MUTAGENICITY TESTS OF MOFEZOLAC (N-22)

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Abstract—1. The reverse mutation test was carried out on mofezolac (N-22) at dose range of 50-5000 μg/plate using Salmonella typhimurium strains, TA100, TA98, TA1535 and TA1537, and Escherichia coli strain WP2uvrA. In all tester strains no significant increases were observed in the number of revertant colonies as compared with solvent control in the absence or presence of mammalian metabolic activation system.

2. The chromosomal aberration test on N-22 was carried out using cultured Chinese hamster lung cells (CHL). The cells were treated with N-22 at the doses of 37.5, 75.0, 150 and 300 μg/ml without S9 Mix and at the doses of 150, 300, 450 and 600 μg/ml with S9 Mix. No significant differences were found in the incidence of structural-and numeral-aberrations of chromosomes as compared with the solvent control in the system without S9 Mix. However, in the system with S9 Mix, structural aberration (11.5%) and numeral aberration (14.2%) of chromosomes were observed in the groups of dosing 600 μg/ml with dose dependency.

3. These results indicate that N-22 has clastogenic activity by the metabolic activation.

Key words : Anti-inflammatory drug, mofezolac, reverse mutation test, chromosomal aberration test.

INTRODUCTION

Mofezolac (N-22) is a new isoxazol derivative which is developed as an oral drug with analgesic and anti-inflammatory effects. In the present study, the mutagenicity of N-22 was investigated by a reverse mutation assay using bacteria and a chromosomal aberration test using cultured mammalian cells.

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MATERIALS AND METHODS

1. Test compound and positive control compounds:

N-22 (Lot. No. 86C1001), which was synthesized in Kotai Kasei Kogyo Co., Ltd., and was supplied by Pharmaceutical Research Laboratory in our company, was used as a test compound. Its chemical structure is shown in Fig. 1 and its chemical name is [3, 4-di (4-methoxyphenyl)-5-isoxazolyl] acetic acid. N-22, which is crystal powder with white, is insoluble in water, but soluble in dimethyl sulfoxide (DMSO). Therefore, N-22 was dissolved in DMSO (Wako Pure Chemical Ind.) on both tests. The solutions were used within 5 days after preparation. In addition, N-22 solution is confirmed to be stable for 7 days at room temperature.

\[
\begin{align*}
\text{CH}_3\text{O} & \quad \text{CH}_{2}\text{COOH} \\
\text{OCH}_3 & \quad \text{N} \\
\end{align*}
\]

Fig. 1. Chemical structure of mofezolac (N-22).

As positive control compounds, 2-nitrofluorene (2NF, Aldrich Chemical Company, INC.), N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG, Nacalai Tesque, INC.), 9-aminoacridine (9AA, Sigma Chemical Co.) and 2-aminoanthracene (2AA, Wako Pure Chemical Ind.) were used in the reverse mutation assay. The concentration of each compound was decided according to the guideline for a reverse mutation test using bacteria proposed by the Labor Standards Bureau in Japan, and were concretely indicated as follows.

<table>
<thead>
<tr>
<th>Compound</th>
<th>TA100</th>
<th>TA1535</th>
<th>TA98</th>
<th>TA1537</th>
<th>WP2 uvr A</th>
</tr>
</thead>
<tbody>
<tr>
<td>non-metabolic activation</td>
<td>ENNG</td>
<td>ENNG</td>
<td>2NF</td>
<td>9AA</td>
<td>ENNG</td>
</tr>
<tr>
<td>3.0</td>
<td>5.0</td>
<td>1.0</td>
<td>80</td>
<td></td>
<td>2.0(μg/plate)</td>
</tr>
<tr>
<td>metabolic activation</td>
<td>2AA</td>
<td>2AA</td>
<td>2AA</td>
<td>2AA</td>
<td>2AA</td>
</tr>
<tr>
<td>1.0</td>
<td>2.0</td>
<td>0.5</td>
<td>2.0</td>
<td></td>
<td>20(μg/plate)</td>
</tr>
</tbody>
</table>

In the chromosomal aberration test, 0.1 μg/ml mitomycin C (MMC, Kyowa Hakko Kogyo Co. Ltd.) and 1000 μg/ml N-nitrosodimethylamine (DMN, Nacalai Tesque, INC.) were used according to the data published by Ishidate (1983).

