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Comparison of Wistar vs. Fischer rat in the incidence of 1,2-dimethylhydrazine induced intestinal tumors

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Background. *Many investigators have observed differences in the susceptibility to induce intestinal tumors by 1,2-dimethylhydrazine (DMH) between various strains of rodents. The results are difficult to compare because of the different regimes used for induction. The purpose of our study was to evaluate the influence of strain on DMH-induced intestinal tumors between Wistar and Fischer rats.*

Materials and methods. *We used 29 Fischer and 30 Wistar male rats that were injected subcutaneously DMH, weekly, at a dosage of 25 mg/kg-body weight for 20 weeks. After 25 weeks from the beginning of the experiment, the animals were sacrificed and autopsied. The complete length of colorectum and all macroscopic changes were examined histologically.*

Results. *The induction of intestinal tumors was 97% in Fischer rats and 100% in Wistar rats. In Wistar rats 184 tumors were found: 133 adenomas, 50 tubular adenocarcinomas and 1 signet-cell carcinoma. 77% of carcinomas were found in colorectum and 23% in the small intestine. In Fischer rats, 126 tumors were found: 94 adenomas, 26 tubular adenocarcinomas, 5 signet-cell carcinomas and 1 mucinous carcinoma; 42% of carcinomas were found in the colorectum and 58% in the small intestine. The strain difference in the incidence of all induced tumors was statistically significant (P=0.001). The differences in the occurrence of the malignant and benign tumors was also significant (P<0.001; P=0.011). Extra intestinal tumors were not found.*

Conclusions. *Wistar rats showed greater percentage of colorectal tumors, and also the distribution of tumors in colorectum resembled more the distribution found in human pathology. That is why we recommend Wistar rat rather than Fischer rat for the research work on the colorectal tumors.*

Key words: intestinal neoplasms – chemically induced; 1,2 dimethylhydrazine; rats, inbred F344; rats Wistar

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Introduction

Colorectal carcinoma (CRC) is one of the leading causes of cancer mortality in the USA.[1] With respect to its incidence as well as mortality rate, CRC takes the second place in Slovenia.[2] This was the reason for much in-

terest in the research of this disease and for highlighting the need for animal models that would be comparable to human disease and would help in the study of etiology, pathogenesis and therapy of the human disease.

Some studies compared the incidence of experimentally induced intestinal tumors between different species of experimental rodents and different strains among the species and demonstrated that susceptibility to carcinogen and the incidence and distribution of tumors which developed is species-, strain-, and sex-dependant.[3-8] Wistar and Fischer rats are among the most commonly used strains of rats in the research of intestinal cancer.[9] The published information on the strain-related differences between them is scarce. Besides the results are difficult to compare because different carcinogenic substances, doses, application regimes and application sites are used. So, we decided that this issue is worth of further studies.

Materials and methods

Animals

We used 29 Fischer (344) and 30 Wistar (Hannover) male rats from The Medical Experimental Center, Ljubljana, Slovenia. They were 8-10 weeks old. The experiment was carried out in accordance with the permission of The Veterinary Administration Board of The Republic Slovenia.

At the onset of the experiment the weight of Wistar rats ranged between 170–340 g and that of Fischer rats between 180–290 g. The experiment was carried out at a room temperature of 20–23°C, humidity 40–70%, and at a natural light cycle. The animals were provided pelleted M-K-02 food (Biotechnical Faculty, Ljubljana) and tap water *ad libitum.*

Carcinogenic agent

CRC was induced by means of 1,2-dimethylhydrazine (DMH) (Fluka Chemie, Switzerland) prepared according to the standard method[10]: 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 dose of DMH was adjusted accordingly, so that it always amounted to 25 mg/kg of body weight. The solution was injected subcutaneously into the skin fold on the hip once weekly throughout a period of 20 weeks. The animals were left to live four weeks after completed DMH injection and thereupon sacrificed by CO_2 inhalation. The body weight was controlled every two weeks.

