Hepatic repercussions of azoxymethane-induced colorectal carcinogenesis

Repercussão hepática da carcinogênese colorretal induzida pelo azoximetano

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ABSTRACT

Objective: To evaluate the hepatic effects of colonic carcinogenesis induced by azoxymethane at different doses and times of exposure in rats. **Methods**: Forty-four Wistar rats were divided into four groups. The animals were eight weeks at the beginning of the experiment. group 1 received 1.0ml of saline intraperitoneally once a week for two weeks. Group 2 received 15 mg/kg of azoxymethane intraperitoneally once a week for two weeks. These animals were killed at the 15th week of the experiment. The animals of group 3 received saline intraperitoneally once a week for two weeks. Group 4 animals received 20mg/kg of azoxymethane intraperitoneally once a week for two weeks. These animals were killed at the 26th week of the experiment. The fragments of liver tissue were stained with hematoxylin and eosin and evaluated microscopically. **Results**: Groups 1 and 2 differed significantly in relation to steatosis, no difference having been found between group 3 and group 4. However, in group 4 we observed pre-neoplastic lesions (foci of altered, clear, vacuolated, basophilic, amphophilic tigroid, oncocytic, small or acidophilus cells, spongiosis and peliosis) and neoplastic lesions (adenomas and colangiomas) containing atypical hepatocytes in between, not identified in group 3. **Conclusion**: In the model of colorectal carcinogenesis, preneoplastic and neoplastic hepatic lesions appear and evolve in proportion to the time of exposure and dose of azoxymethane.

Key words: Colonic neoplasms. Colorrectal neoplasms. Tumor makers, biological. Azoxymethane. Fatty liver.

INTRODUCTION

Colorectal cancer (CRC) is among the most frequent types of malignancies. It is the third most common cancer in the world in both genders, the fourth most common type in men and third in women [1]. Is the fourth most commonly diagnosed cancer in the United States, with 178,000 new cases per year, and a mortality of about 47 per 100,000 inhabitants [2].

The cause of CRC is the result of a complex interaction of external variables, such as environmental and dietary agents, and internal factors, of somatic or hereditary nature [3]. When detected at early stages, it has a greater chance of cure and survival [4].

Considering the importance of neoplastic disease and the need to understand the pathophysiology of the emergence of early lesions, several colorectal carcinogenesis models are udes [5,6]. The model Bird promotes carcinogenesis by 1,2 dimethylhydrazine (DMH) or azoxymethane (AOM) and assesses the formation of aberrant crypts in colonic mucosa of rodents, being widely used in experimental research. The lesions induced by AOM – K-ras, APC, and p53 mutations – are similar to CRC in humans and can also be found in other organs such as liver, small intestine and peritoneum [7-9].

There are few studies on liver injury caused by AOM [10,11] during colorectal carcinogenesis induced according to the Bird model [12-16].

The aim of this study is to evaluate the relationship between dose and exposure time of azoxymethane on the liver repercussions during colonic carcinogenesis in rats.

METHODS

The survey was conducted after approval by the Ethics Committee on Animal Research of the Federal

Work conducted at the Laboratory of Experimental Surgery (LABCEX), Post-Graduation Program in Surgery, Faculty of Medicine, Federal University of Ceará – UFCE, Fortaleza, Ceará State – CE, Brazil.

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University of Ceará (CEPA / UFC), protocol number 09 on February, 3rd 2009, and according to the International Standards for Biomedical Research on Animals.

We used 44 rats (*Rattus norvegicus albinus*, mammalia Rodentia, Muridae) of Wistar strain, obtained from the Central Animal Facility of the Federal University of Ceará, eight-weeks old and weighing 180g. The animals were kept in individual cages with polypropylene lid with zinc galvanized wire, lined with wood shavings, in the laboratory of Experimental Surgery, Department of Surgery, Faculty of Medicine, Federal University of Ceará. They remained housed in appropriate conditions, relative humidity around 50%, and average temperature of 25° C, light-dark cycle of 12/12 hours and ventilation. They received water and food *ad libitum*.

