism of such antibacterial activity have shown that reduction of the nitro group by bacterial pyridine nucleotide-dependent nitroreductases is essential (3, 13, 20). This metabolic pathway involves a 6-electron reduction of the nitroaromatic parent compound to the amine with the formation of reactive nitroso and hydroxylamine intermediates (16). In most cases, the amine product has been isolated.

In addition to their antibacterial activity, these nitroaromatic compounds have also been identified as prokaryotic mutagens (21, 22) and mammalian carcinogens (9). Such extreme forms of toxicity require metabolic activation of the parent nitroaromatic compounds, and this activation is presumably catalyzed by the same enzyme which is responsible for their antibacterial effects (3, 7, 13, 20). Consistent with this hypothesis is the detection of covalently bound drug, presumably from nitroso or hydroxylamine precursors, associated with macromolecules of bacterial origin (20).

Niridazole (Chart 1) is a synthetic nitrothiazole antibiotic which has been used extensively to treat schistosomiasis (18). Recent studies from several laboratories (11, 21, 22) have demonstrated that niridazole is mutagenic for prokaryotic cells. In addition, Urman et al. (27) have shown that the compound is carcinogenic for mice. The metabolism of niridazole by bacteria has not been studied. However, in mammalian microsomal systems, nitroreduction has not been demonstrable under aerobic conditions (5). Under anaerobic conditions, rat liver microsomes can reduce niridazole to a hydroxylamine metabolite, but complete reduction to the amine has not been demonstrated (15). More recently, the aerobic formation of at least 4 niridazole metabolites has been found with rodent liver microsomal systems (5, 6). One of these metabolites was shown to be a glycol, which suggests the formation of a reactive epoxide intermediate during aerobic metabolism (23). It is possible that the formation of this alkene oxide is responsible for the mutagenic and/or carcinogenic effects of niridazole observed under more physiological conditions. Alternatively, the aerobic activation of niridazole to a mutagen and/or a carcinogen may proceed via nitrooxide free radical formation with the subsequent generation of superoxide anion as described for nitrofurazone (19).

The present study was designed to evaluate the role of aromatic nitroreductase activity in the antibacterial and mutagenic effects of niridazole. It was found that nitroreduction was an essential prerequisite for biological activity under both aerobic and anaerobic conditions. Addition of rat liver S-9 had no effect on the dose-dependent mutagenicity of niridazole in sensitive Salmonella typhimurium tester strains. Moreover, tester strains deficient in nitroreductase activity could not be mutagenized by niridazole in the presence of rat liver S-9 and oxygen. The significance of these results for mammalian mutagenesis is discussed.

MATERIALS AND METHODS

Chemicals. Niridazole (Ciba-Giegy, Summit, N. J.), 2-nitrofluorene (Eastman Kodak Co., Rochester, N. Y.), sodium azide (Fisher Scientific Co., Pittsburgh, Pa.), and 2-aminopanthracene (ICN, Irvine, Calif.) were dissolved in sterile dimethyl sulfoxide.
2-Chloroethyl isocyanate was obtained from Fluka for quantitative determination of mutagenic activity. Anaerobic mutagenicity testing was performed by placing the inoculated agar plates into Gas Pak jars (BBL Microbiology Systems, Baltimore, Md.) which were then incubated at 37° in the dark for 14 hr. The plates were then removed from the jars and incubated aerobically at 37° for an additional 34 hr (24).

Mutagenesis Testing. The pour-plate incorporation method for quantitative determination of mutagenic activity was performed as initially described by Ames et al (2). Compounds inducing more than twice the number of spontaneous revertants found on control plates were considered mutagenic. An aerobic mutagenic testing was performed by placing the inoculated agar plates into Gas Pak jars (BBL Microbiology Systems, Baltimore, Md.) which were then incubated at 37° in the dark for 14 hr. The plates were then removed from the jars and incubated aerobically at 37° for an additional 34 hr (24).

Mutagenic Activity of Niridazole in Salmonella Tester Strains. Chart 2 shows the dose-dependent mutagenicity of niridazole for 3 bacterial tester strains. Strain TA1538 appeared to be the most sensitive of the 3 strains. Qualitatively similar results have been reported by McCann et al. (22), suggesting that niridazole acts predominantly as a frame-shift mutagen.

