Micronucleus Formation in Normochromic Erythrocytes from Mice Treated with Newly Synthesized Oxadiazoles

Ahmad Khalil, Ahmed Maslat and Ahmed Fares*

Received on April 9, 2003
Accepted for publication on Sept. 8, 2003

Abstract

Four groups of male albino Balb/c mice (n=4) were exposed for 30 h separately to two newly synthesized oxadiazole derivatives, namely 1, 3-bis[5-benzylthio-1, 3, 4-oxadiazole-2-yl]benzene and 1, 4-bis[5-benzylthio-1, 3, 4-oxadiazole-2-yl]benzene (6.25, 12.5, 25.0, 50.0 mg/kg body weight). Both compounds significantly increased (ANOVA, P = 0.05) the number of micronucleated normochromic erythrocytes at their highest dose (50 mg/kg body weight). Although the second derivative caused significant increases in the number of micronucleated cells at 12.5 and 25.0 mg/kg dose levels, the first failed to do so. The importance of the present results as referred to the practical application of oxadiazole compounds is discussed.

Keywords: Normochromatic erythrocytes; Novel oxadiazoles; Mice; Micronuclei.

Introduction

Oxadiazoles are chemical compounds with five-membered heterocyclic rings, containing one oxygen and two nitrogen atoms. Recently, derivatives of oxadiazoles attracted a special attention due to their wide applications in medicine and agriculture (Jones et al., 1996; Firooz et al., 1995; Mlligan et al., 1993) as well as in industry (Meng and Huang, 2000; Dettet and Schollmeier, 1999; Schulz et al., 1997). The insecticidal activity of some oxadiazole derivatives has been evaluated against the second instar larvae of armyworm *Pseudolatia separata* and *Culex pipens* (Shi et al., 2001; Shiba, 1998). Furthermore, Chen et al. (2000) investigated the fungicidal activity of thiadiazoles in rice sheath bright. Several oxadiazole complexes with Co(II), Ni(II), Cu(II) and Zn(II) have been tested for their fungitoxicity in *Asperigellus niger* and *A. flarius* (Misra et al., 1994). Some oxadiazole derivatives exhibited moderate to good herbicidal pre-emergence activity against *Triticum aestivum* (Cercetto et al., 2000). Other oxadiazole derivatives were found to have a high ovicidal effect in *Tetranychus*

© 2004 by Yarmouk University, Irbid, Jordan.
* Department of Biological Sciences, Yarmouk University, Irbid, Jordan.
Oxadiazoles also have bacteriocidal activity against *Staphylococcus aureus* and *Shigella flexneri* (Kocabalkanli et al., 2001). On the other hand, Maslat et al. (2002) reported that a number of bis-1, 3, 4-oxadiazole derivatives exhibited antibacterial as well as antifungal activity. 5-(3-methylindolyl)-1, 3, 3-oxadiazole-2(3H)-one was shown to be an active inhibitor for the enzyme monoamine oxidase which makes it a potential therapeutic agent for depressive illnesses or Parkinson's disease (Perez et al., 1997; Kruger et al., 1995). 2-azetidinyl-1, 3, 4-oxadiazol was found to have anxiolytic and anti-serotonin properties (Liszkiewicz et al., 1999).

However, some oxadiazole derivatives have been reported to be mutagenic and carcinogenic (Cohen et al., 1975; IARC, 1991). The mechanism of action is not clear. Several 1, 2, 5-oxadiazole N-oxide derivatives have been synthesized and tested for their cytotoxicity in oxia and hypoxia as well as for their ability to bind DNA. They proved to be non-selective, less active and with lower affinity for DNA than the parent compounds (Cerecetto et al., 2000).

In view of the importance of oxadiazoles, we decided to investigate the genotoxicity of two newly synthesized oxadiazoles; particularly 1, 3-bis[5-benzylthio-1, 3, 4-oxadiazole-2-yl]benezene (M1) and 1, 4-bis[5-benzylthio-1, 3, 4-oxadiazole-2-yl]benezene (M2). The chemical structures of these compounds is shown in Figure 1. This study may shed some light on the mutagenic effect of changing the functional group from 1, 3-(meta) to 1, 4-(para) oxadiazoles which has not been investigated previously. For this purpose, the *in vivo* micronucleus (MN) formation test in albino Balb/c mouse peripheral blood normochromatic erythrocytes has been employed. Erythrocytes are considered a sensitive system for evaluating cytogenetic damage since the nucleus of erythroblast is expelled after the last mitosis, yielding DNA-deficient cells.

