The Mutagenic Potential of the Furylethylene Derivative 2-Furyl-1-nitroethene in the Mouse Bone Marrow Micronucleus Test

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The compound 2-furyl-1-nitroethene (G-0) has been tested to determine its ability to induce clastogenic or aneugenic effects in vivo, through the induction of micronucleated polychromatic erythrocytes (MN PCE) in mouse bone marrow. Groups of five CD-1 male mice were administered once intraperitoneally at a dose range of 5–20 mg/kg and bone marrow was sampled at 24 and 48 h after the treatment. G-0 was dissolved in corn oil, thus a vehicle control group received only corn oil at 10 ml/kg. The positive control group was administered with cyclophosphamide (40 mg/kg). All animals dosed with the highest concentration of the test agent (20 mg/kg) showed evident clinical symptoms of toxicity. Although evidences of bone marrow toxicity were observed, no statistically significant increases in the incidence of MN PCE over the vehicle control group were observed at any sampling time with any of the assayed doses of the G-0 compound. Cyclophosphamide treatment increased the incidence of MN PCE in all treated animals, demonstrating the sensitivity of the assay conditions in which it was carried out. From the results obtained, it is concluded that the test agent G-0 is neither clastogenic nor aneugenic in the erythrocytes from the bone marrow of treated mice at the doses tested.

Key Words: micronucleus assay; mice; furylethylenes; polychromatic erythrocytes; bone marrow.

Genetic toxicology studies have given rise to a number of testing procedures, both in vitro and in vivo. They have been designed to assess the effects of chemicals on the genetic material and, consequently, to assess the risk to living organisms including humans. To provide a broad coverage of the mutagenic and presumably carcinogenic potential of a chemical, information on different levels of genotoxic effects, e.g., gene, chromosomal, and cellular apparatus necessary for chromosome segregation, is required. Thus, the micronucleus assay has been widely used to measure genotoxicity, both in vivo and in vitro. The assay, when performed appropriately, detects clastogenicity due to chromosome breakage, and also aneugenicity, due to chromosome lagging resulting from dysfunction of mitotic apparatus (Krishna and Hayashi, 2000).

Some nitro-compounds, among them the 5 nitrofurans, have showed genotoxicity and carcinogenicity in different experimental systems. Although there is also evidence that the furylethylene derivatives with the nitro group outside the furan ring do not show genotoxic effects (IARC, 1974; Jurado and Pueyo, 1995; McCalla, 1983; Ni et al., 1987; Sturdik et al., 1985). We have been studying a novel group of furylethylene derivatives, which have like common characteristic that the nitro group is attached in the exocyclic double bond of the ethylenic chain. In vitro studies, by using both the micronucleus (MN) and the sister chromatid-exchanges (SCE) test in cultured human lymphocytes, have been performed previously. These studies showed negative response in the micronucleus assay, with and without metabolic activation, and a slight increase in the SCE frequency has been observed, mainly in the absence of the S9 fraction. The slight effect disappears in the presence of the metabolic activation system (González Borroto et al., 2001).

To obtain more insight into the genotoxic potential of this group of furylethylene derivatives, and to confirm the results obtained in the in vitro studies, an in vivo mutagenicity study has been carried out by using the mouse bone marrow-micronucleus test. In this study 2-furyl-1-nitroethene was selected as an example to assess the possible in vivo genotoxic potential of this chemical class, by measuring its effects on the formation of micronuclei in polychromatic erythrocytes (PCE) from the bone marrow of treated mice.

MATERIAL AND METHODS

Test compound. The test agent 2-furyl-1-nitroethene (G-0) was synthesized and kindly supplied by the Centro de Bioactivos Químicos, Universidad Central de Las Villas, Cuba. Purity was 96.38%, as established by using high-pressure liquid chromatography (HPLC). Its chemical structure is indicated in Figure 1.

