A role of gender in the occurrence of dimethylhydrazine induced colorectal tumors in Wistar rats

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Human colorectal carcinoma appears more frequently in males. The aim of our study was to evaluate the influence of gender on the induction of colorectal carcinoma by 1,2-dimethylhydrazine (DMH) in Wistar rats. Sixty Wistar rats (30 males, 30 females) were subjected to weekly subcutaneous injections of DMH (20 mg/kg) for 15 weeks. After 25 weeks from the beginning of the experiment the animals were sacrificed and autopsied. All macroscopical lesions were evaluated histologically. Induction of colorectal tumors succeeded in 37% of males and 17% of females. There were 21 tumors of the large bowel found, of these 15 in males and 6 in females. Histologically, males had 11 adenomas, 2 signet-cell carcinomas and 2 adenocarcinomas, while females had 4 signet-cell carcinomas and 2 adenomas. We also found extracolonic tumors, mainly those of the small intestine and of the Zymbal glands. Wistar rats showed lower incidence of DMH-induced colorectal tumors in comparison with other strains of rats. The gender-dependent difference in the incidence of colorectal tumors was found to be statistically marginally significant (p<0.08), whereas the difference in the incidence of all induced tumors between genders was significant (p<0.02). Males showed a greater incidence of colorectal tumors and also a greater histological resemblance to human colorectal tumors than females. That is why we recommend Wistar males rather than females for research on colorectal tumors.

Key words: Colorectal neoplasms-chemically induced; dimethylhydralazines; rats

Introduction

Nowadays, colorectal cancer (CRC) represents one of the most frequent malignant neoplasms of man in the developed world. With respect to its incidence as well as mortality rates, in the United States of America it takes the third place[1] while in Slovenia it is on the second place.[2] It is a well known fact that in humans, males will contract CRC more frequently than females.[1] A few studies that have been carried out on animal models yielded similar results.[3,4,5] The models in those studies differed from each other considerably, so with respect to the type

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and strain of animals used as well as with respect to tumor induction methods. Nowadays, Wistar rats are the strain most commonly used for research purposes. As most authors give preference to male specimens while the published information on sex-related differences in the induction of CRC with DMH is very scarce, we have decided that this issue is worth of further studies.

Materials and methods

Animals

Sixty Wistar rats (provider: Medical Experimental Center, Ljubljana) 9 weeks of age were used. At the onset of the experiment the weight of males ranged between 220-280 g, and that of females between 140-180 g. The experiment was carried out at a

room temperature of 20-23°C, humidity 40-70%, and at a natural day/night cycle. The animals were fed on M-K-02 briquettes (supplier: Biotechnical Faculty, Ljubljana) and tap water.

Carcinogenic agent

CRC was induced by means of DMH (producer: Fluka Chemie, Switzerland) prepared according to the standard method [6,7]: DMH-HCl was dissolved in 0.001 M EDTA and pH value adjusted to 6.5 using 0.1 M NaOH solution. Fresh solutions were prepared once weekly.

Study design

The animals were distributed into groups of 10 per cage. Males and females were kept separately. Every three weeks the animals were weighted and the dose of DMH adjusted accordingly, so that it always amounted to 20 mg/kg of body weight. The solution was injected subcutaneously (s.c.) into the skin fold on the hip once weekly throughout a period of 15 weeks. The animals were left to live 10 weeks after completed DMH injection, and thereupon sacrificed by CO_2 inhalation.

Morphological investigation

On autopsy, all internal organs except the central nervous system were examined. Particular attention was paid to possible presence of tumors in the outer auditory canal. The stomach was opened via the major curve while the intestine was approached longitudinally on the antemesenterial side; after opening, the organs were rinsed with water. The end part of the ileum, large intestine, anus and neoplasms in the small intestine were spread over a polystyrene board, with intestinal mucosa facing upwards. The tissue was fixed in 10% buffered formaldehyde.

Three tissue samples of the large intestine were taken for histological examination from the following sites: the rectum, transversal colon and ascending colon. All the macroscopically visible lesions were sampled as well. The tissue samples were paraffin embedded and cut into 4mm thick histological sections. The sections were afterwards stained by thrichrome method according to Kreyberg. In the cases when histological picture or tumor stage could not be determined from a single section, a stepwise series of sections was made. All intestinal lesions were assessed by histological criteria used in human pathology.[8]

Carcinomas were distributed into three stages according to their phase of development:[9]
– Stage A: tumor tissue is limited to the intestinal wall;
– Stage B: tumor tissue grows through the lamina muscularis propria;
– Stage C: tumor tissue grows through the lamina muscularis propria and disseminates into the lymph nodes and distant organs.