![M1 and M2 chemical structures](image)

**Figure 1.** Chemical formulae of the two oxadiazole derivatives:

- **M1:** 1, 3-bis[5-benzylthio-1, 3, 4-oxadiazol-2-yl]benezene.
- **M2:** 1, 4-bis[5-benzylthio-1, 3, 4-oxadiazol-2-yl]benezene.
Materials and Methods

Chemicals

The two oxadiazole derivatives under investigation were prepared and characterized at Yarmouk University/Department of Chemistry (Tashtoush et al. 2001). They were kindly provided by Professors M. Al-Talib and H. Tashtoush. Prior to the intraperitoneal injection of the mice, the chemicals were dissolved in dimethyl sulphoxide (DMSO). The positive control; mitomycin C (MMC) was manufactured by BDH chemicals (England) and dissolved in distilled water.

Animal Treatment

Twenty four 5-7 week-old male albino Balb/c mice (Average weight 27 gm) were placed in 6 cages (4 animals/each) and maintained on a regular diet ad libitum, water and a 12-h light/dark cycle. The animals were supplied by Yarmouk University Animal House. The animals were intraperitoneally inject using insulin syringes and the final doses of the test chemical were: 6.25, 12.5, 25.0 and 50 mg/kg body weight. The latter dose (50 mg/kg) was chosen because of solubility limitation and toxicity of the solvent. MMC was injected at a dose of 14 mg/kg body weight. The injection volume was 10 ml/kg body weight. These doses were in accordance with the recommendation of the EPA-Gene-Tox Program (Hayashi et al., 1994, 1983; Heddie et al., 1983). The above experiment was performed in triplicate.

Micronucleus Assay

Blood was collected, 30h post injectin, by cutting the posterior part of the tail, in heparinized capillaries. Blood smears were prepared on cleaned prewashed microscopic glass slides. The smears were air-dried and fixed in absolute methanol for 3 min. Four slides were made from each animal and stained in Giemsa solution for 5 min (Schmid et al., 1975). The stained slides were washed in distilled water, cleared in xylene and allowed to air-dry. Two to five fields from each blindly-coded slide were screened and 500 cells (per slide) with well-preserved cytoplasm were scored for micronuclei under the light microscope. Pooled data were analyzed according to Janssen (1982).

Results

Several criteria are considered as evidences for a positive response in the technique, which has been used in the present investigation. One of these is to obtain a reproducible statistically significant positive response at any of the used concentrations of the chemical substance. Another criterion is to establish a dose-response increase in the number of micronucleated normochromatic erythrocytes (MN-NCEs).

As there were no significant differences (ANOVA, P<0.25) neither in the frequency of micronucleated cells between slides prepared from each animal for the same dose nor among slides from replicate experiments at a specific dose, the data were pooled as
presented in table 1. Wilcoxon's test was used to compare the mean frequencies of different treatment groups with the negative control, while Duncan multiple range test was used to determine the means that are significantly different from each other. Our results showed that the two test compounds failed to induce a dose-related increase in the number of MN-NCEs, under the present experimental conditions (Figure 2 and Figure 3). However, the first oxadiazole derivative (M1), at its highest dose (50 mg/kg body weight) significantly increased (P=0.01) the number of MN-NCEs. The mean (±SD) MN frequency ranged from 0.94±0.05 to 1.81±0.27. The highest level of MN-NCEs observed in the negative and positive controls was 1.02±0.14 and 2.74±0.62. Furthermore, there were no significant differences (P=0.01) between the rates of induced MN by M2 at different doses. However, M2 significantly increased the number of MN-NCE (P=0.05) at the following doses: 12.5, 25 and 50mg/kg body weight.

![Graph showing frequency (%) of micronuclei induced in vivo in normochromatic erythrocytes from mice treated with oxadiazole derivative M1.](image)

**Figure 2.** Frequency (%) of micronuclei induced *in vivo* in normochromatic erythrocytes from mice treated with oxadiazole derivative M1.
Micronucleus Formation in Normochromatic Erythrocytes from Mice Treated with Newly Synthesized Oxadiazoles

![Graph showing frequency of micronuclei formation vs dose (mg/kg)]

**Figure 3.** Frequency (%) of micronuclei induced *in vivo* in normochromatic erythrocytes from mice treated with oxadiazole derivative M2.

**Table 1**
Percent of *in vivo* micronucleus formation in normochromatic erythrocytes from mice treated with oxadiazole derivatives M1 and M2.

<table>
<thead>
<tr>
<th>Treatment (mg/kg body weight)</th>
<th>M1 Mean±S.D.</th>
<th>M2 Mean±S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control(^b)</td>
<td>1.02±0.14</td>
<td>0.97±0.06</td>
</tr>
<tr>
<td>Positive control(^c)</td>
<td>2.46±0.13</td>
<td>2.74±0.62</td>
</tr>
<tr>
<td>6.25</td>
<td>0.94±0.05</td>
<td>1.08±0.34</td>
</tr>
<tr>
<td>12.50</td>
<td>1.24±0.09</td>
<td>1.91±0.37</td>
</tr>
<tr>
<td>25.00</td>
<td>1.28±0.17</td>
<td>1.79±0.33</td>
</tr>
<tr>
<td>50.00</td>
<td>1.81±0.27</td>
<td>2.07±0.61</td>
</tr>
</tbody>
</table>

\(^a\) Results of the pooled data from three experiments. 2000 cells were screened in each experiment.

