The Chronological Appearance of Flat Colonic Neoplasias in Rats*

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Abstract. Following weekly s.c. injections of the colonotropic carcinogen 1,2-dimethylhydrazine (DMH), we investigated the occurrence of flat and protruding neoplasias in the colon of rats at various time intervals. Forty-seven DMH-treated rats were sacrificed at the 13th, 15th, 19th, 21st and 22nd weeks. A total of 88 tumors evolved in 35 of the 47 DMH-treated rats. The number of neoplasias/animal was 0.3 at week 13, 1.2 at week 15, 2.0 at week 19, 2.5 at week 21 and 4.0 at week 22. In the right colon, although the percent of flat adenomas was lower than of protruding adenomas, the percent of flat carcinomas was significantly higher than of protruding carcinomas, indicating that flat adenomas progress more rapidly to invasive carcinoma than protruding adenomas in the right colon. The opposite was recorded in the left colon where the percent of protruding adenomas was lower than of flat adenomas, but the percent of protruding carcinomas was higher than of flat carcinomas. During the last experimental week as many as 63% of the protruding carcinomas occurred but only 25% of the flat carcinomas. These experimental results seem to substantiate previous observations in humans suggesting that, in the colonic mucosa, flat and protruding adenomas follow different pathways of neoplastic transformation.

For many years it has been recognized that colorectal carcinomas originate in humans from protruding foci of dysplastic cell proliferation known as adenomatous polyps. About 5% of the adenomas eventually proceed to invasive carcinoma (1). That sequence of events has been referred to as the adenoma carcinoma sequence (2).

In 1956, while describing the histological characteristics of colorectal adenomas in colectomy specimens from patients with familial adenomatous polyposis (FAP), Bussey (3) noticed among multiple protruding adenomas, flat adenomatous lesions. In his monography, Bussey (3) illustrated a "lesion consisting of adenomatous tubules, which have not produced any thickening of the mucosa". Years later, Muto and co-workers (4) detected by colonoscopy – complemented by chromoscopy – similar flat dysplastic lesions in patients without FAP. The authors called these lesions flat adenomas. Some of the flat adenomas reported by Muto had, in addition, an invasive growth. Those significant endoscopic-histological findings led Japanese endoscopists and pathologists to postulate a novel pathway of colorectal carcinogenesis, namely the flat adenoma-carcinoma sequence (5). That pathway of colorectal carcinogenesis attracted worldwide interest and several workers outside Japan confirmed the Japanese experience (6-8). Some authors, however, have questioned the identity of flat adenomas based on the assumption that they may progress to protruding adenomas (9). In this respect, Watari et al. (10) claim that flat adenomas in man change to exophytic over two or more years and that with time 40% of the minute flat adenomas become exophytic.

In experimental animals flat colonic neoplasias have been evoked by the aid of various carcinogens (11-15). In previous experiments in rats using a colonotropic carcinogen, we found in addition to exophytic adenomas and carcinomas, flat adenomas and flat carcinomas (16).

The purpose of the present work was to record the chronological appearance of flat (i.e. non-protruding) and exophytic (i.e. protruding) colorectal neoplasias at various time intervals using an experimental model.

Materials and Methods

Forty-seven Sprague-Dawley rats (Anticimex, Stockholm, Sweden) weighing approximately 200 g were used. The animals were kept 5 rats/cage and were fed with a purine chow diet (R3; Ewos, Astra, Södertälje, Sweden). All rats were injected s.c. with 21 mg/kg of body weight of 1,2-dimethylhydrazine (DMH) hydrochloride salt (MW, 133-02) (Kebo, Stockholm, Sweden) suspended in 1 ml of...
EDTA solution (as a stabilizing agent) once a week. The animals were weighed once in 2 weeks and the DMH dose adjusted accordingly until sacrifice. The experiment was approved by the Ethical Committee of the Karolinska Institute and Hospital, Sweden.

Animals were sacrificed at various time intervals. Group of rats selected at random from the 10 cages were sacrificed at weeks 13 (n=10), 15 (n=11), 19 (n=10), 21 (n=9) and 22 (n=9). They were euthanized with an intramuscular injection of 0.7 ml Hypnoform (Jansen, Belgium); autopsy was performed immediately after.

