UNIVERSIDAD AUTÓNOMA DE QUERÉTARO
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Evaluación radiológica de tumores colónicos químicamente inducidos en ratas Sprague-Dawley

TESIS

QUE COMO PARTE DE LOS REQUISITOS PARA OBTENER EL GRADO DE

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PRESENTA

RICARDO CERVANTES JIMÉNEZ

DIRIGIDA POR

M. en C. Roberto Augusto Ferriz Martínez

SINODALES

M. en C. ROBERTO AUGUSTO FERRIZ MARTÍNEZ _____
DIRECTOR

Dra. MARGARITA TERESA DE JESÚS GARCÍA GASCA _____
SINODAL

Dr. FAUSTO ARELLANO CABRAL _____
SINODAL

Dra. GABRIELA AGUILAR TIPACAMU _____
SINODAL

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EL PRESENTE TRABAJO SE REALIZÓ EN EL LABORATORIO DE BIOLOGÍA CELULAR Y MOLECULAR DE LA FACULTAD DE CIENCIAS NATURALES DE LA UNIVERSIDAD AUTÓNOMA DE QUERÉTARO A CARGO DEL M C ROBERTO FERRIZ MARTÍNEZ Y LA DRA. TERESA GARCÍA GASCA.

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Resumen

En la actualidad existen diferentes tipos de cáncer que afectan al ser humano y que son provocados por diferentes factores, tanto exógenos como endógenos. Actualmente el cáncer de colon se encuentra catalogado con un elevado grado de mortalidad, principalmente en países desarrollados, por lo que la investigación en modelos animales se ha vuelto indispensable en la búsqueda de nuevos tratamientos. El presente trabajo tuvo como objetivo ajustar la concentración de 1,2-dimetilhidracina (DMH) para el desarrollo de tumores de colon y determinar la presencia de tumores previos al sacrificio mediante la observación *in vivo*. Para la determinación radiológica se utilizó un equipo de rayos X (Rx) modificado y sulfato de bario como medio de contraste administrado vía rectal por medio de una cánula. La técnica permitió la observación de tumores de colon antes del sacrificio en animales tratados con 20 y 40 mg/kg peso de DMH. En el grupo control no se observaron tumores, ni en las radiografías ni directamente en el tejido del colon. En el caso del grupo administrado con las dosis de 20 y 40 mg/kg se observó una incidencia del 37.5 y 100% respectivamente de tumores que fueron detectados por radiografía de doble contraste y confirmados durante la disección del colon. La aplicación del tratamiento de 40 mg/Kg presentó una mayor incidencia en el número de tumores y la aplicación del método radiológico ayudó a determinar la ubicación de las lesiones en el tracto colónico de los animales. Esta metodología permitirá llevar a cabo estudios sobre cáncer de colon mediante la determinación de la presencia de tumores en tiempo real lo que permitirá, a su vez, estudiar la eficiencia del cancerígeno, del agente terapéutico y su tiempo de acción. Finalmente, se preparó la publicación de los resultados en la revista American Association for Laboratory Animal Science (JAALAS), la cual tiene un factor de impacto de 0.71 y un tiraje de 6 números al año.

Palabras clave: Cáncer, Colon, Rayos X, 1,2 - dimetilhidrazina

Radiologic evaluation of chemically induced colon tumors in Sprague-Dawley Rats

Cervantes-Jiménez R[1], Jiménez-Mendoza D [2], García-Gasca T[1], Rodríguez-Méndez AJ[3], Ferriz-Martínez RA[1*]

Title: **Radiologic evaluation of chemically induced colon tumors in Sprague-Dawley Rats**

Authors: Cervantes-Jiménez R[1*], Jiménez-Mendoza D [2], García-Gasca T[1], Rodríguez-Méndez AJ[3], Ferriz-Martínez RA[1*]

Institutional affiliations: [1] Facultad de Ciencias Naturales. Universidad Autónoma de Querétaro. P. O. Box 184, Querétaro 76010, Qro., México, C.P. 76230.