All positive control compounds, except MMC and DMN, were dissolved in DMSO, and
Mutagenicity tests of mofezolac (N-22)

had been kept freezing until the use. MMC and DMN were dissolved in and/or diluted with physiological saline (Otsuka Pharmaceutical Co.) just before the use.

2. **Tester strains:**

*Salmonella typhimurium* strains TA100, TA98, TA1535 and TA1537 which were kindly provided by Dr. B. N. Ames and *Escherichia coli* strain WP2uvrA was kindly provided by Dr. M. Ishidate. Bacterial cultures having been stored in a deep freezer (−80°C) was inoculated into 2.5% nutrient broth (Oxoid), and was incubated with gently shaking at 37°C for about 15 hours.

Characteristics of the tester strains, e. g. histidine or tryptophan requirement, UV sensitivity, deep rough (rfa) character and the presence of R-factor plasmid, were checked at the same time of the assay.

3. **Cultured cells**:

In the chromosomal aberration test, CHL/IAU cells, a fibroblast cell line from the lung of a newborn Chinese hamster, were purchased from Dainihon Pharmaceutical Co. Ltd. The cells have been kept and passaged at our laboratory.

4. **Media**:

a. **Reverse mutation test**

The media used for the reverse mutation assay were prepared according to the guideline presented by the Labor Standards Bureau. The minimal glucose agar plate used for the mutagenicity assay contained 2% glucose and 1.5% agar powder in Vogel-Bonner medium E. The mixture of ten volume of 0.6% agar solution containing 0.5% NaCl, and one volume of 0.5mM L-histidine, 0.5mM biotin solution for the assay with *S. typhimurium* strains, while 0.5mM L-tryptophan solution for the assay with *E. coli* strain, were used as a top agar.

b. **Chromosomal aberration test**

The Eagle’s MEM, supplemented with 10% of inactivated newborn calf serum (CS) containing 0.12% NaHCO₃, was used.

5. **S9 Mix**:

a. **Reverse mutation test**

The S9 Mix consists of 4mM NADPH, 4mM NADH, 5mM glucose-6-phosphate, 33mM KCl, 8mM MgCl₂, 100mM sodium phosphate buffer (pH 7.4) and 0.1 ml of S9 fraction per ml of mixture. The mixture was prepared when needed. The S9 fraction was purchased from Oriental Yeast Industry Co. which was prepared from the liver of Sprague-Dawley male rats pretreated with phenobarbital and 5, 6-benzoflavone.

b. **Chromosomal aberration test**

The S9 Mix consists of 4mM NADP, 5mM glucose-6-phosphate, 33mM KCl, 5mM
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MgCl₂, 4mM HEPES-buffer (pH7.2) and 0.3ml of S9 fraction per ml of mixture. The mixture was prepared when needed. The S9 used in chromosomal aberration test is same as one in the reverse mutation assay.

6. Reverse mutation tests:

The reverse mutation assay was carried out according to the preincubation method of Yahagi (1975) which was a modification of that of Ames et al. (1975). Bacterial suspension (0.1ml) was added to a mini-tube containing 0.1ml of test compound solution and 0.5ml of S9 Mix or 100mM sodium phosphate buffer (pH7.4), and the mixture was preincubated at 37°C with gently shaking for 20 min. After 2ml of the top agar kept at about 45°C in a water bath was added to each mini-tube, the content was mixed and spread over each minimal glucose agar plate. Then the plates were incubated at 37°C for 2 days.

The number of revertant colonies was counted using a colony analyzer (Toyo CA-7). In addition, the state of the bacterial growth was observed with binoculars. Three plates per dose were used in all groups, including non-treated, solvent and positive controls. The result was judged as a positive when the mean number of revertants in the N-22 treated groups showed more than 2 times as compared with that in the solvent control and also showed a dose response.

7. Chromosomal aberration test:

The chromosomal aberration test was carried out according to the method of Ishidate (1983). In the method without metabolic activation (in direct method), the cells in 5ml of cell suspension (4 × 10⁶/ml) were seeded in a 60 mm plastic petri plate and cultured for 3 days. The solution of N-22 (25 μl) or MMC (100 μl) was added to the culture. The test compound was allowed to remain in the cultures for 24 or 48 hours. 100 μl of 10 μg/ml colcemid was added to all cultures 2 hours before harvesting. Hypotonic potassium chloride solution (75mM) was added to the cells harvested using 2ml of 0.25% trypsin, and then the cells were incubated for 15 min in a water bath at 37°C. After fixed with Caroy’s fixing fluid, the cell suspension was dropped on slides and stained with Giemsa solution.