The animals were identified, weighed and randomly divided into four groups. After weaning, they were fed standard Biobase Biotec Rats and Mice chow composed of 59% carbohydrate (CHO), 29% protein (NSP) and 12% lipids (LIP).

Groups were composed as follows: Group 1 - (G1) control group with mice not exposed to AOM (n = 9) – animals received an injection of 1.0 ml of sterile 0.9% saline, intraperitoneally, once a week for two weeks and were killed at 15 weeks; Group 2 - (G2) group study with rats exposed to AOM 15 mg / kg (n = 9) – animals injected with AOM 15 mg / kg, intraperitoneally once a week for two weeks and were killed at 15 weeks; Group 3 - (G3) control group with mice not exposed to AOM (n = 14): animals received an injection of 1.0 ml of 0.9% sterile saline solution, intraperitoneally once a week for two weeks and were killed at 26 weeks; Group 4 - (G4) group study with rats exposed to AOM 20 mg / kg (n = 12): animals injected with AOM 20 mg / kg, intraperitoneally once a week for two weeks and were killed at 26 weeks.

At the 15th week (groups 1 and 2) and at week 26 (groups 3 and 4) after the first injection, the animals were anesthetized with 80 mg/kg ketamine and 8 mg/kg xylazine intraperitoneally and set in supine position for surgery. They then underwent laparotomy through a xypho-pubic midline incision exposing the peritoneal cavity to total proctocolectomy and hepatectomy. These organs were weighed, measured and evaluated for the presence of macroscopic lesions. Samples of liver lobes were cleaved and sent for routine histopathology. The colon was opened at the anti-mesocolic edge, washed with saline and extended in kraft paper, folded on its axis concentrically and immersed in 10% formalin solution for fixation and subsequent studies. Then the animals were killed by hypovolemic shock after section of the abdominal aorta.

All organs were cleaved and two fragments of liver (right and left lateral lobe) were removed, as well as the lesions macroscopically observed. The tissues were fixed in 10% buffered formalin for 24 hours and then taken to histotechnical processing. After embedment in paraffin, 5□m thick cuts were made and stained with hematoxylin and eosin (H/E).

The study variables are nominal. We used the chi-square test to compare non-paired samples in contingency tables 2 x 2, comparing groups 1 and 2 and groups 3 and 4 for the presence of hepatic steatosis. The significance level was 5%, which was statistically significant when Á <0,05.

RESULTS

Histopathological examination of the liver showed mild steatosis in all groups. It was less frequent in group 1, in which animals were younger and did not receive AOM.

Groups 1 and 2 differed significantly (Ç □ = 7.54, Á = 0.011) in relation to hepatic steatosis at euthanasia (15 weeks after injection of saline and 15 mg/kg AOM, respectively) (Tables 1 and 2). Groups 3 and 4 did not differ (Ç □ = 2.08, Á = 0.216) in relation to hepatic steatosis at euthanasia (26 weeks after injection of saline and 20 mg/kg AOM, respectively) (Tables 1 and 3).

Premalignant and malignant lesions, however, have only been observed in the rats in from group 4, which received higher dose AOM (20 mg/kg). Of the premalignant lesions, the most common were amphophilic cells, seen in six animals in Group 4 (Figures 1 and 2). The most frequent neoplastic lesions were colangiomas and adenomas. We observed one case of carcinoma in situ and one hepatocellular carcinoma. Spongiosis and peliosis were also common in this group.

DISCUSSION

The focus of aberrant crypts, pre-neoplastic lesions of the colon mucosa, was initially described by Bird [7]. It has the characteristic of being induced by specific carcinogen dose-dependent manner and is seen in the colonic mucosa early in two to four weeks after initiation of dosing. The size and multiplicity of crypts increase with time, and characteristics, such as proliferation and tumor dysplasia, are predictors of outcome [6-9].

