More Than Additive Toxicity of the Combination of 1-Methyl-1-nitrosourea Plus 1,3-Bis(2-chloroethyl)-1-nitrosourea in the Rat

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ABSTRACT

The combination toxicity index of 1-methyl-1-nitrosourea plus 1,3-bis(2-chloroethyl)-1-nitrosourea determined in the rat was 0.32. This overadditive combination toxicity appears mainly to be due to severe damage of the intestinal mucosa as diagnosed by histological examination and to damage of pluripotent stem cells in the bone marrow as could be assessed by the spleen colony technique. The molar ratio of 1-methyl-1-nitrosourea to 1,3-bis(2-chloroethyl)-1-nitrosourea resulting in maximum mortality was about 6. Concomitantly measured DNA interstrand cross-linking in bone marrow cells revealed a slight increase in DNA interstrand cross-linking following both drugs compared to 1,3-bis(2-chloroethyl)-1-nitrosourea alone.

INTRODUCTION

Some human tumor cell lines were reported to be proficient in preventing CNU-induced ISCL by removing O'-chloroethyl DNA monoadducts before cross-links can form (1). Cells able to prevent ISCL were also capable of repairing O'-methylguanine adducts by guanine O'-methyltransferase (2) and were designated as Mer + (methylation repair*). On the other hand Mer- tumor cell lines both were incapable of repairing O'-methylguanine adducts and preventing CNU-ISCL and lacked the repair enzyme O'-methyltransferase (3). Recently Erickson et al. (4) reported that the monoadduct repair system can be inactivated by pretreatment with the methylating agents MNNG or MNU followed by an increase in CNU-ISCL and cytotoxicity to tumor cells. With regard to the potential benefit of the combination of MNNG or MNU plus CNU on in vivo treatment of tumors, knowledge of whole animal and organ toxicity of this combination is indispensable. For this purpose the combination toxicity index of MNU plus BCNU in the rat was determined. Histological examinations were performed to assess the toxic effects in different organs. Furthermore mutagenicity was measured as inhibition of pluripotent (CFU-S) and granulocyte committed (CFU-C) stem cells together with the extent of DNA single-strand breaks (SSB) and ISCL.

MATERIALS AND METHODS

Animals. Male Wistar rats (Charles River Wiga GmbH, Sulzfeld, Federal Republic of Germany) were used at 3 months of age. MNU was kindly provided by Dr. M. Wiessler; BCNU was provided by Dr. G. Eisenbrand (Institute of Toxicology and Chemotherapy, German Cancer Research Center, Heidelberg, Federal Republic of Germany).

Histological Examination. All animals were investigated histologically after spontaneous death. For the investigation of fresh material 4 additional animals were killed by ether anesthesia 5 days after administration of 20 mg MNU/kg plus 7.5 mg BCNU/kg. The following organs were investigated: brain, lung, heart, liver, spleen, bone marrow, kidney, stomach, small intestine, colon.

RESULTS AND DISCUSSION

Determination of CTI. Determination of CTI was preceded by determining the single dose toxicity (evaluation day 90) of the individual drugs using 3 groups of 10 rats each for MNU and BCNU, respectively. For the assessment of the CTI 14 combinations of MNU plus BCNU were investigated using 10 rats per combination. The time interval between MNU and BCNU administration was 2 h. Both drugs were given i.p. In accordance with a method reported by Skipper (5) for quantitatively examining the lethal toxicity of combinations of antitumor drugs in mice, the sums of the decimal fractions of the LD₅₀ of both agents were opposed to the observed mortality. Slightly modifying Skipper's method the LD₅₀(s) of the combination was then calculated by probit analysis (6).

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COMBINATION TOXICITY OF MNU PLUS BCNU

Table 1 Combination toxicity of MNU plus BCNU (single dose)

