INDUCTION OF ABERRANT CRYPTS IN THE COLONS OF RATS BY ALKYLATING AGENTS

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Aberrant crypts are morphologically changed colonic crypts, which appear in colons of mice and rats after treatment with chemical carcinogens and are thought to be early preneoplastic lesions [1]. The method described by Bird [1] enables direct visualisation of these altered crypts in the intact methylene blue-stained colon. They can be distinguished from surrounding normal crypts by increased size, thicker epithelial lining and increased pericryptal zone. This method enables quantitative analysis of the induction of potential preneoplastic lesions in the colon by chemical carcinogens.

The following observations indicate that aberrant crypts might represent preneoplastic lesions in the colon: such crypts are absent in untreated animals; they are induced by colon carcinogens but not noncarcinogens [2]; they appear 2 weeks after carcinogen treatment and persist; they reveal histological changes from mild atypia to dysplasia; these changes increase with time and in response to a high fat diet, a known colon tumor promoter [3]; and they are induced in a species specific and carcinogenic dose-dependent manner [1].

The aim of this work was to study the effect of 4 alkylating agents: 1,2-dimethylhydrazine (DMH); N-nitrosomethylurea (MNU); N-methyl-N-nitro-N-nitrosoguanidine (MNNG) and methyl-methane sulphonate (MMS) on the induction aberrant crypts in the colon of female Wistar rats.
Three of the compounds tested (DMH, MNU, MNNG) are known to induce colon cancer in rats and mice [4, 5, 6].

We have also compared the ability of DMH and MNU to induce aberrant crypts in 3 - 4 week-old and 9 - 10 week-old Wistar rats.

The animals come from the Animal House Colony of the Warsaw Medical School. They were housed in plastic cages with hardwood chip bedding and wire tops. They received standard chow diet (Bacutil, Gdańsk, Poland) and water ad libitum.

DMH or MNU were administered to two groups of rats (at least 6 animals each) twice, 4 days apart, by intragastrical intubation. In each intubation the rats (of both groups) received DMH and MNU at the following dosage levels: 25, 50 or 100 mg/kg body weight, thus the amount given in Fig.1 is the sum of two intubations. MNNG and MMS were administered only to the animals 3 - 4 week-old either by a single intragastric intubation or by two intrarectal installations. The dose chosen was approximately LD₅₀, one-half of the LD₅₀ or LD₅₀ for these compounds. The following solvents were used to dissolve the compounds applied: 0.9% NaCl for DMH, MMS and MNNG for intrarectal treatment, 1% citric acid for MNU and 50% ethanol in 0.9% NaCl for MNNG used in intragastric intubation. Control groups received appropriate solvents (point 0 on Fig.1). Total volumes ranged from 0.1 to 0.4 ml, intrarectally rats received 0.1 ml portions. No serious short-term effect were observed in control groups. Rats were kept on their normal diet for 21 days after the first treatment.

Aberrant crypts were analyzed according to Bird [1]. Rats were killed by cervical dislocation 21 days after the first carcinogen treatment. Their colons were removed, flushed with Krebs-Ringer salt solution, cut open along the longitudinal median axis and fixed flat between filter paper in 10% formalin. The colons (each 8 - 10 cm long) were stored in formalin at room temperature up to one month.

Immediately before analysis the colons were stained with methylene blue (0.2% in Krebs-Ringer solution) for 30 to 60 min to visualise crypt outlines. Then the colons were placed on microscope slides the mucosal side up, and aberrant crypts were scored under a light microscope at the magnification of 25 or 100. The number of aberrant crypts per colon, their location, the number of crypts per focus and the shape of the crypts were recorded. All colons (12 colons for each carcinogen dose) were scored by one observer. Consequently groups were coded and recorded blindly. The
Fig. 1. Induction of aberrant crypt foci by DMH and MNU in the colons of 3 - 4 week-old (▲) and 9 -10 week-old (●) female Wistar rats. The results are expressed as mean values from 2 separate experiments. Each point represents the mean ± S.E. for 12 colons. DMH, 1,2-dimethylhydrazine; MNU, N-nitrosomethylurea
repeated scorers yielded very similar values. The results are expressed as the mean number of aberrant crypt foci per colon ± standard error.