Morphology

During autopsy, all internal organs except the central nervous system were examined. Attention was paid also to the 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 antimesenterial side. After opening, the organs were rinsed with water. The distal part of the ileum, large intestine, anus and neoplasms in the small intestine were spread over a polystyrene board, with intestinal mucosa facing upwards, and fixed in 10% buffered formaldehyde. The total length of colorectum and all macroscopically visible lesions were sampled for the histological examination. The tissue samples were paraffin embedded and cut into 4.5 μm thick histological sections. The sections were stained by Kreyberg trichrome method. In the cases when histological picture or tumor stage could not be determined from a single section, stepwise deeper sections were made. All intestinal lesions were assessed according to histological criteria used in human pathology and the stages of carcinomas defined following Duke's staging system:

- Stage A: tumor is limited to the intestinal wall;

Table 1. Distribution and number of intestinal tumors

	TUMOR LOCATION No (%)			
	Small intestine	**Colon ascendens**	**Colon transversum**	**Colon descendens with rectosigmoid**
FISCHER	21(17)	16(13)	47(37)	42(33)
WISTAR	7(4)	14(8)	57(31)	106(57)

- Stage B: tumor grows through the lamina muscularis propria;

- Stage C: tumor grows through the lamina muscularis propria and disseminates into the lymph nodes;

- Stage D: distant metastases.[11]

Histological criteria for the diagnosis of adenoma were: (1) cytological – increased mitotic activity, 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. Smaller tumor lesions composed of 2-5 crypts (microadenomas), seen only histologically, were also statistically processed.

The criterion for diagnosis of carcinoma was the evidence of tumor growth through the muscularis mucosa. In the case the lesion was suspected of being malignant while there was no clear evidence of tumor growth through the muscularis mucosa, the following additional histological criteria for carcinoma were used: a sharp transition of normal epithelium to severely dysplastic epithelium, the presence of significant necrosis on the surface of tumor and desmoplastic stromal reaction.

Statistical methods

The significance of strain-related difference in the numeric results was tested for the difference between proportions by computer software StatGrafics®Plus.

Results

Number and distribution of intestinal tumors

All animals survived throughout the duration of the experiment. In the intestine of Fischer and Wistar rats 126 and 184 tumors were found, respectively. The tumors were induced in 97% of Fischer rats and 100% of Wistar rats, while the tumors of the colorectum were induced in 48% of Fischer and in 83% of Wistar rats (Table 1). The strain difference in the incidence of all induced tumors was statistically significant (P=0.001). Extraintestinal neoplasms were not found.

The microadenomas that were evaluated by the systematic histological examination of the whole length of the colorectum represented 70% of tumors in Fischer and 60% of tumors in Wistar rats.

In Fischer rats, 25% of the induced tumors were carcinomas that were mostly found in the small intestine (58%), followed by the as-

Table 2. Histological types and stages of intestinal tumors according to Duke's system

	HISTOLOGIC TYPES OF TUMORS												
	Adenomas	**Tubular adenocarcinomas**				**Signet-ring cell carcinomas**				**Mucinous carcinomas**			
		A	B	C	D	A	B	C	D	A	B	C	D
FISCHER	94	19	7	0	0	3	2	0	0	0	1	0	0
WISTAR	133	41	8	0	1	1	0	0	0	0	0	0	0

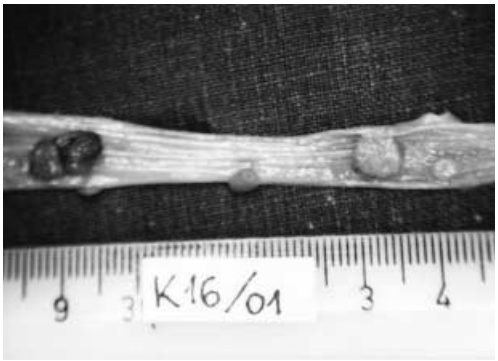

Figure 1. Segment of the large bowel with five tumors giving the appearance of chain-like arrangement.

cending colon (23%), descending colon with rectosigmoid (16%) and the transverse colon (3%).

In Wistar rats, 30% of tumors were carcinomas and were located in the transverse colon (39%), descending colon with rectosigmoid (37%), small intestine (14%) and ascending colon (10%). The most of the small intestinal tumors were found in duodenum.

Macroscopic appearances and histological examination

Macroscopically, tumors grew as plaques or as polypoid lesions on stalk or formed "napkin ring" masses. In 73% of Wistar rats, multiple colorectal tumors were found. The majority of those tumors was strung closely together and gave the appearance of "chain of tumors" (Figure 1). The latter consisted of 3-8 tumors and were mostly located in the transverse and the descending colon with rectosigmoid. Five Wistar rats presented such chains, while none was found in Fischer rats.