[2] División de Investigación y Posgrado, Facultad de Ingeniería, Universidad Autónoma de Querétaro, P. O. Box 184, C.P. 76010, C.P. 76230.

[3] Facultad de Medicina. Universidad Autónoma de Querétaro. P. O. Box 184, Querétaro 76010, Qro., México, C.P. 76230.

[*]Corresponding author. Email: raffer712701@gmail.com

Running head: 1,2-DMH and X-ray observation of the tumors in rats SD

Abbreviations: CRC, colorectal cancer; DMH, 1,2-dimethylhydrazine; Rx, X-ray technique.

Abstract

Nowadays there are different types of cancer that affect humans and are caused by different factors, both exogenous and endogenous. Currently, colon cancer is catalogued with a high degree of mortality, mainly in developed countries therefore, research in animal models has become essential in the search for new treatments. The present study aimed to adjust the concentration of 1,2-dimethylhydrazine (DMH) for colon tumors development. Besides, the presence of tumors was determined by using modified X-ray equipment (Rx) with barium sulfate as contrast medium, which was administered rectally by a cannula. This technique allowed the observation of colonic tumors of DMH (20 or 40 mg/kg of body weight) treated animals before sacrifice. The control group did not show tumors neither by Rx nor in the dissected colon tissue while treated animals showed tumors by Rx that were confirmed when colon was dissected. An incidence of 37.5 and 100% of tumors was observed for 20 and 40 mg DMH/kg of body weight, respectively. This methodology will allow to perform studies on colon cancer by determining the presence of tumors in real time that will enable, in turn, to study the efficiency of carcinogens and therapeutic agents.

Keywords: Cancer, Colon, X-ray, 1,2 - dimethylhydrazine.

Introduction

Cancer is a chronic disease characterized by abnormal cell proliferation with changes in genotypic and phenotypic properties. [1] Among the different types of cancer, colorectal cancer (CRC) is caused by endogenous and exogenous factors [2, 3, 4, 5] with a high incidence of mortality in developed countries, with increased incidence in developing countries, mainly in recent decades. For this reason, there is an increased interest in taking advance of animal models in order to test new therapies. [6] Some treatments have focused on induce tumors using different carcinogenic compounds [7, 8] in order to induce tumors in localized areas however, in most cases, detection of the developed tumors can be possible until animals are sacrificed.

Particularly, 1,2 dimethylhydracine (DMH) has been widely used as colon carcinogen and the formed adenocarcinomas can be observed through the entire rat colon after several weeks of treatment. [9, 10] DMH is a precancerous compound that is metabolized to its active form, azoxymetanol, that induces tumorogenesis in laboratory animals as in the case of some species of susceptible rats. [11, 12] It produces adenomatous lesions based on cell proliferation and epithelial dysplasia, which can range from mild to severe where the adenomas are precursor lesions of colorectal adenocarcinomas. [13] Although DMH is an effective carcinogenic compound, treated rats appear asymptomatic the most of the time until the disease is unmasked, commonly after several months, and the expected results are not always achieved.

In order to observe and analyze the lesions or tumors, different radiological techniques have been developed. They help to identify different types of conditions, including the analysis of tumors in various tissues [14], using contrast media that improve these observations at a low cost. [15] It is necessary to standardize techniques for the observation of tumors using contrast

media such as barium sulfate in experimental animals. [16] The observation of colon tumors before animals sacrifice will allow determining the tumor size, localization but also the effectiveness of a specific treatment. By using this technique, it will be possible to improve time and resources in the study of colon cancer in rats.

Materials and Methods

Animal model and treatments

Male Sprague Dawley rats of 5 weeks old were used. Three groups of 8 animals were separated into individual cages with food (Nutricumus, Rodent Laboratory Chow 5001) and water *ad libitum*. Circadian cycle was adjusted to 12 h of light and 12 h of darkness. After one week of adaptation, 3 treatment groups were formed. The control group was injected subcutaneously with the vehicle (0.9% NaCl/2 mM EDTA) once a week for eight weeks. Groups 2 and 3 were treated with a subcutaneous injection of 20 or 40 mg DMH/ kg of body weight once a week for 8 weeks. The animals were kept under continual observation of weight and food consumption for 10 weeks more after DMH treatments.