In the metabolic activation method, one ml of S9 Mix and 30 μl of N-22 solution or 120 μl of 50mg/ml DMN was added to 3 day-old cultured cells. Cells were treated for 6 hours and washed with Dulbecco’s phosphate buffer solution (pH 7.4), and then recultured with a new culture medium. Chromosomal preparations were made by the same way as the direct method after 18 hours.

The cells in 2 dishes per group treated with test chemicals, and 2 slides of chromosomal preparations were made from each dishes. A hundred well-spread metaphases per each group were analyzed, and structural aberrations of chromosomes were observed by a blind method. Structural aberrations were classified as chromatid- and chromosomal-type
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including gaps, breaks, exchanges, and others. The cell having any type of these aberrations was recorded as an aberrant cell. We used to score a gap only when a clear discontinuity existed and the chromosome or chromatid is not broken at the discontinuous point. Polyploid cells were also counted as numeral aberrations.

The results from the score of structural aberrations were separately evaluated from that of the numeral aberrations. The final judgement was made on the incidence of total cells with chromosomal aberrations excluding gaps. The final evaluation on the outcome of each test was given as follows:

Negative  (−) less than 4%
Inconclusive (±) From 4% to less than 8%
Positive    (+) 8 % or more

For the numeral aberrations, the final judgement was given as follows:

Negative  (−) less than 5%
Inconclusive (±) From 5% to less than 10%
Positive    (+) 10% or more

Furthermore, the mutagenicity of the test chemical was evaluated in consideration of dose dependence.

8. Dosage:

In the reverse mutation assay, a preliminary test was carried out using S. typhimurium strain TA100 without S9 Mix to estimate the dosages of N-22 to be used. Since no antibacterial effects were observed at 5000 µg/plate, the dose was used as maximum and 6 more diluted doses of 2500, 1000, 500, 250, 100 and 50 µg/plate were used.

In the chromosomal aberration test, the doses were decided in the direct method and in the metabolic activation method, separately. The survival ratio of cells treated with the test compound for 48 hours in the direct method or for 6 hours following recovery time of 18 hours in the metabolic activation method, was measured using “Monocellater” (Olympus Tokyo). The concentration showing 50% inhibition of cell growth was estimated to be about 151 µg/ml (equivalently to be 445 µM) in the direct method and to be about 465 µg/ml in the metabolic activation method (Fig. 2). From these results, the maximum dose of N-22 was decided to be 300 µg/ml, which was about twice of the 50% inhibition dose, and the below doses were set as 150, 75, 37.5, 18.8 and 9.38 µg/ml in the direct method. In the metabolic activation method, the doses were selected to be 900, 600, 450, 300, 150 and 75.0 µg/ml, but the chromosomal preparations at 900 µg/ml could not observed in preliminary observation owing to the toxicity. Only the preparations at 300, 150, 75.0 and 37.5 µg/ml were observed in the direct method, while at 600, 450, 300 and 150 µg/ml in the metabolic activation method.
RESULTS

1. Reverse mutation tests:

Results of the reverse mutation test are shown in Tables 1 and 2. The background lawn of bacterial growth was sparse at 1000 µg/plate and more in TA1537 strain, and at 5000 µg/plate in TA1535 strain. However, the revertant colonies did not increase as compared with those of the solvent controls with or without S9 Mix. On the other hand, the number of revertant colonies in positive controls increased remarkably.

