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Effects of Quinoline and 8-Hydroxyquinoline on Mouse Bone Marrow Erythrocytes as Measured by the Micronucleus Assay

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Both quinoline and 8-hydroxyquinoline (HOQ) were tested for their genotoxicity in CD_1 male mice by using a bone marrow micronucleus assay. Mice were intraperitoneally treated in single injections with three dose levels (25, 50, and 100 mg/kg) of each chemical with corn oil as solvent vehicle. Bone marrow was sampled at 24, 48, and 72 h postinjection. Quinoline resulted in a significant dose-related increase in the number of micronucleated polychromatic erythrocytes (MPCE) at the 24 h sampling time for all doses tested. The high dose (100 mg/kg) and the medium dose (50 mg/kg) also induced statistically significant increases ($P < .05$) in the number of MPCEs at 48 h interval. The ratios of polychromatic to normochromatic erythrocytes at the 24 h sampling time were lower for the treated than the control animals. Although HOQ resulted in some increases in the number of MPCEs over the control, this compound induced a statistically significant increase in the number of micronucleated normochromatic erythrocytes (MNCEs) at all three doses following 24 h treatment. Both low and medium doses also induced a higher incidence of MNCEs at the 48 and 72 h sampling times. No data were available for the high dose at these times. The cytotoxic effect of this compound was expressed as low PCE/NCE ratios with all doses at 24 h after injection and as a high mortality rate in animals treated with the high dose (100 mg/kg).

Key words: genotoxicity, clastogens, carcinogen, short-term assay, industrial chemical

INTRODUCTION

Many chemical compounds present in our environment or used by man for different purposes are mutagenic. The identification of these mutagenic

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compounds is of prime importance to avoid or limit unnecessary exposure to them. Therefore, many short-term tests (STTs) have been developed to detect these potentially hazardous chemicals. Since many of the cancer-causing agents are mutagenic, and mutation is likely to be involved in the initiation of carcinogenesis, many in vivo and in vitro STTs were used for the prediction of chemical carcinogenicity [de Serres and Ashby, 1981; McCann and Ames, 1976; Purchase et al., 1976]. Although the results obtained with such STTs as predictors of carcinogenicity are controversial at present [Brockman and DeMarini, 1988; Tennant et al., 1987], the ability of these STTs to detect genotoxic agents is well documented. These genotoxic agents may play important roles in the cause and/or progression of some human diseases other than cancer [Hartman, 1983; Hartman and Morgan, 1985].

The micronucleus assay (MNA) in mammalian bone marrow is one of the STTs used for the detection of genotoxic agents [Schmid, 1975, 1976; Heddle et al., 1983]. The principal value of MNA is for the in vivo system which may pick up genotoxic effects at the chromosomal level not covered by other STTs or in vitro assay systems [Jenssen and Ramel, 1980]. The available information indicates that the MNA is useful for the detection of clastogens as well as spindle poisons [Racine and Matter 1984].

Quinoline is a major industrial chemical and a common environmental pollutant. It is mainly used for food flavoring, in the manufacture of dyes, and as an antimalarial agent. Its monohydroxy derivative 8-hydroxyquinoline (HOQ) is used as fungicide, bactericide, and disinfectant. Quinoline was reported to be carcinogenic [Hirao et al., 1976; LaVoie et al., 1987], while HOQ gave no evidence of carcinogenicity in rodents [Gold et al., 1987; Haseman et al., 1987], although it has a colchicinelike effect on chromosomes [Grant et al., 1981] by damaging the spindle apparatus and preventing the completion of mitotic cycle beyond the metaphase. The present study was aimed at determining the ability of these two related compounds to induce micronuclei in CD_1 male mouse bone marrow erythrocytes.

MATERIALS AND METHODS

Chemicals

Quinoline (benzo[B]pyridine; CAS No. 91-22-5; MW = 129.15) and HOQ (oxybenzopyridine; CAS No. 148-24-3; MW = 145.16) were obtained from Radian Corporation (Austin, TX). The structure of these two chemicals is presented in Figure 1. Triethylenemelanine (TEM) was purchased from Polysciences Laboratories (Warrington, PA).