X-rays

Nine weeks after the beginning of treatment, the rats were fasted for 24 hours in order to avoid the presence of feces in the colon behavior during exposure to X-rays. Subsequently, the animals were anesthetized with 40% chloroform. Was introduced 3 ml solution of 60% barium sulfate by cannula latex balloon 2-way type, from 25,123 12fr/5ml connected to a 5 ml syringe. Radiographs were taken dividing into five segments the caudal area (Figure 1) in order to cover different areas of the intestine. A Satelec X-Mind DC generator (Acteon Equipment, France) with a DG-073B-DC double anode tungsten tube (Toshiba Electron

Tubes and Devices Co., LTD, Japan) under 70 kV and intensity of 8 mA and a S10835CMOS image sensor for X-ray imaging (Hamamatsu City, Japan) were used (Figure 2). The active area of the X-rays was 2.58 x 3.6 cm, with an optical resolution of 16.7 line pairs and pixel size of 30 microns. For all radiographs were taken rat-sensor distance of 4 cm and an exposure time of 0.200 seconds. [17]

Histopathological analysis

At the end of the experiment the animals were sacrificed by decapitation, the colon was dissected and tumors were fixed in paraformaldehyde-10% PBS, dehydrated in ethanol solutions, xylene / ethanol 1:1, concentrated xylol and paraffin. [18] Tissues were included in paraffin blocks and serial sections of 5-7 μm were cut and mounted on high attachment slides (SuperFrost, Fisher, Pittsburgh, PA) in a hot water bath and 0.03% gelatin. The slides were deparaffinized with 100% xylene, and rehydrated in absolute ethanol, 2 x 5 min, 96% ethanol 2 x 5 min, 70% ethanol x 5 min, 50% ethanol x 5 min and distilled water x 5 min and finally equilibrated in water (Merck, Darmstadt, Germany) in 0.5% ethanol, with potassium sulfate to 10% aluminum and 0.25% red mercury II oxide (Sigma Aldrich, St. Louis, MO), washed in water for 1 minute and then dipped quickly five times in ammonia water (1% NH_4OH) and washed again with water for 1 min. The preparations were incubated for 15 seconds with 0.25% eosin (Sigma Aldrich, St. Louis, MO) in 60% acidified alcohol, washed with water and dehydrated in an ethanol gradient (50-100%, 1 min each time). Samples were immersed in xylene 3 times before they were permanently mounted in Entellan (Merck, Pennsylvania, USA). Classification of lesions was done according to Angeles (2002). [13]

Statistical Analysis

Body weight and food consumption changes were analyzed by t test ($p \leq 0.05$) using the SPSS 16.0 software.

Results

No significant differences between body weight and food consumption were observed though the experiment (Figure 3). Results of the Rx showed that no tumors were found in the control group (Figure 4), whereas the groups treated with 20 or 40 mg DMH/kg of body weight showed clearly visible tumors that were highlighted by the barium sulfate. Tumors observed by Rx were confirmed during the colon dissection, a total of 2 tumors were found in the group treated with 20 mg DMH/kg of body weight and 8 tumors for the group treated with 40 mg DMH/kg of body weight.

Histopathological Analysis

The control group showed cryptic foci with healthy tissue characteristics, while for the groups treated with 20 and 40 mg/kg of DMH different types of injuries were detected. The 20 mg/kg group showed two low-grade lesions, (Table 1). For the group treated with 40 mg/kg a total of 8 lesions were found, 6 low-grade and 2 high-grade lesions (Table 2).

Discussion

No differences we observed between groups for food intake and body weight changes. These results are similar to that reported by Reynoso et al. [19] Tumors formation occurred in all animals treated with 40 mg DMH/kg of body weight whereas in the group treated with 20 mg DMH/kg of body weight, only two rats presented tumors.