Mouse bone marrow micronucleus test. CD-1 male mice, supplied by Charles River France, were maintained on A04C diet (Usine d’Alimentation Rationnelle, UAR, France) and water ad libitum, and kept under controlled conditions (22 ± 2°C, 30–70% relative humidity, and a 12-h light/dark cycle). Animals were 6–7 weeks old when used for the determination of the doses in a preliminary dose-range-finding study; as well as in the main study for the micronucleus test. The test agent was dissolved in corn oil (Sigma-Aldrich,
Spain) and the solution was administered intraperitoneally at the volume of 10 ml/kg of body weight. The negative control group received corn oil-only, while the positive control group was treated with cyclophosphamide (CP) at the concentration of 40 mg/kg, dissolved in physiologic solution.

In the preliminary dose-range finding experiment, animals were treated with G-0 in doses ranging from 10 to 100 mg/kg, and clinical signs of toxicity and deaths were recorded 24 h later. According to the symptoms of systemic toxicity elicited by G-0 and exhibited by animals, a series of three doses from 5 to 20 mg/kg was chosen to assess the micronuclei induction.

The micronucleus assay was conducted using a method based on the OECD and European Union guidelines (European Union, 1992; OECD, 1997). Briefly, groups of five males were treated with G-0 (5, 10, or 20 mg/kg body weight). Only male mice were used, because in a previous study there was no marked sex-related difference in toxicity for this class of furylene derivatives (Ramos et al., 1997). Separated groups of animals were killed at 24 and 48 h after dosing, by cervical dislocation. In the first sample time-period, animals dosed with the three doses (5, 10, or 20 mg/kg) were used; in the second sampling time, only animals treated with the high dose (20 mg/kg) were used. Femurs were removed and bone marrow collected in tubes containing 2 ml of fetal calf serum and centrifuged 5 min at 180 × g. Two smears were prepared and allowed to air dry, prior to fixation and staining with acridine orange solution. One drop of 0.04 mM acridine orange solution in So¨rensen’s phosphate buffer is placed on the fixed cells under a coverslip. Observations were made within a day by using an Olympus fluorescent microscope equipped with blue excitation and 515–530 nm barrier filter (Krishna and Hayashi, 2000).

Slides were coded and scored blind; 2000 polychromatic erythrocytes (PCEs) by animal were examined for the presence of micronuclei, which means 10,000 PCEs scored per dose-group. Due to the fact that normochromatic erythrocytes (NCE) have a lack of fluorescence or are viewed opaquely with blue excitation and 515–530 nm barrier filter (Krishna and Hayashi, 2000).

The normality of the distribution of the frequency of micronucleated PCE (MNPl/1000) scores was assessed by means of the Kolmogorov-Smirnov test of goodness of fit. The incidences of micronuclei in micronucleated PCE (MNPCE/1000) scores was assessed by means of the Student’s t-test. The PCE/NCE ratio in control and treated groups was compared by the Student’s t-test. Statistical decisions were made with a significance level of 0.05 (Crebelli et al., 1999; Jones et al., 2001).

RESULTS

During a preliminary dose range-finding experiment, animal dosed with 20 mg/kg exhibited clinical signs of toxicity (including irritation, slight ataxia, palpebral ptosis and hunched posture). Animals dosed above this dose exhibited the same toxic signs but in a severe manner and, consequently a dose of 20 mg/kg was chosen as the highest dose for the main study.

In the main study, animals treated with the high dose (20 mg/kg), showed clinical signs of systemic toxicity, such as piloerection, palpebral ptosis, dyspnoea, and hunched posture. The symptoms confirmed the absorption of the test compound. Taking into account that bone marrow is a well-perfused tissue, and that levels of test substance in blood or plasma will be similar to those observed in bone marrow, we assumed that the test compound reached the target cells. Although, during the preliminary study, slides from animals dosed over the highest selected dose (20 mg/kg) were stained and observed, a reduction in the number of fluorescent PCEs was detected, indicating bone marrow toxicity. Besides, a decrease in the PCE/NCE ratio was obtained in a dose-dependent manner when the treatment and control groups were compared. The reduction is observed in both sampling times, and the values for doses of 10 and 20 mg/kg reached statistical significance.