Table 1

<table>
<thead>
<tr>
<th>Organism</th>
<th>Total isolates tested</th>
<th>Niridazole</th>
<th>Desnitro niridazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>19</td>
<td>32</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Salmonella</td>
<td>3</td>
<td>16</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Aerobacter</td>
<td>2</td>
<td>32</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>2</td>
<td>64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>2</td>
<td>32</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Serratia</td>
<td>2</td>
<td>32</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Proteus</td>
<td>3</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Providencia</td>
<td>2</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>1</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Neisseria meningitidis</td>
<td>1</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Haemophilus influenzae b</td>
<td>1</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
</tbody>
</table>
Two additional tester strains (TA98 and TA100) were studied because of their increased sensitivity and spectrum of response (22). Niridazole was more effective in causing reversion to histidine independence in strain TA98 than it was in the parental strain TA1538 (Table 2; Chart 2). Likewise, TA100 displayed greater niridazole sensitivity than did its parent strain TA1535 (Table 2; Chart 2). This mutagenic activity in strain TA100 suggests that niridazole may also act to cause base substitutions. At any given dose, the number of revertants per plate was substantially greater with TA100 than it was with TA98 (Table 2); however, the percentage of increase above background was similar in the 2 strains. In all subsequent work, TA98 and TA100 were used because of their greater sensitivity to niridazole than were the TA1535 and TA1538 Salmonella tester strains.

Bacterial killing was apparent in both strains at doses of niridazole above 0.33 μg. The killing was first assessed by visual inspection of the agar plates. Bacterial colonies appearing abnormally small or plates containing regions of opacity were considered suggestive of killing. Colonies were then chosen from these regions and streaked upon fresh minimal medium. Killing was confirmed by the failure of these selected colonies to grow.

**Effect of O2 and Rat Liver S-9 on the Mutagenic Activity of Niridazole.** Chart 3 depicts the effects of O2 and the addition of an S-9 fraction derived from Aroclor-treated rats on the mutagenicity of 0.33 μg of niridazole. Similar effects were observed at all doses except when bacterial killing was apparent. Anaerobiosis increased the number of revertants by 37% and 93% in strains TA98 and TA100, respectively.

Under aerobic conditions, the addition of rat liver S-9 resulted in a 43% increase in the number of TA98 revertants and a 13% increase in TA100 revertants. Anaerobic incubation in the presence of S-9 increased the number of revertants by 63% in TA98, but similar treatment of TA100 resulted in a slight (15%) decrease.

None of the observed changes was statistically significant except for the effect of anaerobiosis on the number of TA100 revertants in the absence of S-9 (p < 0.05). These results suggest that rat liver enzymes can contribute very little to the observed mutagenicity of niridazole when drug concentrations are maintained below that which causes bacterial killing.

**Roles of Aromatic Nitro Group in Niridazole Mutagenesis.** The desnitro derivative of niridazole was synthesized in order to evaluate the role of the aromatic nitro group in the antibacterial and mutagenic effects of niridazole. As shown in Table 1, the nitro group is required for antibacterial activity. Chart 4 depicts the mutagenic effects of desnitro niridazole in Salmonella tester strains TA98 and TA100. Over a 4-log dose range, no bacterial killing and/or mutagenic activity could be demonstrated.

**Role of Aromatic Nitroreductase Activity in the Antibacterial and Mutagenic Activity of Niridazole.** To assess the role of bacterial nitroreductase in the antibacterial and mutagenic effects of niridazole, several niridazole-resistant but histidine-dependent strains were developed from both TA98 and TA100. One mutant line from each strain was then selected for further study. These were designated TA98NR101 and TA100NR3, respectively.

Bacterial nitroreductase activity was assessed under both aerobic and anaerobic conditions using nitrofurazone as the substrate. Anaerobiosis resulted in an approximate 2-fold stim-

---

**Table 2**

<table>
<thead>
<tr>
<th>Compound</th>
<th>TA98</th>
<th>TA100</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>21</td>
<td>139</td>
</tr>
<tr>
<td>2-nitrofluorene</td>
<td>780</td>
<td>148</td>
</tr>
<tr>
<td>Sodium azide</td>
<td>21</td>
<td>134</td>
</tr>
<tr>
<td>2-Aminoanthracene</td>
<td>14</td>
<td>134</td>
</tr>
<tr>
<td>Niridazole</td>
<td>0</td>
<td>178</td>
</tr>
<tr>
<td>0.01</td>
<td>32</td>
<td>100</td>
</tr>
<tr>
<td>0.50</td>
<td>32</td>
<td>100</td>
</tr>
<tr>
<td>1.00</td>
<td>32</td>
<td>100</td>
</tr>
<tr>
<td>3.00</td>
<td>32</td>
<td>100</td>
</tr>
<tr>
<td>10.00</td>
<td>32</td>
<td>100</td>
</tr>
</tbody>
</table>