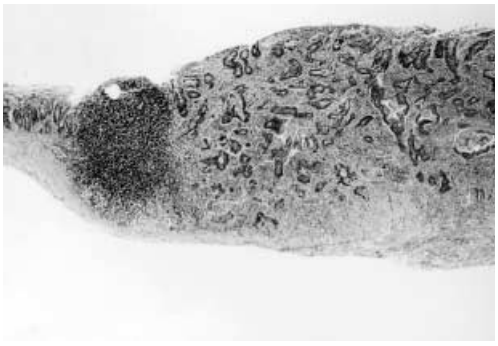

Figure 3. Adenocarcinoma stage Dukes B. On the left site there is a large bowel wall with a lymphatic follicle next to tumor tissue that invades the whole bowel wall (40X magnification).

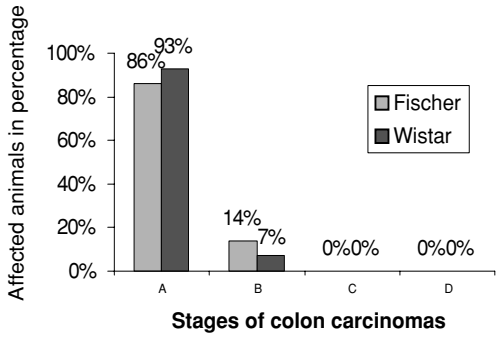

Figure 2. Comparison of colonic tumor stages in Wistar and Fisher rats.

In the review of histological samples in Wistar rats 133 adenomas, 50 tubular adenocarcinomas and 1 signet-ring cell carcinoma were found. In Fischer rats, the histological examination revealed 94 adenomas, 26 tubular adenocarcinomas, 5 signet ring-cell carcinomas and 1 mucinous carcinoma (Table 2).

Most tubular adenocarcinomas were well-differentiated lesions. They grew mostly as polypoid or papillary growths into the lumen. On the contrary, signet-ring cell carcinomas were mostly small, plaque-like lesions with prominent invasion into the deeper levels of the bowel wall. In both strains, signet-ring cell carcinomas were found in the ascending colon with only one tumor being located in the small intestine.

The difference in the occurrence of the malignant and benign tumors between the strains was statistically significant ($P<0.001$; $P=0.011$).

We also found a relation between intestinal lymphoid tissue and tumor location. More than a half of the carcinomas were found in the vicinity of the lymphoid follicles. In one Wistar rat, intussusception connected with tumor in the transverse colon was observed.

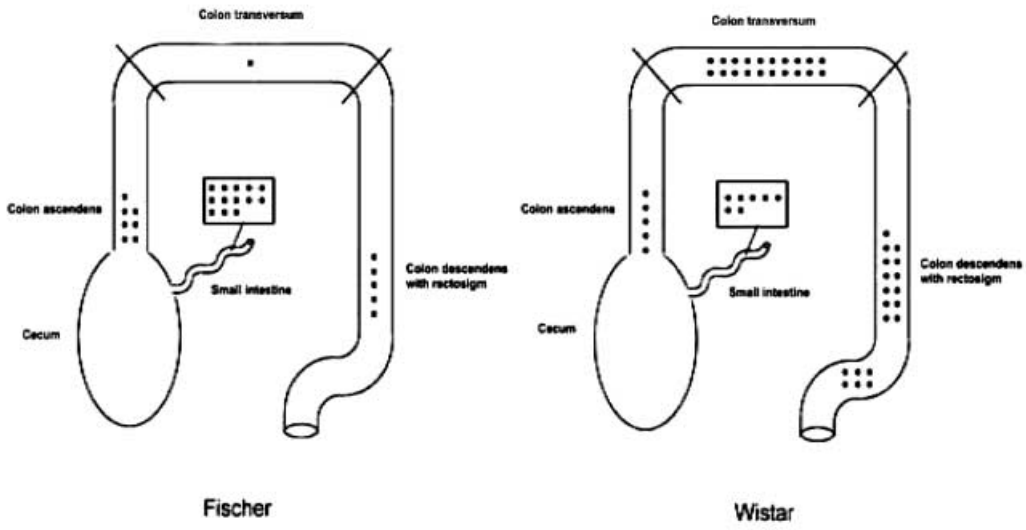

Figure 4. Distribution of intestinal carcinomas in Fischer vs. Wistar rats (each spot represents one tumor).