2. Chromosomal aberration test:

Results of the chromosomal aberration test are shown in Tables 3 and 4. In the direct method, the incidence of cells with structural aberrant (excluding gaps) were 0–1.0% in N–22 treated groups, and the incidence of aberrant cells including gaps were 0–3.8%. The incidences of cells having numeral aberrations (polyploidy) were 0–2.4%. No significant increases were found in the incidence of aberrant cells between the N–22 treated groups and the solvent control. In the metabolic activation system with S9 Mix, the incidence of structural aberrations increased dose-dependently and the maximum incidence (excluding gaps) was 11.5% at 600 µg/ml. The numeral aberrant cells also increased dose-dependently, and the maximum incidence was 14.2% at 600 µg/ml. In contrast, the incidence of aberrant cells increased greatly in positive controls in both direct and metabolic
Table 1. Reverse Mutation Tests on N-22 in *S. typhimurium* and *E. coli* Strains in the absence of S9 Mix.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Dose (µg/plate)</th>
<th>Revertants/plate (mean ± S.D.)</th>
<th>TA100</th>
<th>TA1535</th>
<th>TA98</th>
<th>TA1537</th>
<th>WP2uvrA</th>
</tr>
</thead>
<tbody>
<tr>
<td>NONE</td>
<td>...</td>
<td>94.6 ± 2.30</td>
<td>9.4 ± 2.13</td>
<td>15.0 ± 3.50</td>
<td>3.7 ± 0.83</td>
<td>19.4 ± 3.20</td>
<td></td>
</tr>
<tr>
<td>DMSO</td>
<td>...</td>
<td>97.0 ± 2.94</td>
<td>12.3 ± 1.85</td>
<td>19.8 ± 2.16</td>
<td>5.7 ± 1.02</td>
<td>15.4 ± 2.94</td>
<td></td>
</tr>
<tr>
<td>N-22</td>
<td>50</td>
<td>99.7 ± 6.11</td>
<td>10.6 ± 0.20</td>
<td>17.5 ± 0.90</td>
<td>3.6 ± 0.64</td>
<td>12.1 ± 0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>105.6 ± 3.41</td>
<td>11.4 ± 4.27</td>
<td>16.7 ± 4.04</td>
<td>5.4 ± 1.94</td>
<td>19.1 ± 3.53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>95.1 ± 19.31</td>
<td>6.6 ± 2.20</td>
<td>16.2 ± 1.10</td>
<td>4.5 ± 2.19</td>
<td>16.2 ± 0.70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>102.8 ± 6.93</td>
<td>8.6 ± 1.44</td>
<td>16.0 ± 3.66</td>
<td>5.7 ± 0.98</td>
<td>22.3 ± 2.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>97.2 ± 14.27</td>
<td>10.9 ± 2.13</td>
<td>16.2 ± 3.32</td>
<td>3.6 ± 2.11</td>
<td>19.8 ± 2.74</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2500</td>
<td>95.4 ± 7.49</td>
<td>7.8 ± 0.50</td>
<td>19.9 ± 2.00</td>
<td>1.6 ± 1.33</td>
<td>26.2 ± 3.66</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5000</td>
<td>84.2 ± 6.10</td>
<td>7.2 ± 4.77</td>
<td>18.1 ± 1.94</td>
<td>1.3 ± 1.15</td>
<td>19.0 ± 2.51</td>
<td></td>
</tr>
<tr>
<td>ENNG</td>
<td>2</td>
<td>......</td>
<td>......</td>
<td>......</td>
<td>......</td>
<td>631.4 ± 48.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1166.2 ± 127.03</td>
<td>......</td>
<td>......</td>
<td>......</td>
<td>......</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>......</td>
<td>1688.8 ± 241.85</td>
<td>......</td>
<td>......</td>
<td>......</td>
<td></td>
</tr>
<tr>
<td>2NF</td>
<td>1</td>
<td>......</td>
<td>......</td>
<td>158.2 ± 33.67</td>
<td>......</td>
<td>......</td>
<td></td>
</tr>
<tr>
<td>9AA</td>
<td>80</td>
<td>......</td>
<td>......</td>
<td>1243.6 ± 189.18</td>
<td>......</td>
<td>......</td>
<td></td>
</tr>
</tbody>
</table>

a : mean ± S. D. of 3 plates. b: not tested.