The AOM is a metabolite of DMH, whose mechanism of induction of preneoplastic lesions is attributed to increased expression of c-fos gene and reduced c-myc gene, as well as the mutated K-ras gene alterations similar to those observed in spontaneous carcinogenesis in humans [17,18]. AOM is usually preferred over DMH due to being more powerful and requiring few reactions to its activation. It is activated in the liver by N-oxidation, generating reactive compounds essential for chemical carcinogenesis (metilazoximetanol ion and methyl diazoxide) being brought to the colon into the bloodstream or via bile as a glucuronide conjugate. After

Table 1- Liver injury by azoxymethane.

Injuries	Groups	G1	G2	G3	G4
Steatosis	Light	4	8	7	9
	Moderate		2	1	1
	Severe				
Premalignant lesions	Clear				2
	Vacuolated				2
	Basophilic			1	1
	Intermediate				
	Anphophilic		1		6
	Tigroid				1
	Oncocytic				2
Spongiosis				7	
Peliosis				5	
Colangioma				6	
Adenoma				3	
Carcinoma in situ				1	
Hepatocellular Carcinoma					1

activation, the methylated DNA is mainly at positions N7-guanine and O6-guanine [19].

In the present study, preneoplastic lesions in the liver appeared and evolved proportionally to the dose and duration of exposure to the carcinogen.

AOM causes hepatocyte proliferation. This condition is still considered preneoplastic, as hepatocytes do not seem to have any degree of autonomous growth, and called foci and nodules of altered hepatocytes. Three hepatocytic lineages can be identified during the development of tests for liver tumors in rodents: glycogenolyitic-basophilic, anphophilic-basophilic and xenomorphic-basophilic [20,21].

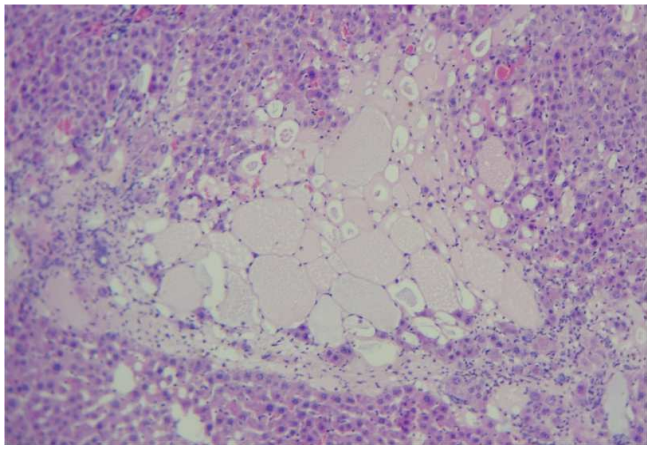

Figure 1 – *Spongiosis – HE 10 x.*

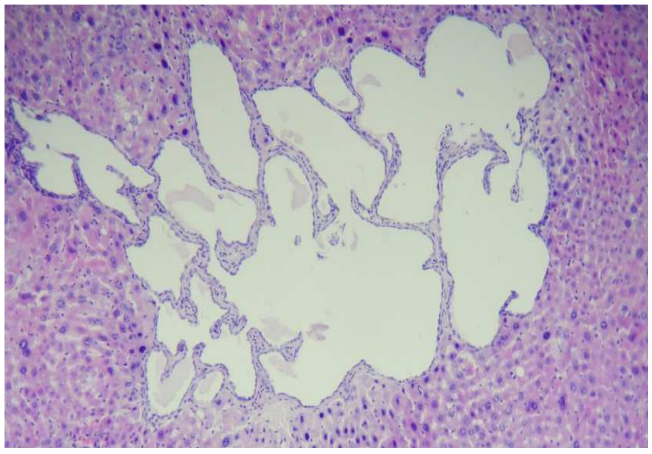

Figure 2 – *Cystic Colangioma – HE 10 x.*

Preneoplastic (foci of altered, clear, vacuolated, basophilic, amphophilic, tigroid, oncocytic, small and acidophilus cells, with spongiosis and peliosis) and neoplastic

Table 2- Hepatic steatosis in groups 1 and 2.