The colons were dissected and fixed immediately in milapot (90 ml of 5 mol acetic acid, 510 ml of methanol, 400 ml of DW). After overnight fixation, the colons were divided into two segments: the right colon (comprising the cecum, the ascending colon and the transverse colon) and the left colon (comprising the descending colon and rectum). The gross appearance of all visible lesions was described, measured and their exact location in the colon was recorded.

All lesions (polyps or suspected polyps) were either biopsied with a skin biopsy forceps or dissected with the aid of scissors and scalpel and processed for routine microscopy. The remnant colons were Swiss-rolled and examined microscopically.

**Glossary**

**Adenoma:** Grossly visible lesion showing a group of dysplastic crypts, or grossly invisible lesion (detected at microscopy) having \( \geq 5 \) dysplastic crypts. Adenomas were subdivided into flat (i.e. non-protruding) and protruding. In well-oriented sections, flat adenomas were regarded as lesions with a group of dysplastic glands having a height not exceeding twice the thickness of the surrounding normal mucosa. Protruding adenoma were regarded as lesions with a group of dysplastic glands having a height exceeding twice the thickness of the surrounding normal mucosa.

**Adenocarcinomas:** Lesions with neoplastic glands invading through the muscularis mucosae into the submucosal layer or beyond. Adenocarcinomas were subdivided into non-lymphoid-associated carcinomas (NLACs) and lymphoid-associated carcinomas (LACs): NLACs were either flat (i.e. non-protruding) or protruding adenocarcinomas. LACs were subdivided according to their microscopic pattern into glandular and signet ring cell carcinomas. LACs grew within the confines of a lymphoid plaque or beyond. Due to difficulties in interpretation, LACs were not sub-classified into either flat or protruding.

**Figure 1.** Flat adenoma in the colon of a rat following dimethylhydrazine injections (H&E x1).

**Figure 2.** Protruding adenoma in the colon of a rat following dimethylhydrazine injections (H&E x1).
Figure 3. Flat non-lymphoid-associated adenocarcinoma in the colon of a rat following dimethylhydrazine injections (H&E x1).

Figure 4. Protruding non-lymphoid-associated adenocarcinoma in the colon of a rat following dimethylhydrazine injections (H&E x1).

Figure 5. Lymphoid-associated adenocarcinoma in the colon of a rat following dimethylhydrazine injections (H&E x1).
Results

A total of 88 tumors evolved in 35 of the 47 DMH-treated rats. Of the 88 tumors, 54 were adenomas, 16 were NLACs and 18 were LACs. Of the 54 adenomas, 31 were flat (Figure 1) and 23 were protruding (Figure 2). Of the 16 NLACs, 8 (50%) were flat (Figure 3) and 8 (50%) protruding.

No tumors were found in the rectum.

Chronological appearance of colonic tumors. Adenomas: Table I shows that at week 13, only one adenoma (i.e. 1.9% of all 54 adenomas found in this series) had developed. The percent of adenomas increased (when compared to week 13) at 15, 19, 21 and 22 weeks. The highest percent of adenomas (43%) was found at the end of the experiment, at week 22.

Adenocarcinomas: Table I shows that the first carcinoma (i.e. 6% of all 16 carcinomas found in this series) occurred at week 15. The percent of carcinomas increased (when compared to week 15) at 19, 21 and 22 weeks. The highest percent of carcinomas was found at the end of the experiment, at week 22.

No tumors were found in the rectum.

Localization of flat colonic tumors (Table III). Of the thirty-one flat adenomas, thirteen (42%) were found in the right colon and the remaining eighteen (58%) in the left colon. Of the twenty-three protruding adenomas, fourteen (60%) were found in the right colon and the remaining nine (40%) in the left colon.

Of the total eight flat NLACs, seven (87.5%) were recorded in the right colon and one (12.5%) in the left colon. Of the eight protruding NLACs, four (50%) were found in the right and the remaining four (50%) in the left colon.