* * *

*Killing observed; see text for details.*
Likewise, for TA100, there were 120 and 106 revertants in the absence of S-9 fraction from Aroclor-treated rats. In the absence of drug, there were 21 and 30 aerobic and 23 and 27 anaerobic TA98 revertants in the absence (•, O) and presence (•, D) of S-9 fraction from rat liver. Studies were performed both in the presence (A, A) and absence (O, O) of S-9 fraction from Aroclor-treated rats. In the absence of drug, TA98 and TA100 tester strains. Salmonella tester strains TA98 (top) and TA100 (bottom) were plated on minimal nutrient agar containing 1 to 100 µg of desnitro niridazole. Plates were incubated for 24 hr at 37° either aerobically (A, •) or anaerobically (A, O). Studies were performed both in the presence (•, D) and absence (O, O) of S-9 fraction from rat liver. In the absence of drug, there were 21 and 30 aerobic and 23 and 27 anaerobic TA98 revertants in the absence and presence of S-9. Likewise, for TA100, there were 120 and 106 aerobic and 139 and 188 anaerobic revertants.

Niridazole nitroreduction was assessed under anaerobic conditions (Table 3). Reductase activity was detected in both TA98 and TA100 but was not demonstrable in either of the resistant mutants. This suggested that an inability to reductively activate niridazole was, at least in part, responsible for the resistance of strains TA98NR101 and TA100NR3. Table 3 also shows that rat liver S-9 contains a substantial amount of anaerobic niridazole nitroreductase activity. Nevertheless, S-9 did not significantly enhance the number of niridazole-induced reversions in TA98 and TA100 (Chart 3).

Table 4 shows the mutagenic response of the nitroreductase-deficient strains to standard chemical mutagens in the presence and absence of rat liver S-9. In the absence of S-9, both TA98NR101 and TA100NR3 gave the expected response to 2-aminoanthracene (Table 2), and the addition of S-9 fraction increased the number of revertants in both strains. With sodium azide, strain TA100NR3 gave results indistinguishable from those obtained with strain TA98 (Table 2). The response of TA98NR101 to 2-nitrofluorene was considerably blunted when compared to that of the parental strain TA98 (Table 2), consistent with the observed deficiency in nitroreductase. Nevertheless, the number of revertants was substantially increased (≥5-fold) by the addition of rat liver S-9.

Under aerobic conditions, the nitroreductase-deficient mutants were resistant to both the antibacterial effects of niridazole over a 4-log dose range (data not shown) and the mutagenic effects of niridazole over a 2-log dose range (Chart 6). At niridazole levels of > 10 µg/plate, a marked increase in the number of revertants was noted in both mutants. Similarly, the addition of S-9 had no effect on the number of revertants until drug levels of > 10 µg/plate were obtained. At 100 µg/plate, the addition of S-9 increased the number of revertants in TA98NR101 and TA100NR3 by 45 and 35%, respectively.

Studies with the reductase-deficient mutants under anaerobic conditions were hampered by the extensive bacterial killing evident at niridazole concentrations of > 10 µg/plate. In TA98NR101, the addition of S-9 in the presence of niridazole at 10 µg/plate (the highest concentration yielding no apparent killing) enhanced the number of revertants by 90%. Similarly, in TA100NR3, the addition of S-9 in the presence of niridazole at 3 µg/plate resulted in a 98% enhancement. Thus, at sufficiently high drug concentrations in the presence of strict anaerobiosis, a contribution of the mammalian enzyme may be appreciated.

![Chart 4. Dose-dependent effect of desnitro niridazole on reversion in TA98 and TA100 tester strains. Salmonella tester strains TA98 (top) and TA100 (bottom) were plated on minimal nutrient agar containing 1 to 100 µg of desnitro niridazole. Plates were incubated for 24 hr at 37° either aerobically (A, •) or anaerobically (A, O). Charts were performed both in the presence (•, D) and absence (O, O) of S-9 fraction from rat liver. In the absence of drug, there were 21 and 30 aerobic and 23 and 27 anaerobic TA98 revertants in the absence and presence of S-9. Likewise, for TA100, there were 120 and 106 aerobic and 139 and 188 anaerobic revertants.](chart4)

![Chart 5. Nitroreductase activity in strains TA98, TA100, and their niridazole-resistant mutants. Bacterial nitroreductase activity was measured under aerobic (•, •) and anaerobic (O, O) conditions in strain TA98 (•, D) and its niridazole-resistant mutant TA98NR101 (•, D) (left) and strain TA100 (•, O) and its niridazole-resistant mutant TA100NR3 (•, O) (right). Each 2-ml reaction mixture contained 1.23 ml of a 5-hr log-phase culture, 20 µg of nitrofurazone, and 14 mg of glucose. Assays were performed at 37°, and the rate of nitroreduction was determined as the rate of increase in absorbance at 400 nm.](chart5)
DISCUSSION

