Genotoxicity of the Antihypertensive Drugs Hydralazine and Dihydralazine

Abstract. *The genotoxicity of the antihypertensive agents hydralazine and dihydralazine was tested in mammalian cells and bacteria. Both drugs elicited DNA repair in rat hepatocyte primary cultures. In the Ames test, both with and without an S-9 fraction, hydralazine was mutagenic in strains TA100 and TA1537, whereas dihydralazine was weakly mutagenic in strain TA1537. These findings support the observation that hydralazine is carcinogenic in mice. The carcinogenicity of many chemicals results from interaction with DNA. Since these studies demonstrate that hydralazine and dihydralazine damage DNA in mammalian cells, these drugs should be viewed as potential human carcinogens.*

Hydralazine (HZ) is a cyclic hydrazine that has been widely used since 1951 as an antihypertensive agent. It is one of the medications used in the National Heart, Lung, and Blood Institute Hypertension Detection and Followup Program (*1*). In Europe, the dihydrazine derivative dihydralazine (DHZ) is more widely used.

In 1978, Toth (*2*) reported an increased incidence of lung tumors in mice exposed to HZ. Subsequently, HZ was shown to be weakly mutagenic in the Ames *Salmonella* microsome test (*3*). In vitro alteration of the pyrimidine bases of DNA by HZ has also been demonstrated (*4*). In the studies reported here, we have examined the activity of HZ and related compounds in a new short-term test for chemical carcinogens that we developed, the hepatocyte primary culture/DNA (HPC/DNA) repair test (*5*).

The HPC/DNA repair test assays for the potential of chemicals to produce DNA damage as measured by an autoradiographic determination of the incorporation of [^{3}H]thymidine during DNA repair in freshly isolated hepatocytes in primary monolayer cell culture. The use of intact liver cells in this test provides a target cell with broad capability for carcinogen metabolism. This test has displayed a high degree of sensitivity and specificity for chemical carcinogens from a variety of structurally different classes, requiring different pathways of metabolic activation (*5–7*). In the HPC/DNA repair test, both HZ and DHZ reproducibly elicited DNA repair (Table 1). The nuclear ring system of HZ, phthalazine, was inactive, and therefore the production of DNA damage by HZ and DHZ must be attributed to the hydrazine side chain.

It has been postulated that all compounds positive in the Ames test and in the HPC/DNA test are most likely carcinogenic (*7*). Therefore, we reexamined the mutagenicity of HZ and DHZ in the Ames test, using the plate incorporation assay described by Ames and his associates (*8*). We found that HZ was mutagenic to strains TA100 and TA1537, both with and without an added S-9 liver enzyme preparation (Table 2). It was not mutagenic to strains TA1535, TA1538, or TA98. We found that DHZ was only weakly mutagenic to strain TA1537, both with and without an S-9 liver fraction. Phthalazine was nonmutagenic. The weak mutagenicity of these compounds in the Ames test as compared to their definite positive result in the HPC/DNA repair test at lower concentrations is in agreement with similar findings for some other chemicals examined with these two tests (*9*).

The genotoxicity of HZ and DHZ to both bacteria and mammalian cells indicates that the carcinogenicity of HZ (*2*) probably results from damage to DNA, as seems to be the case for many chemical carcinogens. A separation of chemical carcinogens into two principal categories, genotoxic and epigenetic, based upon mechanisms of action has been proposed (*7, 10*). Genotoxic carcinogens, which damage DNA, have a number of important characteristics including carcinogenicity at low doses, carcinogenicity in some cases with single exposures, additive or synergistic effects with other genotoxic carcinogens, and potentiation by cocarcinogens or promoters. Thus, genotoxic carcinogens, such as HZ, must be regarded as a potential hazard even at relatively low levels of exposure.