Staging of the intestinal carcinomas

In Wistar rats, the majority of tumors (82%) were found in stage A, 16% were in stage B (Figure 3), and 2% in stage D according to Duke's system (Table 2). Only 3% of tubular adenocarcinomas stage A were found in the small intestine, while 46% were found in the transverse colon, 41% in the descending colon with rectosigmoid and 10% in the ascending colon. The adenocarcinomas stage B were found mostly (63%) in the small intestine, 25% in the descending colon with rectosigmoid and 12% in the transverse colon. Only one signet-ring cell carcinoma was found in the ascending colon. In one rat, we found a carcinosis of the liver and peritoneum (Dukes D). The comparison of stages of only colorectal carcinomas between different strains of rats is shown in Figure 2.

In Fischer rats, 68% of carcinomas were stage A, 32% stage B, while other stages were not found; 56% of adenocarcinomas stage A and 75% stage B carcinomas were found in the small intestine, while others were located in the descending colon with rectosigmoid (22%), transverse (10%) and the ascending colon (10%). The adenocarcinomas in stage B were similarly distributed: small intestine (75%), descending colon with rectosigmoid (12.5%), and ascending colon (12.5%). The signet-ring cell carcinomas presented 12.5% of all carcinomas in Fischer rats and all were found in the ascending colon. The distribution of induced intestinal carcinomas in Fischer and Wistar rats is schematically presented in Figure 4.

Discussion

DMH injected subcutaneously is one of the most effective CRC inducers in small rodents. This substance has been studied in large-scale experiments[12-19], but the incidence of experimentally induced tumors in different strains of animals was not clearly defined. No data can be found in literature comparing DMH-induced tumors in Wistar and Fischer strains, although those are most often used for experimental purposes.[9]

The intestinal tumors were induced in 97% of Fischer and 100% of Wistar rats. Though there was no significant difference in the share of animals affected with tumors between the two strains , the incidence of intestinal tumors was significantly higher in Wistar rats.

Nevertheless, we have to emphasize that the microadenomas containing 2-5 aberrant crypts were also included in analysis. Microadenomas presented almost 70% of all tumors found and their frequency supports the likelihood that CRC develop from adenomas.[5,6,21-23] Because of the inclusion of microadenomas, the total number of induced tumors in our study somewhat exceeded the number of tumors induced by the same dose and number of applications by other authors.[6,7,23-25]

Fischer rats developed markedly less carcinomas than Wistar rats; 58% of them were found in the small intestine, others were equally distributed in the ascending and descending colon. There were, however, less tumors found in the transverse colon than reported by other authors.[9,23-25] The tumors of the small intestine, which were mainly well differentiated adenocarcinomas, developed most often in the proximal part of the small intestine. Macroscopically, both strains developed polypoid, cauliform lesions and also ring-like lesions with elevated edges that were comparable with human disease. Sessile tumors exhibiting endophytic growth pattern were rare.

In our study, the histological types of intestinal tumors in rats are consistent with those of other authors who report the greatest number of well differentiated adenocarcinomas and some signet-cell carcinomas, while poorly differentiated adenocarcinomas were rarely found.[20-23,26]

Our results of tumor stage analysis were comparable with those obtained by other authors, according to which a majority of colorectal tumors (75%) were in stage A.[20-23,26] Our comparison of carcinoma stage by strain has shown noteworthy differences. Fischer rats developed twice as much stage B tumors than Wistar rats. The tumors found in Fischer rats were showing more invasiveness and were usually growing deeper in the bowel wall. Likewise some other authors, we also found a case of stage D tumor, with peritoneal carcinosis and distant metastases.[5, 20-22]

The analysis of the small intestinal tumors revealed differences between the two strains in regard to stages: well or moderately differentiated adenocarcinomas stage B predominated in Wistar strain and well differentiated adenocarcinomas stage A in Fischer rats. Most of the macroscopically visible small intestinal tumors were located in the proximal part (duodenum, proximal jejunum), which is consistent with the carcinoma of the small intestine in humans.

An association of DMH-induced rat colorectal tumors with colorectal lymphoid follicles was observed previously, but not quantified. Our experiment revealed that more than 50% of carcinomas developed in the immediate proximity of the intestinal lymphoid tissue. This is supposed to be an immunologic answer to antigenic components present in the tumor and simultaneously because of the more rapid replication of the epithelial cells in the vicinity of lymphatic tissue.[27-29]

Tumors found in both Wistar and Fischer strain histologically resembled those found in human pathology. Wistar rats have developed a greater incidence of colorectal tumors and distribution of tumors resembled more the distribution as it is seen in human pathology than those in Fischer rats. Therefore we recommend Wistar rats rather than Fischer rats for the research work on the colorectal tumors.

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