<table>
<thead>
<tr>
<th>Group</th>
<th>MNU (mg/kg)</th>
<th>BCNU (mg/kg)</th>
<th>MNU/BCNU molar ratio</th>
<th>Sum of the decimal fractions of the LD₅₀ of both agents</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.5 (24)*</td>
<td>0.03</td>
<td>0.03</td>
<td>0.3</td>
<td>0.55*</td>
</tr>
<tr>
<td>2</td>
<td>5 (49)</td>
<td>0.06</td>
<td>0.06</td>
<td>0.8</td>
<td>0.49*</td>
</tr>
<tr>
<td>3</td>
<td>10 (97)</td>
<td>0.12</td>
<td>0.12</td>
<td>0.17</td>
<td>0.29</td>
</tr>
<tr>
<td>4</td>
<td>10 (97)</td>
<td>0.12</td>
<td>0.12</td>
<td>0.34</td>
<td>0.38</td>
</tr>
<tr>
<td>5</td>
<td>10 (97)</td>
<td>0.12</td>
<td>0.12</td>
<td>0.34</td>
<td>0.38</td>
</tr>
<tr>
<td>6</td>
<td>20 (194)</td>
<td>0.24</td>
<td>0.24</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>7</td>
<td>20 (194)</td>
<td>0.24</td>
<td>0.24</td>
<td>0.34</td>
<td>0.17</td>
</tr>
<tr>
<td>8</td>
<td>20 (194)</td>
<td>0.24</td>
<td>0.24</td>
<td>0.34</td>
<td>0.17</td>
</tr>
<tr>
<td>9</td>
<td>27.25 (265)</td>
<td>0.33</td>
<td>0.33</td>
<td>0.22</td>
<td>0.34</td>
</tr>
<tr>
<td>10</td>
<td>30 (291)</td>
<td>0.36</td>
<td>0.36</td>
<td>0.22</td>
<td>0.22</td>
</tr>
<tr>
<td>11</td>
<td>30 (291)</td>
<td>0.36</td>
<td>0.36</td>
<td>0.22</td>
<td>0.22</td>
</tr>
<tr>
<td>12</td>
<td>40 (388)</td>
<td>0.48</td>
<td>0.48</td>
<td>0.22</td>
<td>0.22</td>
</tr>
<tr>
<td>13</td>
<td>40 (388)</td>
<td>0.48</td>
<td>0.48</td>
<td>0.22</td>
<td>0.22</td>
</tr>
<tr>
<td>14</td>
<td>50 (485)</td>
<td>0.60</td>
<td>0.60</td>
<td>0.90</td>
<td>0.69*</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, µmol/kg.

Marrow as main targets of toxicity. In the small intestine necroses of the intestinal villi and extended ulcerations of the mucosa were observed. In the colon an ulcerative mucosal and hemorrhagic colitis predominated. In the bone marrow a pronounced atrophy of hemopoietic elements was the main finding. Further analysis of myelotoxicity points out that especially the CFU-S are damaged by the combination of MNU plus BCNU. Thus combination of 100 µmol MNU/kg (10.3 mg/kg) plus 50 µmol BCNU/kg (10.7 mg/kg), corresponding to a sum of the decimal fractions of the LD₅₀ of 0.49, depressed CFU-S survival to 6.8 ± 3.2% compared to 10.2 ± 2.5% following single-dose treatment with 200 µmol BCNU/kg (42.8 mg/kg) alone. The results given in Table 2 indicate that the granulocyte committed (CFU-C) stem cells are not damaged to the same extent as the CFU-S.

Comparing the results following 200 µmol MNU/kg or 50 µmol BCNU/kg separately on the one hand and 200 µmol MNU/kg plus 50 µmol BCNU/kg combined on the other hand reveals the more than additive effect of both compounds on CFU-S and the merely additive effect on CFU-C. Specifically, the observed level of CFU-S is 3.8 times lower while the observed level of CFU-C is only 1.4 times lower than expected on an additive basis.

Results of alkaline elution (evaluated 20 h after treatment) following 200 µmol MNU/kg and 50 µmol BCNU/kg yielded 173 ± 3.0 and 32 ± 1.0 rad equivalents SSB and 0 and 10.5 ± 6.5 rad equivalents ISCL, respectively. Combined administration of both doses revealed 135.5 ± 7.5 rad equivalents SSB and 22 ± 3.0 rad equivalents ISCL. The data indicate an increased frequency of BCNU induced cross-links at this time point due to MNU pretreatment while at the same time the SSB frequency is decreased.

In vitro experiments indicated peak cross-linking levels 6 to 8 h after exposure to CNU's (12). After in vivo treatment of rats with BCNU, however, this peak was extended to a plateau between 6 and 16 h followed by a small decrease 24 h after treatment (13). We determined cross-linking levels 20 h after treatment at the end of this plateau. Rat bone marrow cells exhibited cross-linking without MNU pretreatment. Therefore it is likely that the marrow cells have only limited repair capacity in comparison to MER+ cell lines of human origin (1).

The pronounced damage of the normal intestinal mucosa...
the rat by the combination treatment is apparently due to the same mechanism, inhibition of monoadduct repair systems by MNU. In view of the successful sensitization of human colon tumor cell lines by the methylating agents MNU, MNNG, or streptozotocin to killing by CNUs (14, 15), knowledge of the sensitivity of normal mucosa cells of human colon to the combination treatment would be of special interest.

To our knowledge, there exist only a few reports in the literature on the combination toxicity of other methylating agents plus CNUs. Skipper, for instance, reported that 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide plus CCNU resulted in a CTI of 0.45, while 5-[3,3-bis(2-chloroethyl)-1-triazeno]-imidazole-4-carboxamide plus CCNU revealed a CTI of 1.1 (additive) (5). Whether a comparable mechanism is responsible for the more than additive combination toxicity of the dimethyl analogue plus CCNU must, however, be proved.

In conclusion, the combined administration of the methylating agent MNU plus BCNU is accompanied by a pronounced combination toxicity, especially on the intestinal tract and on the bone marrow. The same overadditive toxicity might occur with combinations of other methylating agents plus CNUs so that eventual clinical trials should be performed with the respective precautions.

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REFERENCES

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