Niridazole is an effective anthelmintic agent which has been widely used during the past 20 years in the clinical treatment of infestations due to Schistosoma mansoni, Schistosoma haematobium, and Dracunculus (18). Although it has been largely replaced by several newer antiparasitic agents, recent reports of its profound antiinflammatory and immunosuppressive properties have generated renewed interest in its biodisposition (6, 29). Thus, reports of bacterial mutagenicity (11, 21, 22) and mammalian carcinogenicity (27) suggest not only that the several hundred thousand patients treated with the drug might be at increased risk for cancer but that the development of a valuable new immunosuppressive agent might be stifled in its infancy.

In this report, we have examined the role of the aromatic nitro group in the biological toxicity of niridazole. In addition, we have reevaluated the effectiveness of mammalian liver enzymes in the metabolic activation of niridazole to a bacterial mutagen.

Previous studies by Watson (28) and Collins (10) have shown that niridazole is an effective antibacterial agent. This is confirmed here for several gram-negative species (Table 1). The data in the present study suggest that, as with nitrofurans (20), this nitrothiazole requires the presence of an aromatic nitro group (Table 1) and its metabolic activation in situ by the bacteria (Table 3) in order to exert antibacterial effect. Since the activated nitro group on niridazole is relatively more oxygen labile than is the comparable substituent of the nitrofurans (6, 15), its greater effectiveness against anaerobic species was predictable.

As a bacterial mutagen, niridazole is intriguing in its ability to cause both frame-shift and base substitution mutations (Table 2). Similar observations were made by McCann et al. (22). McCalla et al. (21) also demonstrated alkaline-labile lesions in DNA from cells of strain TA1975 after exposure to niridazole. Whether the molecular events associated with these 2 processes are the same or different has not yet been elucidated; however, the present study indicates that the nitro group is required for both frame-shift and base substitution-type mutagenicity. Thus, desnitro niridazole is ineffective in eliciting histidine-independent revertants over a wide dose range (Chart 4). Moreover, both types of mutation were also found to depend on bacterial nitroreductase activity. In nitroreductase-deficient mutants derived from TA88 and TA100, the mutagenic potency of niridazole was reduced more than 10-fold (Table 2; Chart 6).

The nitroreductase-deficient mutants used in the present study were stable mutations of the parent strains which retained the R factor for ampicillin resistance. They were generated under aerobic conditions, and thus, it is not unreasonable that, in at least one strain (TA100NR3), the aerobic nitroreductase activity was more profoundly inhibited than was the anaerobic enzyme activity. Similar resistance has been reported for strains of Escherichia coli lacking one of the soluble nitroreductases (3). As in the case of the E. coli mutants, our strains showed decreased sensitivity to other nitro-containing compounds (e.g., nitrofurantoin and metronidazole).4

Several possible mechanisms could account for the observed mutagenicity of higher doses of niridazole in nitroreductase-deficient mutants. The simplest would be to attribute the effect to residual nitroreductase activity. In this case, our inability to detect anaerobic niridazole nitroreductase activity probably reflects the fact that the activity in parental strains approaches the limit of detectability of the assay. However, this alternative is rendered less probable by the observation that, at 100 µg/plate, both mutant strains show the same number of his revertants that were seen in parental strains at 0.33 µg/plate (Charts 2 and 6) despite the fact that there was more residual reductase activity present in TA100NR3 than there was in TA88NR101 (Chart 5).

Since all of the biochemical determinations in the present study were made using intact bacteria, it is possible that mutations affecting bacterial permeability might contribute to the niridazole resistance of the mutant strains. Such a mutation would have to be rather specific for nitroaromatic compounds since these tester strains have essentially no cell wall. Also, the mutants are as sensitive as TA88 and TA100 to other standard mutagens (Table 2 versus Table 4). Moreover, the

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (µg/plate)</th>
<th>S-9</th>
<th>TA88NR101</th>
<th>TA100NR3</th>
<th>revertants/plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>-</td>
<td>15</td>
<td>162</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>21</td>
<td>195</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Aminoanthracene</td>
<td>1.00</td>
<td>15</td>
<td>171</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>170</td>
<td>466</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium azide</td>
<td>1.00</td>
<td>-</td>
<td>407</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Nitrofluorone</td>
<td>10.00</td>
<td>-</td>
<td>78</td>
<td></td>
<td>382</td>
</tr>
<tr>
<td>+</td>
<td>382</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Niridazole</td>
<td>0.33</td>
<td>-</td>
<td>16</td>
<td></td>
<td>154</td>
</tr>
<tr>
<td>+</td>
<td>26</td>
<td>230</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

relative resistance of the TA98NR101 strain to 2-nitrofluorene quantitatively reflects the relative deficiency in nitroreductase activity characteristic of this strain (Table 4; Chart 5). This is consistent with the differences in sensitivity between the mutants and the parental strains to the mutagenicity of nitroaromatics resulting wholly from differences in nitroreductase activity.