\(^b\) DMSO: dimethyl sulfoxide, negative control (10ml/kg).

\(^c\) MMC: mitomycin C, positive control (14mg/kg).
Discussion

The current study provides interesting results regarding the genotoxicity assessment of two novel oxadiazoles using the MN test. Both compounds were found to be weak mutagens. Very few mutagenic data concerning oxadiazoles are available in the published literature for comparison. Several oxadiazole derivatives were reported carcinogenic in mammalian systems. Trans-5-amino-3-[2-(5-nitro-2 furyl)vinyl]-1, 2, 4-oxadiazole was shown to be carcinogenic in hematopoietic system of rat and mouse and in stomach (CPDB, 200). 2-amino-5-(5-nitro-2 furyl)-1, 2, 4-oxadiazole was found to be carcinogenic in kidney, lung, mammary glands and stomach of rat (IARC, 1991; Cohen et al., 1975).

The exact mechanism of action of oxadiazoles is not clear. However, three observations were made in the present investigation. Binucleated erythrocytes were very rare, the size of micronuclei was relatively small and the time needed for the appearance of MN was relatively long. In a preliminary study, MN were not observed when the sampling time was less than 24 hours. Our observations suggest that the oxadiazole derivatives investigated in this study could act as clastogenes. To clarify whether the spindle apparatus is also affected needs the use of antikinetochrome antibody staining in future studies (Stopper et al., 1994). In para monocyclic aromatic amines, mutagenic activity depends not only on the chemical groups present but also on their position relative to one another (Temcharion et al., 1994). However, 5, 5-dimercapto-bis-[1, 3, 4-oxadiazol-2-yl] butane was found to be mutagenic using Ames test (Maslat et al., 2002). Shahin (1989) noted that the position, size and the chemical nature of substituent groups all influence the mutagenicity. The present findings show that both positions (para and meta) of the core benzene ring in the studied oxadiazole derivatives are nearly equal in their properties. This is extremely important since the oxadiazole ring can be a carrier of essential functional groups (Nicolaides et al., 1996, Kruger et al., 1995, Castro et al., 1993) which make them potent drugs. Thus, it might be worthy to replace the benzene ring with an oxadiazole in sulfa drugs for a possibly safer use. Confirmation and/or extending the present finding using other genetic end points in different biological systems both in vivo and in vitro deserves further investigation, especially if such compounds are to be considered for any application in medicine, industry or agriculture.

Acknowledgements

We wish to thank Mr. Issa Al-Jahamani for his excellent technical assistance.
تنوك النوى الصغيرة في خلايا الدم الحمراء طبيعة التكوين المأخوذة من الفئران بعد معاملتها بمركزين جديدين من الأوكسيدازول

أحمد خليل، أحمد مسلط وأحمد فارس

ملخص

عرضت أربع مجموعات من ذكور الفئران المهيئ (البيضاء)، لمدة ثلاثين ساعة لأحد مركزين من مركبات الأوكسيدازول المستحضرة حديثاً (M1 و M2). وتراوحت الجرعات المستخدمة بين 6.25 و 50 ملغم من المركب لكل كيلوغرام من وزن الفئران. وقد وجدت زيادة ذات دلالة إحصائية في عدد خلايا الدم الحمراء الطبيعية الناتجة عن نوى صغيرة بعد حقن الفئران بالجرعة العالية (50 ملغم) من المركبين. وبالرغم من أن الجرعتين المتوسطتين (2.5 و 25 ملغم) من المركب الثاني (M2) أحدثت زيادة واضحة إحصائياً في عدد الخلايا الحاملة للنوى الصغيرة، إلا أن هاتين الجرعتين من المركب الأول لم تؤديا إلى النتيجة ذاتها.

References


Carcinogenic Potency Data Base (CPDB) A summary table of chemicals in the carcinogenic potency data base: Results for positivity, potency and target sites. 2000 In: http://potencyberkley.edu/chemicalsummary.html


Khalil, Maslat and Fares


Mullican, M., Wilson, M., Conner, D., Kostlan, C., Shriei, D. and Dyer R. Design of 5-1, 3, 4-thiadiazoles, 1, 3, 4-oxadiazoles and 1, 2, 4-triazoles as orally-active, non-ulcerogenic antiinflammatory agents. *J Med Chem.* 36 (1993) 1090.