The antihypertensives HZ and DHZ are administered at doses well within the range in which other genotoxic carcinogens have produced cancer in humans

Table 1. DNA repair elicited by hydralazine and its analogs in the HPC/DNA repair test. The test was run according to the procedures of Williams (*5*). Hepatocytes were isolated from adult Fisher F344 rats by in situ perfusion of the liver with a calcium- and magnesium-free salt solution and then with collagenase in Williams medium E. The liver was removed, and the cells were detached by combing. After centrifugation, the cells were resuspended and plated. After 1.5 hours of attachment in culture, hepatocytes were exposed to the test compounds plus [^{3}H]thymidine for incorporation during repair. Incubation was carried out overnight, and then the cells were fixed and processed for emulsion autoradiography. Nuclear grains of DNA repair were counted electronically on a counter (Artek 880). One sample of HZ was obtained from Laboratories Eurobio, Paris, France, and a second was purchased from Sigma Chemical, St. Louis. We obtained DHZ sulfate from Dr. A. Bonapace, Milan, Italy. Chemical analyses to assess requirements of purity and conformity were carried out at the Centre de Recherches Clin-Midy, Montpellier, France. Phthalazine was purchased from Aldrich Chemical, Milwaukee, Wisc.

Compound	DNA repair (grains per nucleus)						
	0	$5 \times 10^{-5}M$	$5 \times 10^{-4}M$	$10^{-3}M$	$5 \times 10^{-3}M$	$10^{-2}M$	$5 \times 10^{-2}M$
Hydralazine	0.0	1.3 ± 1.3	12.1 ± 2.3	23.0 ± 6.8	Toxic	Toxic	
Dihydralazine	0.0		4.8 ± 1.3	8.9 ± 2.5	Toxic		
Phthalazine	0.0				0.9 ± 0.3	0.0	Toxic

Table 2. Mutagenesis induced by hydralazine and its analogs in the *Salmonella* microsome test. The assay was performed by the plate incorporation method described by Ames and his associates (*8*), using strains TA100, TA1537, TA1535, TA1538, and TA98. Fifty microliters of S-9 fraction prepared from Aroclor 1254-induced rats were used on each plate. The number of revertants represents an average for three plates after subtracting the number of spontaneous revertants in the presence of S-9 alone (TA100 = 200; TA1537 = 7). The DHZ sulfate was dissolved in S-9 mix buffer (8 μM $MgCl_2$, 33 μM KCl, and 100 μM Na_3PO_4 at *p*H 7.4). The HZ hydrochloride and phthalazine were dissolved in distilled water. A concentration of 1 mg per plate equals $6.25 \times 10^{-3}M$ HZ, $5.26 \times 10^{-3}M$ DHZ, and $7.69 \times 10^{-3}M$ phthalazine. None of these compounds were cytotoxic at this concentration.

Compound	Concentration per plate	Average number of induced revertants per plate			
		TA100		TA1537	
		S-9	No S-9	S-9	No S-9
Hydralazine	1 mg	285	350	24	25
Dihydralazine	1 mg	21	4	7	4
Phthalazine	1 mg	0	0	0	0

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upon long-term exposure (*11*). We know of no direct evidence for the carcinogenicity of HZ in humans (*12*), although specific epidemiologic studies are not available (*13*). Both HZ and its metabolites are rapidly excreted, primarily in the urine (*14*). An important enzyme in the metabolism of HZ is *N*-acetyltransferase (NAT) (*15*), which has a polymorphic distribution in humans (*16*), individuals being either rapid or slow acetylators. The development of HZ toxicity (*17*) seems to be more frequent in slow acetylators (*18*). Therefore, we examined the relationship between the genotoxicity of HZ and NAT activity in hepatocytes isolated from rats and from rabbits, a species exhibiting an acetylator polymorphism like that of humans (*19*). In rat or slow acetylator rabbit hepatocytes, the half-life of sulfamethazine, the substrate for NAT used in determining acetylator phenotype, was similar and over 15 times that of rabbit hepatocytes from a rapid acetylator (*20*). We found that HZ was genotoxic in rabbit hepatocytes with a slow rate of acetylation but not in those with a rapid rate of acetylation (*20*), although all hepatocyte preparations responded to the positive control aromatic amine carcinogen 2-aminofluorene. These observations suggest that rapid acetylation may diminish the genotoxic effect of HZ. Thus, slow acetylators in the human population may be more susceptible to the genotoxic effects of these hydrazine drugs. We conclude that HZ and DHZ are potential human carcinogens and that evidence for or against their actual carcinogenicity must be vigorously pursued.