Alternatively, the results could be interpreted as consistent with the appearance of a new nitroreductase in the mutants which has a marked increase in the Km for nitroaromatic substrates. Finally, a new mechanism of mutagenesis not involving the nitro group cannot be ruled out. Further work is in progress to distinguish among these possibilities.

The lack of effect of rat liver enzyme on bacterial mutagenesis by niridazole has been reported previously (11). However, since these studies were performed under aerobic conditions at low niridazole concentrations, it was felt that the role of mammalian enzymes should be reevaluated in light of our present understanding of niridazole metabolism. Under aerobic conditions, niridazole can be converted to at least 4 polar metabolites (5). One of these, the 4,5-glycol, indicates that an epoxide intermediate is formed during aerobic metabolism (23). However, in neither TA98 nor TA100 did the addition of rat liver S-9 significantly increase the yield of his* revertants (Chart 3). This lack of effect may be explained by the difference between the observed Km for niridazole in liver microsomal preparations (=25 μM) and the maximum nonbactericidal concentration used in the plate incorporation assay (=0.8 μM). However, if this were the only explanation, a marked enhancement would then be expected at the levels of drug used with the nitroreductase-deficient mutants (=230 μM). Since no real enhancement was noted (Chart 6), we conclude that, under aerobic conditions, either mammalian liver enzymes cannot form a bacterial mutagen or the mutagen once formed cannot enter the bacteria. Further studies are necessary to distinguish between these possibilities.

Under anaerobic conditions, mammalian nitroreductase can form an oxygen-labile hydroxyxylamine from niridazole (15). With TA98 and TA100 tester strains, this pathway could not significantly enhance the mutagenicity of niridazole (Chart 3). In fact, only under the extreme conditions typified by anaerobiosis plus high drug concentration plus the absence of bacterial nitroreductase could any effect of S-9 be demonstrated.

From the above discussion, one might question the idea that niridazole poses a risk to those individuals taking the drug. Nevertheless, at least one group of investigators has demonstrated niridazole carcinogenicity in mice and hamsters (8, 27). Their study involved continuous feeding of the drug to the animals for 40 to 70 weeks. It is not clear from their data, however, that the 5- to 7-day course of therapy (the usual dose is 25 mg/kg/day) generally used to treat schistosomiasis poses a similar threat. Since the drug has a relatively short half-life in animals (14) and in humans and since the DNA lesions caused by the drug in bacteria are readily repaired upon removal of the agent (21), the true carcinogenic potential of the drug remains unknown. More intensive investigations under physiologically relevant and clinically pertinent conditions must be performed to assess the true risk of niridazole exposure.

One possible area of interest for future investigations relates to the possible involvement of intestinal microorganisms in the metabolic activation of niridazole to eukaryotic mutagens and/or carcinogens. Such a mechanism has been reported recently to account for the presence of mutagenic activity in urine of animals receiving the antiparasitic agent 4-isothiocyanato-4'-nitrophenylamine (4) despite its lack of mutagenic activity in vitro in the presence of rat liver enzymes. Similarly, reactive niridazole metabolites formed by intestinal microorganisms may be absorbed and result in genetic damage in distant tissues and organs. The intestinal flora have been found to contribute significantly to the formation of the immunosuppressive metabolite of niridazole found in the urine of niridazole-treated animals.

The present study demonstrated that the mutagenicity of a nitro-containing compound, niridazole, for tester microorganisms was dependent upon specific bacterial nitroreductase activity that was not present in the mammalian activation mixture. These results suggest that mutagenicity testing with standard tester microorganisms overestimates the potential long-term hazards of nitro-containing compounds. More specifically, many of these compounds may be mutagenic for bacteria yet incapable of being converted to DNA-modifying agents in eukaryotic cells under physiological conditions.

REFERENCES

17. Grunberg, E., and Titsworth, E. H. Chemotherapeutic properties of hetero-


Niridazole Mutagenesis

Relative Importance of Bacterial and Mammalian Nitroreductases for Niridazole Mutagenesis


Updated version Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/40/12/4599

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.