Animals

Six- to 8-wk-old male CD_1 mice obtained from Charles River Breeding Laboratories (Wilmington, MA) were housed five per cage and acclimatized for 5–7 d prior to dosing. Bedding consisted of hardwood chips and excelsior nesting material. Water and purina laboratory rodent chow were given ad libitum.

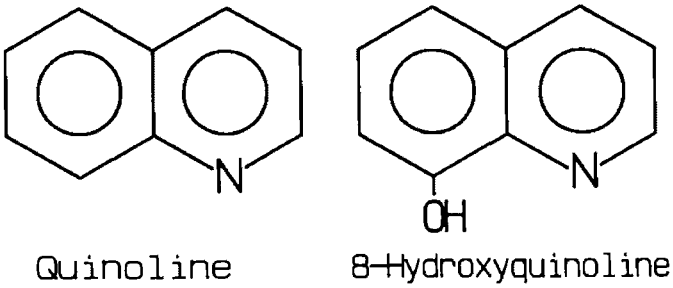

Fig. 1. Chemical structures of quinoline and 8-hydroxyquinoline.

Chemical Treatment

Each chemical was tested in three different doses (25, 50, and 100 mg/kg). Selection of these dose levels was based on the information from in vivo sister chromatid exchange studies sponsored by The National Toxicology Program (Shelby, personal communication). Chemicals were intraperitoneally administered as single injections in a volume equivalent to 10 ml/kg body weight. Corn oil was used as vehicle for both test chemicals. In both experiments, TEM, dissolved in sterile phosphate buffered saline, was used (0.5 mg/kg) for positive controls. At least 15 animals were treated for each dose level for each chemical. Five animals were used for negative and positive controls, respectively, for each experiment.

Sampling and Bone Marrow Preparation

Three posttreatment times, i.e., 24, 48, and 72 h, were selected for both test compounds while positive and negative controls were sampled once only at 24 h posttreatment. At each sampling time, animals were sacrificed by cervical dislocation. Femura were removed and the marrow was flushed from bones with 3 ml of fetal bovine serum into a 15-ml centrifuge tube. Tubes were then shaken and centrifuged at 1,000 rpm for 5 min.

Preparation and Staining of Slides

Following centrifugation, all but a small portion of the supernatant was discarded and the cells were resuspended in the remaining serum. Four replicate slides were prepared from each animal by using the slide-drawn smear method. Overnight air-dried smears were fixed for 15 min in absolute methanol and stained by using a May-Gruenwald-Giemsa staining technique [Schmid, 1976].

Scoring of Slides

From the coded slides, a total of 2,000 polychromatic erythrocytes (PCEs) per animal were scored for the presence of micronuclei (MN). As measurement of the toxic effect of the test compound to the erythropoietic tissue, the PCE normochromatic erythrocytes (NCE) ratios were calculated by scoring the number of NCEs encountered during screening the first 1,000 PCEs. Since HOQ is known to inhibit the spindle in plants, the number of MNCEs among 2,500 NCEs was also scored for animals treated with this compound. Scoring was based on the number of micronucleated cells regardless of the number of micronuclei per cell. A PCE (or NCE) with micronucleus, regardless of the

number, was counted as one MPCE (or MNCE). The criteria for the identification of micronuclei were those of Schmid [1976].

Statistical Analysis

The two-tailed Student's t-test was performed to compare MPCE values between treated and control animals. The Cochran-Armatage two-tailed trend test was used to determine the dose-response relationship [Snedecor and Cochran, 1967]. The zero dose was not used in this test.