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20. In collaboration with Dr. W. Weber of the University of Michigan, we compared the NAT activity and genotoxicity of HZ in hepatocytes from rapid and slow acetylator rabbits to that in rat hepatocytes. For the HPC/DNA repair test, we followed the procedures of Williams (*5*), using $10^{-3}M$ HZ. Hepatocytes were freshly isolated from adult Fisher F344 rats or New Zealand white rabbits and exposed to the test compound as described in Table 1. A positive result of 24.8 ± 5.5 grains per nucleus was obtained in rat hepatocytes. The slow acetylator rabbit cells gave a value of 17.7 ± 4.0, whereas the rapid acetylator rabbit hepatocytes at the highest nontoxic dose showed only a borderline result of 4.8 ± 2.4. For NAT activity, hepatocytes were incubated with $10^{-4}M$ sulfamethazine (SMZ) per milliliter of medium. Since the decrease in the SMZ concentration is proportional to the increase in *N*-acetyl SMZ, one can determine the rate of acetylation by measuring the disappearance of SMZ from the medium [A. C. Bratton and E. K. Marshall, *J. Biol. Chem.* **218**, 537 (1939); D. J. Hearse and W. W. Weber, *Biochem J.* **132**, 519 (1973)]. The logarithm of the SMZ concentration was plotted against time and the half-life determined by regression analysis. The half-life of SMZ was 50 hours for rat hepatocytes, 49 hours for hepatocytes from a slow acetylator rabbit, and only 3 hours for hepatocytes from a rapid acetylator rabbit.
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Genetic Mosaics of *Caenorhabditis elegans*: A Tissue-Specific Fluorescent Mutant

Abstract. *Genetic mosaics can be generated by x-irradiation in the simple nematode* Caenorhabditis elegans. *A mutation in the gene* flu-3 *alters the characteristic autofluorescence of intestinal cells under ultraviolet light and can be used as a cell- and tissue-specific marker. Embryos heterozygous for* flu-3 *give rise to adults with patches of these altered intestinal cells. The previously established intestinal cell lineage in* Caenorhabditis elegans *and the distribution and sizes of the fluorescent patches are consistent with a somatic segregation of the* flu-3 *allele.*

Genetic mosaics are useful for studying cell lineage and cellular interaction in development (*1*). Several interesting problems in development such as the construction of fate maps, estimation of number of primordial cells, analysis of cell differentiation, pattern formation, sex-determination, and behavior have been studied by means of mosaics and chimeras in *Drosophila* and mouse (*2*). The free-living soil nematode *Caenorhabditis elegans*, with its simple cellular anatomy, is particularly amenable to genetic manipulation (*3*, *4*). Here we demonstrate the use of a fluorescent marker to detect genetic mosaics in the intestinal tissue of *C. elegans*. A mutation in the gene *flu-3* gives rise to an altered intestinal autofluorescence which is both purple and more intense than that of the wild type. The mutant fluorescent phenotype is fully recessive with respect to the wild type (*5*, *6*).

Developing embryos heterozygous for the fluorescent marker were exposed to x-irradiation in the hope that it would enhance the frequency of mosaics, since we did not observe any spontaneous occurrence of mosaics among about 20,000 unirradiated heterozygous *flu-3* animals.

Males homozygous for the recessive fluorescent marker *flu-3 (t301)II* were crossed with dumpy hermaphrodites *dpy-5(e61)I* so that we would be able to distinguish heterozygous outcross progeny from any hermaphrodite progeny produced by self-fertilization (*3*). The non dumpy progeny resulting from cross-fertilization are heterozygous for both the morphological *dpy* marker and for the fluorescent marker *flu-3*. They are easily distinguishable from the dumpy

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