Detection of colon tumors with Rx allow us to found a large number of tumors in the cross sections of the ascending colon and in animals treated with 40 mg of DMH which determines a higher incidence of these areas. Two of the 9 lesions found in animals were of adenoma type; these kinds of lesions are the most common in the human sigmoid and rectum. [20] In the case of the treatment with 20 mg of DMH, lesions were located in the ascending colon with high degree of premalignant and neoplastic character.

Barium sulfate cause no adverse effects in colon therefore, the implementation of this contrast medium make this technique inexpensive and feasible for *in vivo* tumors observation. [21] Barium sulfate was also used orally to determine the activity of drugs in the digestive tract with good results, [22] so this type of imaging technique can help in monitoring and pinpoint the precise location of irregularities in the colon, as is the case of polyps and tumors formation.

Previous studies have shown the importance of knowing the shapes, size and stage of tumors in order to establish control methods. It is necessary to implement economic techniques that can help get a timely and appropriate diagnosis to improve the work with laboratory animals. Some limitations of using this technique are the animals' manipulation and the anesthesia so it is important to train the personnel that will handle the animals in order to avoid damage. Some level of practice is also needed as well of the implementation of the Rx equipment.

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Figures and tables

Figure legends

Figure 1. Segmentation of the rat colonic tract. The colon was divided into five parts: left upper segment (LUS), right upper segment (RUS), left lower segment (LLS), lower right segment (LRS), middle segment (MS).

Figure 2. X-ray equipment. 1) Image sensor for X-ray, 2) adjustable lever and 3) tungsten tube**.**

Figure 3. Body weight (a) and food intake (b) of DMH treated and not treated rats. Rats were intraperitoneally administrated with DMH (20 or 40 mg/kg) every week for 8 weeks and kept in observation under 10 weeks more.

Figure 4. Rx determination of colon tumors and their confirmation by colon dissection. a) Control; b) Control dissection, healthy tissue; c) Control X-ray zone C; d) Control dissection, healthy tissue; e) LUS tumor localization, f) tumor of 0.7 mm in diameter located in the LUS zone, g) radiograph of the right upper caudal section with two tumors; h) dissection of the RUS section, two 3 mm tumors are observed.

Figure 5. Hispathological analysis for colon lesions and tumors. Confirmation of tumors in different tissues obtained from treatments of 20 and 40 mg of DMH by histopathological changes (10X). a) Colon form healthy control animal b) Tissue with low-grade lesion c) Tissue with high-grade lesion d) Neoplasm.

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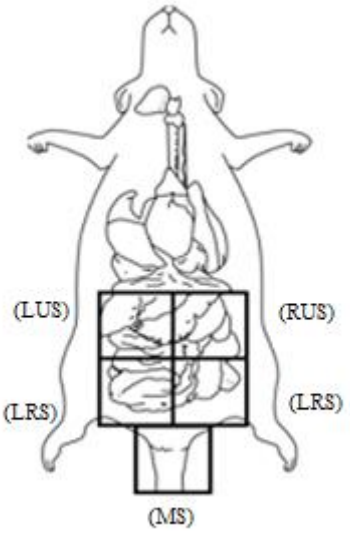

Figure 1.

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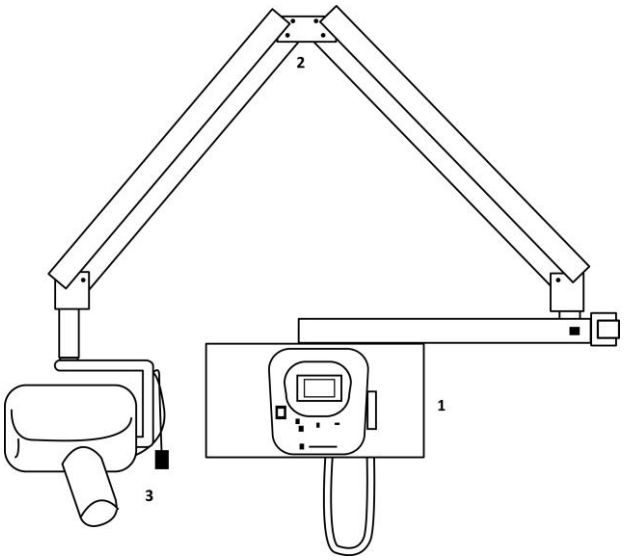