RESULTS

The effects of different dose levels of quinoline (25, 50, and 100 mg/kg) on the frequency of MPCEs in CD_1 male mouse bone marrow at three posttreatment intervals (24, 48, and 72 h) are shown in Table I. At 24 h, all three doses resulted in a significant increase in the numbers of MPCEs, while at 48 h, the medium and high doses resulted in significant elevations. The increase in MPCEs is significantly dose-related. The repeat experiment, at 24 h sampling time, gave similar results (Table I). The toxicity of this compound was also seen 24 h after treatment, as judged from PCE/NCE ratios in treated animals. These ratios appeared lower than those from control animals, but the response was not dose related. At 48 and 72 h after treatment, however, the ratios from treated animals were relatively higher than that from the control.

The results obtained with HOQ are presented in Table II. The high dose

TABLE I. Micronuclei Induced in Bone Marrow Polychromatic Erythrocytes (PCEs) of CD_1 Male Mice After Single Ip Treatment With Quinoline

Sampling time (h)	Dose (mg/kg)	MPCE/1,000 PCE ± S.E.[a]	PCE/NCE[b]
24	0	3.0 ± 0.3	0.82
	25	4.7 ± 0.5*	0.69
	50	9.2 ± 1.7**	0.61
	100	12.5 ± 1.7**	0.79
48	25	3.9 ± 0.2	0.82
	50	7.0 ± 0.5**	1.08
	100	8.3 ± 0.5**	1.04
72	25	2.8 ± 0.4	1.33
	50	2.5 ± 0.4	1.27
	100	3.0 ± 0.6	1.08
24[c]	0	2.4 ± 0.3	0.85
	25	4.9 ± 0.4**	0.69
	50	6.9 ± 0.3**	0.72
	100[d]	7.9 ± 0.6**	0.75[e]

[a] Total MPCE/10,000 PCE (2,000 PCEs were scored for each of 5 animals).
[b] Based on 5,000 PCEs.
[c] Results from repeat experiment.
[d] Only 4 animals were analyzed.
[e] Based on 4,000 PCEs.
* $P < .05$ (compared to zero dose).
** $P < .01$ (compared to zero dose).

TABLE II. Micronuclei Induced in Bone Marrow Erythrocytes of CD_1 Male Mice After Single Ip Treatment With 8-Hydroxyquinoline

Sampling time (h)	Dose (mg/kg)	MPCE/1000 PCE ± S.E.[a]	MNCE/1000 NCE ± S.E.[b]	PCN/NCE[c]
24	0	2.5 ± 0.2	1.5 ± 0.2	1.04
	25	2.9 ± 0.4	3.0 ± 0.3**	0.64
	50	3.6 ± 0.4	3.2 ± 0.4**	0.69
	100[d]	4.7 ± 0.8*	5.3 ± 0.6**	0.59
48	25	4.0 ± 0.6*	3.6 ± 0.1**	0.85
	50	3.4 ± 0.3*	3.5 ± 0.2**	1.08
72	25	2.5 ± 0.3	2.2 ± 0.2*	0.85
	50	3.0 ± 0.2	3.0 ± 0.2**	0.92
24[e]	0	2.7 ± 0.3	1.6 ± 0.1	1.04
	25	2.7 ± 0.3	2.7 ± 0.2**	0.69
	50[f]	3.0 ± 0.2	4.4 ± 0.4**	0.59

[a] The number is based on 10,000 PCEs (2,000 PCEs were scored for each of 5 animals).
[b] The number is based on 12,500 NCEs (2,500 NCEs were scored for each of 5 animals).
[c] Based on 5,000 PCEs.
[d] Only 3 animals were analyzed.
[e] Results from repeat experiment.
[f] Only 4 animals were analyzed.
* $P < .05$.
** $P < .01$.