Table 2. Reverse Mutation Tests on N-22 in *S. typhimurium* and *E. coli* Strains in the presence of S9 Mix.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Dose (µg/plate)</th>
<th>Revertants/plate (mean±S.D.ª)</th>
<th>TA100</th>
<th>TA1535</th>
<th>TA98</th>
<th>TA1537</th>
<th>WP2 uvr A</th>
</tr>
</thead>
<tbody>
<tr>
<td>NONE</td>
<td>...</td>
<td>96.9±12.61</td>
<td>9.8±4.72</td>
<td>25.5±1.60</td>
<td>11.6±4.20</td>
<td>18.6±6.61</td>
<td></td>
</tr>
<tr>
<td>DMSO</td>
<td>...</td>
<td>88.1±25.77</td>
<td>8.4±2.13</td>
<td>31.1±3.31</td>
<td>7.5±2.01</td>
<td>16.3±1.60</td>
<td></td>
</tr>
<tr>
<td>N-22</td>
<td>50</td>
<td>125.8±19.75</td>
<td>9.6±1.15</td>
<td>26.9±4.90</td>
<td>8.2±1.66</td>
<td>16.6±3.46</td>
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</tr>
<tr>
<td></td>
<td>100</td>
<td>114.7±13.44</td>
<td>7.2±1.33</td>
<td>26.4±7.91</td>
<td>11.2±1.22</td>
<td>16.4±2.30</td>
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</tr>
<tr>
<td></td>
<td>250</td>
<td>118.4±8.77</td>
<td>8.0±2.00</td>
<td>25.8±6.43</td>
<td>10.6±1.81</td>
<td>19.6±1.40</td>
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<tr>
<td></td>
<td>500</td>
<td>94.6±16.32</td>
<td>8.7±3.52</td>
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<td>10.1±2.20</td>
<td>15.0±7.76</td>
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</tr>
<tr>
<td></td>
<td>1000</td>
<td>101.3±12.62</td>
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<td>9.2±3.11</td>
<td>20.3±3.93</td>
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</tr>
<tr>
<td></td>
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<td>79.7±1.92</td>
<td>7.6±2.77</td>
<td>23.2±4.04</td>
<td>9.1±3.72</td>
<td>21.9±9.59</td>
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</tr>
<tr>
<td></td>
<td>5000</td>
<td>85.3±3.80</td>
<td>6.4±1.17</td>
<td>27.4±2.25</td>
<td>7.8±4.39</td>
<td>17.6±4.24</td>
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</tr>
<tr>
<td>2AA</td>
<td>0.5</td>
<td>...</td>
<td>...</td>
<td>262.1±32.04</td>
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<td>...</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1198.0±163.37</td>
<td>...</td>
<td>...</td>
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</tr>
<tr>
<td></td>
<td>2</td>
<td>...</td>
<td>118.8±20.68</td>
<td>...</td>
<td>230.4±42.71</td>
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<tr>
<td></td>
<td>20</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>241.9±9.41</td>
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</tr>
</tbody>
</table>

ª: mean±S.D. of 3 plates. b: not tested.
Abbreviation: DMSO; dimethyl sulfoxide, 2AA; 2-aminoanthracene.
### Table 3. Chromosomal Aberration Test on N-22 in CHL Cells in the absence of S9 Mix.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Time (hr)</th>
<th>Dose (μg/ml)</th>
<th>Scored Cell No.</th>
<th>Chromosomal aberrant (%)</th>
<th>Judge-ment</th>
<th>Polyploid (%)</th>
<th>Judge-ment</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td>ctg</td>
<td>ctb</td>
<td>cte</td>
<td>csb</td>
<td>cse</td>
</tr>
<tr>
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<td>24-0*</td>
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<td>200</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>DMSO</td>
<td>24-0</td>
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<td>200</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N-22</td>
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<td>0</td>
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<tr>
<td></td>
<td>24-0</td>
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<tr>
<td></td>
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<td>200</td>
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<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>24-0</td>
<td>300</td>
<td>200</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MMC</td>
<td>24-0</td>
<td>0.1</td>
<td>200</td>
<td>4.5</td>
<td>8.0</td>
<td>32.5</td>
<td>0</td>
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<tr>
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<td>48-0</td>
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<td>200</td>
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<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>MMC</td>
<td>48-0</td>
<td>...</td>
<td>200</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MMC</td>
<td>48-0</td>
<td>37.5</td>
<td>200</td>
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<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>MMC</td>
<td>48-0</td>
<td>75.0</td>
<td>200</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MMC</td>
<td>48-0</td>
<td>150</td>
<td>200</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MMC</td>
<td>48-0</td>
<td>300</td>
<td>200</td>
<td>2.9</td>
<td>1.0</td>
<td>0</td>
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</tr>
<tr>
<td>MMC</td>
<td>48-0</td>
<td>0.1</td>
<td>200</td>
<td>11.0</td>
<td>46.5</td>
<td>79.5</td>
<td>0</td>
</tr>
</tbody>
</table>

*a: Treatment time ~ Expression time.