Histological criteria for diagnosis of adenoma were 1) cytological (multiplied mitoses, polymorphism, and hyperchromatism of the nuclei, basophilia of the cytoplasm, decreased mucine excretion), and 2) histological (stratification of the nuclei, irregular proliferation of the glandular formations). The criteria for diagnosis of carcinoma was the evidence of tumor growth through the muscularis mucosae.

Statistical methods

The significance of sex-related difference in the numeric results was tested by Pearson's Chi-square test.

Results

Ninety-five percent of animals survived throughout the duration of the experiment. One male was sacrificed 7 weeks before the end of experiment because of a large tumor of the Zymbal gland while another two males died spontaneously due to intestinal tumors (1 in the large and 1 in the small intestine) 10 and 1 days respectively before the end of experiment.

During the experiment the animals were fed normally, their body weight was increasing by advancing age. In the last two weeks, the body weight of five males was found to have decreased, and the presence of carcinomas of the Zymbal gland or intestine was confirmed in all of them.

Tumors of the large intestine and rectum

Twenty-one tumors were found in the large intestine, 15 of these in males and 6 in females (Table 1). Tumors developed in 37% of males and 17% of females, i.e. in 27% of all animals. Three males (10%) and one female (3%) had multiple primary tumors.

Sex-related difference in the occurrence of all colonic tumors was borderline significant (p<0.08).

Table 1. The number of tumors induced in the large intestine

Sex	Histological type of tumor			
	Adenoca.	Signet-cell ca.	Adenoma	Total
Males	11	2	2	15
Females	0	4	2	6
T o t a l	11	6	4	21

An analysis of sex-related differences in the number of colorectal carcinomas also showed a borderline significance (p<0.08).

A majority of the induced tumors were found in the transverse colon (30%), followed by the ascending colon (17%), rectosigmoid (10%) and descending colon (7%) (Figure 1).

Most tumors in males were polypoid (Figure 2); a majority of them were situated in the transverse colon (73%), and none in the rectosigmoid. Intussusception in the transverse and descending colon respectively was observed in two males.

In females, the tumors induced in the large intestine were rare and relatively evenly distributed (Figure 1). Three females presented with tumors in the rectum. There were neither tumors in the descending colon nor intussusception observed. Macroscopically, most tumors in females were flat. On a subsequent histological examination they were recognized as signet-cell carcinomas.

In the review of histological samples 21 tumors were analysed (Table 1) as follows: there were 11 adenocarcinomas (Figure 3), 6 signet-cell carcinomas and 4 adenomas.

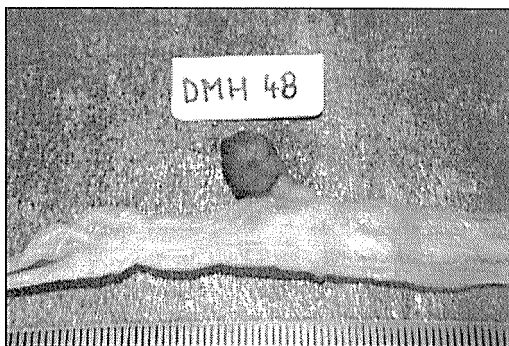All the neoplasms were macroscopically visible. Further microscopic examination also revealed the

Figure 2. Polypoid tumor of the large intestine. The tumor is attached with a short stem. A prestenotic dilatation of the intestinal wall is visible on the right.

Figure 3. A well-differentiated adenocarcinoma which infiltrates to the serosis

presence of adenomas consisting of a single or a few glandular formations.