Of the eighteen LACs, fourteen (78%) were present in the right colon and the remaining four (22%) in the left colon.

Discussion

Following weekly injections of DMH, Suzuki and Umehara (17) sacrificed two rats each at 6, 8, 10, 14, 18 and 22 weeks. Those authors found neither grossly visible tumors, nor dysplasia in the 6 rats killed before the 14th week of treatment (we found one flat adenoma and two LACs at week 13). However, cytophotometric DNA analysis disclosed a significant increase in proliferative activity of the mucosa 4 weeks before the appearance of histological dysplasia and 8 weeks before the appearance of grossly visible tumors. Their results (17) indicated that biochemical changes precede the development of visible lesions (i.e. adenomas or carcinomas). The colonic neoplasias recorded in that work were not subclassified into flat or protruding (17).

In the present investigation we found in DMH-treated rats that colonic adenomas increased from 2% at week 13 to 43% at week 22, NLACs from 6% at week 15 to 37.5% at week 22 and LACs from 11% at week 13 to only 17% at week 22. Thus, adenomas and LACs evolved one week earlier than NLACs. With increasing DMH doses, the number of adenomas and NLACs steadily increased, whereas the number of LACs were less influenced by the cumulative DMH doses.

When the profile of adenomas was analysed separately at various time intervals, it was found that flat adenomas increased from 3% at week 13 to 29% at week 22, whereas protruding adenomas showed a more dramatic increase from 4% at week

<table>
<thead>
<tr>
<th>Week</th>
<th>Adenomas</th>
<th>Carcinomas**</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>1 (1.9%)</td>
<td>1 (1.4%)</td>
</tr>
<tr>
<td>15</td>
<td>6 (11.1%)</td>
<td>1 (6.3%)</td>
</tr>
<tr>
<td>19</td>
<td>15 (27.8%)</td>
<td>4 (25.0%)</td>
</tr>
<tr>
<td>21</td>
<td>9 (16.7%)</td>
<td>5 (31.3%)</td>
</tr>
<tr>
<td>22</td>
<td>23 (42.6%)</td>
<td>6 (37.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>54 (100%)</td>
<td>16 (100%)</td>
</tr>
</tbody>
</table>

** Lymphoid-associated carcinomas (LACs) are not included
15 to nearly 57% at week 22. These findings suggest that despite the fact more flat adenomas (n=31) than protruding adenomas (n=23) developed between 13 and 22 weeks of DMH treatment, protruding adenomas notably developed during the last experimental week. Since in the last experimental week a similar increase for flat adenomas was not registered, it would appear that flat adenomas are less dose-dependent lesions than protruding adenomas. One possible consequence of these findings may be that flat and protruding adenomas follow different pathways of neoplastic transformation.

In the literature there are experimental and clinico-genetic data that support that assertion. In a previous experiment in DMH-treated rats (18), we found that flat adenomas expressed less human tumor-associated antigens (CO17-1A, Ga 73-3, BR55, GICA 19-9 and CA-50) than protruding adenomas. In another experiment (19), we found 25% flat adenomas in DMH-treated animals, but none in Glu-1-treated rats; all Glu-1-induced adenomas were protruding. The possibility that the chemical composition of the carcinogen had played a role in the evolution of flat adenomas was entertained in that work (19). More recently, Iishi et al. (20) found that the administration of azoxymethane and pravastatin – an inhibitor of ras p 21 isoprenilation – resulted in a significant increase of flat colonic adenomas, indicating that the ras oncogene may be a factor in the development of flat adenomas in rats. In humans, Suzuki et al. (21) found that Bcl-2, Bak and p53 (i.e. apoptosis-related proteins) were significantly lower in flat than in protruding colorectal neoplasias. More recently Richter et al. (22) found that chromosomal losses on chromosome 17p and 20 and gains in chromosomes 2q, 5, 6, 8q and 12q occurred exclusively in flat colorectal adenomas. While studying angiogenesis in colorectal adenomas we found (23) that the number of microvessels, evidenced by Factor VIII immunostain, were normal to slightly increased in flat adenomas but moderately to markedly increased in protruding adenomas. Although the causes for the failure of flat adenomas to generate microangiogenesis remained elusive, it suggested that the stroma of the host reacted differently when harboring either a flat or a protruding adenoma.