DMH and MNU induced aberrant crypts in Wistar rats in the dose-dependent manner (Fig. 1).

We also examined the effect of animal age on aberrant crypts induction by DMH and MNU. It was found that 9 - 10 week-old rats were 4 - 7 times more sensitive to aberrant crypts induction by DMH and MNU than young (3 - 4 week-old) ones (Fig.1).

This is in agreement with the suggestion that this result might be explained by the proposed hypothesis that aberrant crypts are early preneoplastic lesions in the colon, since carcinogenic processes are more frequent in older organisms than in younger ones.

In this work we also present investigations on the induction of aberrant crypts by MNNG and MMS. MNNG, known to induce colon cancer when administered intrarectally to Fisher rats in a total dose of 40 mg/kg [7], was found to induce 3.8 aberrant crypts per colon in 3 - 4 weeks old Wistar when administered intrarectally twice in a total dose of 50 mg/kg (Table 1). MNNG was found not to induce colon tumors when administered intragastrically in a single dose of 250 mg/kg to rats [8]. In our studies it was found that after a single intragastrical intubation, MNNG (125 and 250 mg/kg) induced a very small number of aberrant crypts per colon in a dose-independent manner (Table 1).

MMS, a direct mutagen but not carcinogen, induced a very small number of aberrant crypts per colon (0.2 ± 0.2) when administered intragastrically, and in 3 - 4 week-old female Wistar rats at the LD50 (250 mg/kg) dose. When administered intrarectally, MMS was also ineffective in aberrant crypt induction, since only 0.2 ± 0.2 aberrant crypts per colon were found at the lower dose studied (Table 1).

These results confirm the observation that there is a good agreement between the carcinogenicity of chemical compounds and their ability to induce aberrant crypts in rat colons. This observation also supports the hypothesis [1] that aberrant crypts are preneoplastic genetic lesions of which only some will develop into cancers.

Aberrant Crypt Assay [1] seems to be of value as a short-term model test on rat colon for carcinogenic properties of chemical compounds and their modifiers. Further investigations are necessary to explain the mechanism of aberrant crypt transformation into cancer.
Table 1

*Induction of aberrant crypt foci in colons of 3 - 4 week-old female Wistar rats by MNNG and MMS*

The results are expressed as mean values ± S.E. from 2 separate experiments (6 rats per group)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Route of administration</th>
<th>Dose mg/kg</th>
<th>Number of AC foci/colon ±S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>intragastrical</td>
<td>2 × 0.2 ml</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>intrarectal</td>
<td>2 × 0.1 ml</td>
<td>0</td>
</tr>
<tr>
<td>50% Ethanol in 0.9% NaCl</td>
<td>intragastrical</td>
<td>1 × 0.2 ml</td>
<td>0</td>
</tr>
<tr>
<td>MNNG</td>
<td>intragastrical</td>
<td>1 × 125</td>
<td>0.44 ± 0.44</td>
</tr>
<tr>
<td>MNNG</td>
<td>intrarectal</td>
<td>1 × 250</td>
<td>0.11 ± 0.11</td>
</tr>
<tr>
<td>MMS</td>
<td>intragastrical</td>
<td>1 × 125</td>
<td>0</td>
</tr>
<tr>
<td>MMS</td>
<td>intrarectal</td>
<td>1 × 25</td>
<td>3.80 ± 1.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 × 250</td>
<td>0.20 ± 0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 × 30</td>
<td>0.20 ± 0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 × 60</td>
<td>0</td>
</tr>
</tbody>
</table>

MNNG, N-methyl-N'-nitro-N-nitrosoguanidine; MMS, methyl-methane sulphonate; AC, aberrant crypts.

**REFERENCES**