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244 **Figure 2.**

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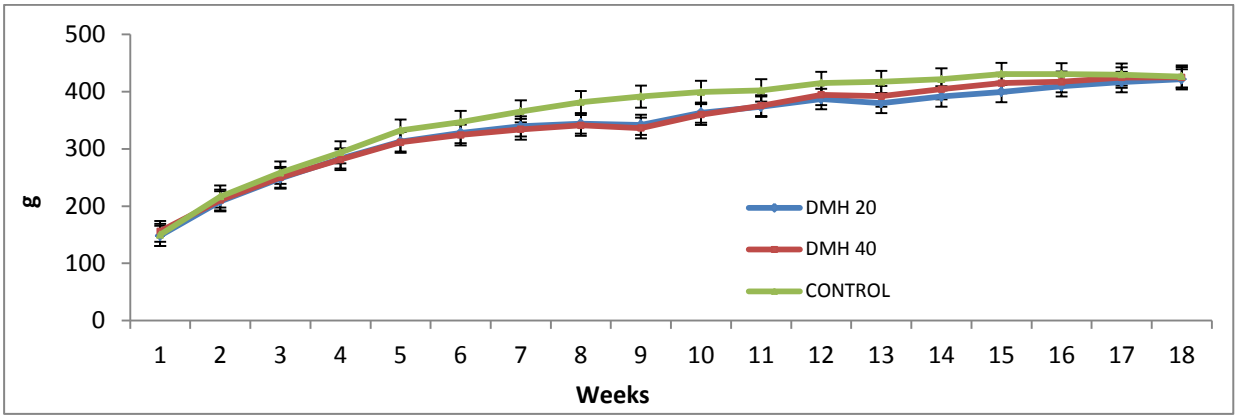

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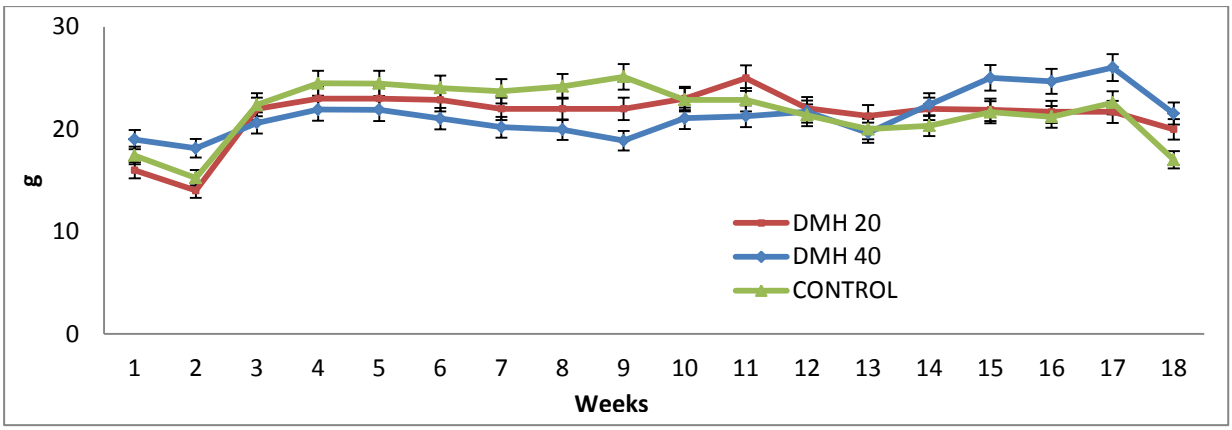