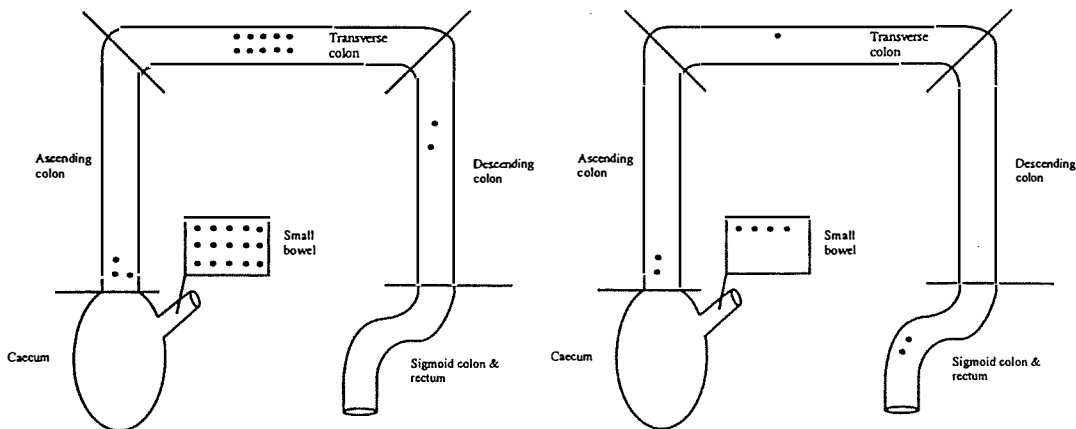The majority of polypoid tumors were found to be well or moderately differentiated adenocarcino-

Figure 1. Graphic presentation of tumors induction sites in the intestine of males (left) and females (right). The points within the intestine represent individual tumors.

mas. Poorly differentiated adenocarcinomas were rare. In contrast to that, signet-cell carcinomas were mostly flat lesions which infiltrated the wall.

The sex-related differences in the number of tumors induced in the large intestine were not statistically significant. In males 15 tumors were found in the colon and rectum as follows: there were 11 adenocarcinomas, 2 signet-cell carcinomas and 2 adenomas. In females 6 tumors were induced: 4 signet-cell carcinomas and 2 adenomas.

When determining the stage of colorectal carcinomas, the majority of tumors (71%) were found to be stage A (Figure 4). Many smaller, well-differentiated stage A tumors could be interpreted as adenomas, and therefore step-wise sections had to be made in a few cases in order to ascertain the invasion through the *muscularis mucosae*.

The comparison of stage distribution by sex did not show statistically significant differences (p<0.14). In males the majority of carcinomas (77%) were stage A while the rest were stage B, the latter being invariably situated in the transverse colon. None of the males presented with a stage C tumor. Most tumors in females were stage A, all of them were signet-cell carcinomas. One female had a stage C tumor. Histologically, this was a signet-cell carcinoma with evidence of lymph node involvement and carcinosis of the liver and peritoneum.

Other tumors

Apart from tumors of the large intestine, there were also 19 tumors of the small intestine and 5 tumors of the Zymbal gland found along with 1 hemangioma of the liver and one hepatocellular carcinoma.
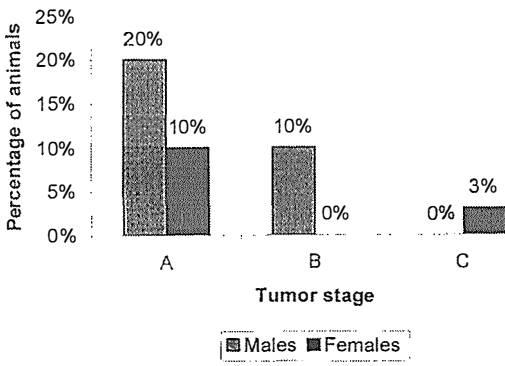

Figure 4. Comparison of grades of large intestine carcinomas.

The tumors of the small intestine were mostly large, polypoid and macroscopically similar to those found in the large intestine, although they were histologically different: a half of these tumors were signet-cell carcinomas, the majority (80%) of them being stage B. The rest were adenocarcinomas exhibiting focally increased mucine production. Tumors of the small intestine occurred in one third of males (33%); 80% of these presented with signet-cell carcinomas while the rest had moderately differentiated adenocarcinomas. Three males had multiple primary tumors, and two showed evidence of intussusception. In females, tumors of the small intestine were found in 13%. A half of these were adenocarcinomas, while the other half were signet-cell carcinomas; 75% of all tumors were in an advanced stage B.

The sex-related differences in the incidence of small-intestinal tumors were found borderline significant (p<0.07). The sex-related difference in the number of all tumors induced was statistically significant (p<0.02).

Discussion

While CRC is a relatively frequent tumor in humans, its spontaneous occurrence in rats is rare.[10] DMH is one of the most effective CRC inducers in small rodents.[11] This substance has been studied in large-scale experiments,[12] and the tumors induced have been compared with those occurring naturally in humans[10,13,14], although any sex-related differences were not clearly defined.