(100 mg/kg), however, was very toxic and resulted in death of many mice several hours after injection. Thus only 24 h data could be obtained for this dose. There was a slight increase in the number of MPCEs in treated animals over controls. The increases were statistically significant at 24 h for the medium and high doses and at 48 h for the low and medium doses. There was a significant dose-related response only at 24 h ($P < .01$). This was not confirmed with the repeat 24 h data. The numbers of MNCEs induced by all doses over all three sampling times were small but significantly different from the controls, and there was also a significant dose-related trend at 24 h, which was confirmed with the repeat 24 h data. The peak incidence of induced MNCEs was at or close to 48 h after treatment. The PCE to NCE ratios for treated animals were much lower than controls, particularly at 24 h sampling time.

DISCUSSION

In rodent carcinogenicity studies, quinoline has been found to be carcinogenic in both mice and rats. Quinoline induced hepatic tumors in newborn male CD_1 mice and leukemia or lymphoma in female mice [LaVoie et al., 1987]. Quinoline also is hepatocarcinogenic in rats [Hirao et al., 1976]. The available mutagenicity data seem to indicate that quinoline is a promutagen. It was found mutagenic in a *Salmonella* histidine reversion system only when Arcolor-induced rat liver homogenate also was present in the incubation mixture [Epler et al., 1977; Hollstein et al., 1978; Seixas et al., 1982; Yoshikawa et al., 1982]. Quinoline has been shown to induce unscheduled DNA synthesis in primary cultures of rat hepatocytes and to bind to RNA, DNA, and certain

polynucleotides in the presence of NADPH and rat liver microsomes [Tada et al., 1980]. Quinoline also produced nucleic acid adducts only when tested in the presence of liver extract [Kubinski et al., 1981]. Although reported to be negative in the chromosomal aberration test in in vitro studies in Chinese hamster cells [Ishidate and Odashima, 1977], quinoline induced DNA single-strand breaks in the alkaline elution/rat hepatocyte assay [Sina et al., 1983]. In the present study this compound was found to induce micronucleus formation in the bone marrow of CD_1 male mice. This result is in agreement with the aforementioned reports about carcinogenicity and mutagenicity of quinoline.

The carcinogenicity data on HOQ is not clear. It has been reported to be a weak carcinogen [Shubik and Hartwell, 1969]. However, in two recent rodent carcinogenicity studies conducted by the National Toxicology Program, this compound was judged to show no evidence of carcinogenicity [Gold et al., 1987; Haseman et al., 1987]. This compound is known to have colchicinelike effects and is recommended as a spindle inhibitor in plant chromosomal studies [Grant et al., 1981].

HOQ has been treated in several in vitro assays for genotoxicity. It was found to be mutagenic in *Salmonella* when assayed by the histidine reversion test [Epler et al., 1977; Talcott et al., 1976; Tennant et al., 1987] and was effective in inducing chromatid aberrations in human leukocytes [Epler et al., 1977]. It was also reported to be mutagenic, either with or without exogenous activation, in mouse lymphoma cells, and to cause chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells [Tennant et al., 1987]. In addition, HOQ produced nucleic acid adducts when tested with the DNA-cell-binding assay [Kubinski et al., 1981]. In the present study, HOQ induced a significant number of MNCEs, although it did not produce high numbers of MPCEs over the three sampling times tested. This may indicate that HOQ affects the later stages of the cell cycle. The micronucleated cells resulting from HOQ treatment had probably passed the PCE stage 24 h after treatment and thus could not be detected as MPCEs. This finding agrees with the hypothesis that the proportion of micronucleated NCEs increases if the agent acts on G_2 chromosomes or on the spindle apparatus [Schmid, 1976] and that the incidence of more than one MNCE per thousand PCE indicates an effect on cell stages past the S phase [Maier and Schmid, 1976]. The results obtained with HOQ, therefore, parallel those of in vitro genotoxicity assays and can be reconciled with the fact that this compound is used as a spindle inhibitor. However, the relationship of HOQ to carcinogenicity remains unclear.

The toxicity of HOQ is much higher than that of quinoline. This was evident, since many mice died several hours after injection of the 100 mg/kg dose level of HOQ, while the same dose level of quinoline did not produce any animal mortality.

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