248 **Figure 3.**

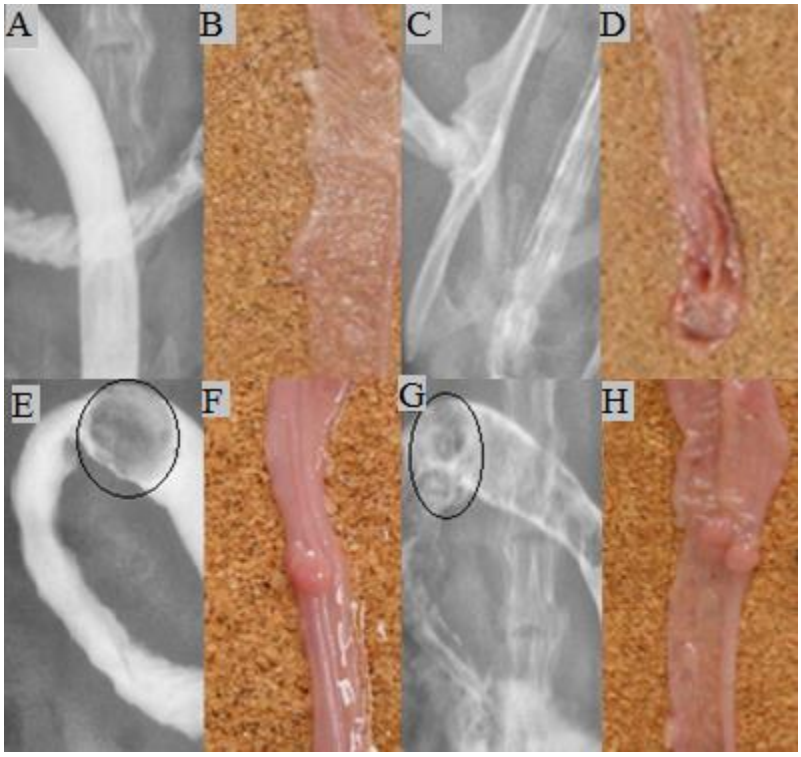

Figure 4.

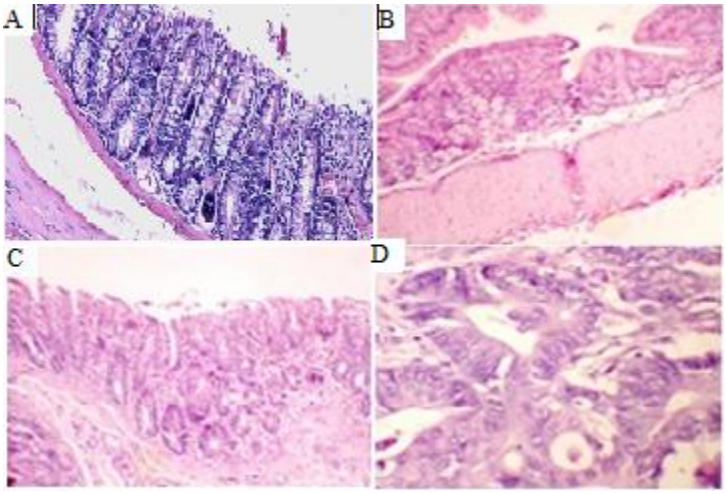

Figure 5.

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254 **Table 1.** Relationship of tumors and its location in the various areas flow, number of tumors

255 localized per rat and lesions size with the treatment of 20 mg/kg weight**.**

Rat	Tumor number	Location	Diameter (cm)
1	0	-	-
2	1	up	0.6
3	0	-	-
4	1	up	0.5
5	0	-	-
7	0	-	-

256

Table 2. Relationship of tumors and its location in the various areas flow, number of tumors localized per rat and lesions size with the treatment of 40 mg/kg weight.

Rat	Tumor number	Location	Diameter (cm)
1	1	cross	0.7
2	1	cross	0.4
3	2	up/cross	0.3/0.3
4	2	up/cross	0.3/0.5
5	1	cross	0.1
6	1	cross	0.3