Some authors claim to have established a lower incidence of DMH induced tumors in Wistar rats as compared to other strains of rats.[15] These findings have also been confirmed by our results showing that tumor induction was successful in 27% of the experimental animals only. The occurrence was considerably lower in females than in males, although the difference was only borderline significant.

Histological examination revealed a few adenomas in the large intestine, which consisted of one or more glands. These lesions were not macroscopically evident. Nevertheless, this finding is interesting as it confirms probable development of carcinoma from adenoma [16,17], and points out the similarity with colorectal carcinogenesis in humans. Such adenomas were also reported by other authors[18] although they were not evaluated quantitatively. Un-

doubtedly, there would have been even more adenomas found in our study, had we decided to examine the entire intestine by systematic microscopy. This will be the subject of our further research.

Our data on the sites of primary tumor induction in males are consistent with those of other authors, who report the greatest number of tumors in the transversal colon.[19] Comparable with our results, other authors also found rare tumors in the ascending and descending colon but not in the caecum.[19] It is of interest to note that not a single tumor of the rectum could be found in males. Consistent with this observation, other authors also report rare tumor occurrence in this site (5%).[7,20] In females as well tumors of the wide intestine were induced in the same sites as reported by other authors,[12] the only exception being the ascending colon where the numbers of induced tumors reported by other authors are somewhat higher.

Likewise other authors, macroscopically we also found prevailingly polypoid tumors.[10,12] There was however, sex-related difference found with respect to the site of their occurrence. Thus males had a prevailing majority of polypoid tumors in the central part of the colon. Sessile tumors exhibiting endophytic growth were extremely rare. The latter were found prevailingly in the proximal part of the ascending colon. Intussusception, which was observed in a few animals, has also been reported by other authors.[6,10] The site of origin is just as usual in the transverse colon, which is consistent with the sites of origin of polypoid tumors.[6] It is well known that large polypoid tumors are associated with more aggressive invasion of the intestinal wall.[19] This is responsible for hardening of the wall and consequential invagination into the distal part of the intestine.

In females, most tumors were situated in the rectum and proximal part of the ascending colon. Macroscopic differences were evident as well: a majority (70%) of tumors were sessile and wall-invading. Intussusception was not noted. This could partly be attributable to the fact that those tumors were relatively small.

Histologically, our number of adenocarcinomas was smaller than those reported by other authors who used other strains of rats and the same dose of DMH.[6,7] The descriptions of histological pictures of tumors in males were consistent with our results, although the rate of signet-cell carcinomas in our series was greater (13% of all tumors). According to some authors, the occurrence of the latter tumors is attributable to a higher dose of DMH.[21] It has been found that all those tumors were flat and that they occurred in the proximal part of the ascending colon. This finding is consistent with the reports of other authors.[13,22] The majority of polypoid tumors that were found in the transverse colon were adenocarcinomas. Their histological picture depended on the tumor size. Thus, smaller tumors mostly showed a well differentiated adenocarcinomatous component with a minimal or no mucinous component, whereas larger polypoid tumors showed a greater rate of mucinous component, which has also been described by some other authors.[19]

Our results of tumor stage analysis were comparable with those obtained by other authors, according to which a majority of tumors (71%) were in stage A.[9] Our comparison of carcinoma stage by sex has not shown statistically significant differences, however it seems noteworthy that none of the females presented with a stage B tumor. The fact that most of those tumors were signet-cell carcinomas renders this information all the more interesting. A majority of authors[10,16] believe that this very type of tumors is the most aggressive. Stage was also associated with the degree of differentiation of colorectal tumors: the majority of stage B tumors were moderately or poorly differentiated carcinomas. Stages were rarely described in experimental studies with DMH, the only exception being individual cases of stage C with carcinosis and distant metastases.[6,16,23].

Few authors studied the origin of sex-related differences in the occurrence of DMH induced tumors. The majority of investigations were centred on the study of the influence of sex hormones.[24] A stimulating effect of male sex hormones on tumor induction with DMH was established.[5]

Our results have shown that males developed significantly more colorectal tumors than females. Furthermore, the tumors in males were histologically more similar to colorectal tumors in humans. Therefore, we recommend male rather than female Wistar rats to be used for experimental work with the mentioned tumor model.

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