Abbreviation: ctg; chromatid and chromosomal gap, ctb; chromatid break, cte; chromatid exchange, csb; chromosomal break, cse; chromosomal exchange, TAG; total cells which have chromosomal aberrants including ctg, TA; total cells which have chromosomal aberrants excluding ctg, DMSO; dimethyl sulfoxide, MMC; mitomycin C.
Table 4. Chromosomal Aberration Test on N-22 in CHL Cells in the presence of S9 Mix.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>S9 Mix</th>
<th>Time (hr)</th>
<th>Dose (µg/ml)</th>
<th>Scored Cell No.</th>
<th>Chromosomal aberrant (%)</th>
<th>Judge-ment</th>
<th>Polyploid</th>
<th>Judge-ment</th>
</tr>
</thead>
<tbody>
<tr>
<td>NONE</td>
<td></td>
<td>6-18*</td>
<td>200</td>
<td></td>
<td></td>
<td>-</td>
<td>1.0(0.0)</td>
<td>-</td>
</tr>
<tr>
<td>DMSO</td>
<td></td>
<td>6-18</td>
<td>200</td>
<td></td>
<td></td>
<td>-</td>
<td>0.5(0.0)</td>
<td>-</td>
</tr>
<tr>
<td>N-22</td>
<td>+</td>
<td>6-18</td>
<td>200</td>
<td></td>
<td></td>
<td>-</td>
<td>0.5(0.0)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>6-18</td>
<td>450</td>
<td></td>
<td></td>
<td>-</td>
<td>2.5(0.5)</td>
<td>-</td>
</tr>
<tr>
<td>DMN</td>
<td>+</td>
<td>6-18</td>
<td>1000</td>
<td></td>
<td></td>
<td>-</td>
<td>5.0(0.0)</td>
<td>-</td>
</tr>
</tbody>
</table>

a; Treatment time - Expression time, b; Values in parenthesis are % of endoreduplication or type.
Abbreviation: ctg; chromatid and chromosomal gap, ctb; chromatid break, cte; chromatid exchange, csb; chromosomal break, cse; chromosomal exchange, TAG; total cells which have chromosomal aberrants including ctg, TA; total cells which have chromosomal aberrants excluding ctg, DMSO; dimethyl sulfoxide, DMN; N-nitrosodimethylamine.
Mutagenicity tests of moezolac (N–22)

activated method. This indicates that N–22 is weakly clastogenic only after the metabolic activation.

A metaphase in the control group is shown in photo. 1, and metaphases having structural- or numeral aberration are shown in photos. 2 and 3, respectively.

Photo. 1. A normal metaphase of CHL cell treated with DMSO together with S9 Mix for 6 hr.

Photo. 2. An abnormal metaphase of CHL cell treated with 600 μg/ml N 22 together with S9 Mix for 6 hr.
DISCUSSION

Bacterial mutagenicity assays were widely used for the mutagenicity tests on chemicals including clinical drugs. However, chromosomal aberrations can be detected only in mammalian cell systems. Therefore, it has been recommended to use these 2 tests as a minimum battery for mutagenicity tests in vitro (Ishidate, 1988).

N-22 showed no mutagenicity in reverse mutation assay with and without S9 Mix, nor in the chromosomal aberration test without S9 Mix. However, structural and numeral aberrations were noted in about 10%, respectively, at the dose of 600 μg/ml (1.77mM) of N-22 in the chromosomal aberration test with S9 Mix.

N-22 has two methoxyphenyl groups. Biotransformation study with rats showed that the methoxy groups of N-22 were converted to hydroxyl group by the demethylation. The demethylated N-22 was conjugated and finally excreted into urine or bile (Masuda, et al., in preparation). Kato et al. (1983) reviewed that formaldehyde is generally produced in the process of demethylation of methoxy groups. Therefore, the demethylation of N-22 is also thought to produce formaldehyde. Concerning an in vitro mutagenicity of formaldehyde, positive results were obtained by chromosomal aberration tests using fibroblast cell of human skin (Levy et al., 1983) and by sister chromatid exchange tests using cultured Chinese hamster ovary cells (Natarajan et al., 1983). Ishidate (1983) reported that
Mutagenicity tests of mofezolac (N-22)

formaldehyde induced structural aberration (14%) and numeral aberration (11%) at 0.5mM and 0.25mM, respectively, in CHL cells. Therefore, chromosomal aberrations induced by N-22 after metabolic activation in the present test were thought to be caused by formaldehyde which was produced in the process of demethylation. In addition, no mutagenicity of formaldehyde was shown in in vivo tests, micronucleus test and chromosomal aberration test (Natarajan et al., 1983). N-22, as well as formaldehyde, is thought not to have mutagenicity owing to oxidation and biotransformation of N-22 in vivo.

REFERENCES