Earlier animal experiments (19) suggested that the chemical composition of the carcinogen may play a role in the development of either flat or protruding adenomas. The question arises as to whether in man also different environmental carcinogens might lead to the development of either flat or protruding adenomas. It should be mentioned that the degree of dysplasia was found to be different in flat adenomas from Japanese and Swedish patients (24) suggesting that geographic-environmental influences may be at stake.

Watari et al. (10) claim that flat (non-polypoid) tumors in man change to exophytic over two or more years and that with time 40% of the minute flat adenomas become exophytic. In the present model there appears to be no indication that flat adenomas evolved into protruding (i.e. exophytic) adenomas. In fact, the dramatically increased percentage of protruding adenomas during the last week of the DMH treatment was not

### Table II. The occurrence of colorectal neoplasms correlated to the duration of 1,2 dimethylhydrazine (DMH) treatment.

<table>
<thead>
<tr>
<th>Week at sacrifice following weekly DMH injections</th>
<th>Adenomas</th>
<th>Carcinomas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protruding</td>
<td>Flat (i.e. non-protruding)</td>
</tr>
<tr>
<td>Week 13</td>
<td>1 (3.2%)</td>
<td>2 (11.1%)</td>
</tr>
<tr>
<td>Week 15</td>
<td>1 (4.3%)</td>
<td>5 (16.1%)</td>
</tr>
<tr>
<td>Week 19</td>
<td>6 (26.1%)</td>
<td>10 (32.3%)</td>
</tr>
<tr>
<td>Week 21</td>
<td>3 (13.0%)</td>
<td>6 (19.4%)</td>
</tr>
<tr>
<td>Week 22</td>
<td>13 (56.5%)</td>
<td>9 (29.0%)</td>
</tr>
<tr>
<td>All weeks</td>
<td>23 (100%)</td>
<td>31 (100%)</td>
</tr>
</tbody>
</table>

*LACs: Lymphoid-associated carcinomas.

### Table III. Distribution of flat and protruding neoplasms in the right and in the left colonic segments in 49 Sprague-Dawley rats injected weekly with 1,2 dimethylhydrazine.

<table>
<thead>
<tr>
<th>Localization</th>
<th>Protruding (i.e. non-protruding)</th>
<th>Flat (i.e. non-protruding)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right colon</td>
<td>14 (60.1%)</td>
<td>13 (41.9%)</td>
</tr>
<tr>
<td>Left colon</td>
<td>9 (39.9%)</td>
<td>18 (58.1%)</td>
</tr>
<tr>
<td>Total</td>
<td>23 (100%)</td>
<td>31 (100%)</td>
</tr>
</tbody>
</table>

*LACs: Lymphoid-associated carcinomas.
compensated for by a substantial decrease in the proportion of flat adenomas during the same experimental week.

In the right colon of rats the percent of flat adenomas was lower than for protruding adenomas but the percent of flat carcinomas was significantly higher than for protruding carcinomas. Those results indicate that, for unknown reasons, flat adenomas progress more rapidly to invasive carcinoma than protruding adenomas in the right colon. The opposite was recorded in the left colon where the percent of protruding adenomas was lower than for flat adenomas but the percent of protruding carcinomas was higher than for flat carcinomas. Interestingly, the majority (62.5%) of the protruding NLACs evolved during the last experimental week. On the other hand, the rate for flat NLACs at week 22 was only 25%. These experimental results seem to substantiate previous observations in humans (4,6,9,10,22), suggesting that flat neoplasias might proceed to carcinoma through a trail that is at variance with that followed by protruding adenomas.

Another finding that emerged from this work is that 12 of the 47 animals remained tumor-free. It is evident that even within the same strain of animals there are individuals with an apparently tenacious resistance to developing colonic tumors despite weekly treatment with a colonotropic carcinogen. The causes for this remarkable resistance in some rats of the same strain deserve to be further investigated.

Acknowledgements
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References