The results of the micronucleus test with the furylene derivative selected for this study are summarized in Table 1. The incidence of MNPl in each treatment group, the PCE/NCE ratio, as well as the number of micronucleated PCEs/ scored PCEs are shown. Only one scorer analysed all the slides.

Despite the evident signs of acute toxicity shown by the animals, mainly at the high dose, and the reduction in the

![FIG. 1. Chemical structure of the 2-furyl-1-nitroethene (G-0).](image-url)
PCE/NCE ratio, the results show that the MNPCE frequency in male mice bone marrow was not affected by treatment with any of the selected doses of the test agent G-0, in either of the sampling times, 24 and 48 h.

The incidence of MNPCE in vehicle-treated mice (1.6 MNPCE/1000 PCEs) in both sampling times was within the accepted spontaneous range for this mouse strain (Krishna et al., 2000; Salamone and Mavournin, 1994). A statistically significant increase in the incidence of MNPCE over the control value was observed following treatment with the positive control substance cyclophosphamide (17.6 MNPCE/1000 PCE, \( P \leq 0.001 \)). This data confirmed the sensitivity of the experimental protocol followed in the detection of genotoxic effects.

**DISCUSSION**

Nowadays, some nitrofurans continue being used as antimicrobial agents in human and veterinary medicine, even though mutagenic and carcinogenic properties have been shown for several of these compounds (Hamilton-Miller, 1997; IARC, 1974; Jurado and Pueyo, 1995; McCalla, 1983; Ni et al., 1987).

The G-0 compound is not properly a nitrofuran, but the furylethylene derivatives, with the nitro group located outside the furan ring, are isomers of position of the 5-nitrofurylethylene derivatives, and it has been indicated that such structural differences can produce changes in their genotoxic behaviour (Estrada, 1998). It has also been reported that only those derivatives with the nitro group coupled in position 5 of the furan ring are mutagenic, and that the substitution of this functional group results in the loss of mutagenic activity (Estrada, 1998). It has also been reported that only those derivatives with the nitro group coupled in position 5 of the furan ring are mutagenic, and that the substitution of this functional group results in the loss of mutagenic activity (Estrada, 1998). It has also been reported that only those derivatives with the nitro group coupled in position 5 of the furan ring are mutagenic, and that the substitution of this functional group results in the loss of mutagenic activity (Estrada, 1998). It has also been reported that only those derivatives with the nitro group coupled in position 5 of the furan ring are mutagenic, and that the substitution of this functional group results in the loss of mutagenic activity (Estrada, 1998). It has also been reported that only those derivatives with the nitro group coupled in position 5 of the furan ring are mutagenic, and that the substitution of this functional group results in the loss of mutagenic activity (Estrada, 1998). It has also been reported that only those derivatives with the nitro group coupled in position 5 of the furan ring are mutagenic, and that the substitution of this functional group results in the loss of mutagenic activity (Estrada, 1998).

The results of these studies are summarized in Table 2. Recently, Slapsyte et al. (2002) reported the results of cytogenetic testing of a classical 5-nitrofuran, the nitrofurantoin, in human lymphocytes *in vitro* at concentrations of 20 and 40 \([\mu M]\); in this study chromosome aberrations and SCE were observed. At the same time, these authors studied the effects of the therapy with nitrofurantoin used for a long-term antimicrobial prophylaxis of urinary tract infection in children treated for a period of 1–12 months. No statistically significant increases in chromosome aberrations and SCE frequencies were found in the treated children, indicating a possible *in vivo* detoxification.

In the present study, no effects on the induction of MNPCE were observed with the test agent in a range of doses from 5 to 20 mg/kg in treated mice. Our previous *in vitro* results and the *in vivo* results reported here, as well as the other data mentioned above, would indicate/confirm the detoxification exerted by the metabolism on the possible genotoxicity of this class of chemicals. On the other hand, the global results of *in vitro* and *in vivo* studies with the test agent G-0, and other related derivatives, indicate the possibility that there is a safety margin for the use of these novel compounds in human and veterinary medicine. Nevertheless, additional mutagenicity studies measuring different levels of DNA damage are still necessary.

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**REFERENCES**