		Hepatic lesions		
		Without steatosis (%)	With steatosis (%)	Total (%)
Groups	Group 1	5 (55.6)	4 (44.4)	9 (100)
	Group 2	0 (0)	10 (100)	10 (100)
Total		5 (26.3)	14 (73.7)	19 (100)

X^2 = 7.54, ρ = 0.011 – corrected by the test of Fisher

Table 3- Hepatic steatosis in groups 3 and 4.

		Hepatic lesions		
		Without steatosis (%)	With steatosis (%)	Total (%)
Groups	Group 3	6 (42.9)	8 (57.1)	14 (100)
	Group 4	2 (16.7)	10 (83.3)	12 (100)
Total		8 (30.8)	18 (69.2)	26 (100)

X^2 = 2.08, ρ = 0.216 – corrected by the test of Fisher

(adenoma and cholangioma) lesions can be distinguished from the non-transformed tissue surrounding them by changes in the expression of various enzymes, including adenosine triphosphatase (ATPase), glucose-6-phosphatase, γ-glutamyl transpeptidase (γGT) and placental glutathione S-transferase (GST-P); these are commonly used as malignant disease markers [22-24].

Steatosis was seen more frequently in group 2 than in group 1. However, the difference between groups 3 and 4 was significant. On the other hand, group 4 animals with displayed preneoplastic and neoplastic lesions, possibly due to longer AOM exposure (11 weeks longer). Morphometric studies showed that foci of vacuolated cells (fat cells) can develop cell glycogen and gradually evolve into mixed cells and hepatic malignancy [22].

Comparing the groups 1 (15 weeks) and 3 (26 weeks), steatosis was more evident in the latter, where the only variable was time (11 weeks). The liver of senile animals can present with degeneration, with areas of fatty, lighter, vacuolated and ballooned cells, with bulkier and irregular nuclei [25].

In this study, AOM caused preneoplastic and neoplastic liver injury, proportional to the dose and duration of exposure, similar to the findings of Bird in colorectal carcinogenesis. However, more studies are necessary to strengthen these findings.

In conclusion, in the model of colorectal carcinogenesis involving rats, preneoplastic and neoplastic liver injuries appear and evolve in proportion to the duration of exposure and the dose of AOM.

RESUMO

***Objetivo**: Avaliar as repercussões hepáticas da carcinogênese colônica induzida por diferentes doses e tempos de exposição ao azoximetano em ratos Wistar. **Métodos**: Quarenta e quatro ratos foram distribuídos em quatro grupos. Os animais tinham oito semanas no início do experimento. No grupo 1, receberam 1.0mL de solução salina intraperitonealmente uma vez por semana por duas semanas. No grupo 2, receberam 15 mg/kg de azoximetano intraperitonealmente uma vez por semana por duas semanas. Esses animais foram mortos na 15ª semana do experimento. Os animais do grupo 3 receberam solução salina intraperitonealmente uma vez por semana por duas semanas. Os animais do grupo 4 receberam 20mg/kg de azoximetano intraperitonealmente uma vez por semana por duas semanas. Esses animais foram mortos na 26ª semana do experimento. Os fragmentos de tecido hepático foram corados pela hematoxilina e eosina e avaliadas microscopicamente. **Resultados**: Grupo 1 e grupo 2 diferiram significantemente em relação a esteatose, mas não houve diferença entre o grupo 3 e o grupo 4. No entanto, no grupo 4 foram observadas lesões pré-neoplásicas (focos de células alteradas, claras, vacuoladas, basofílicas, anfofílicas, tigróides, oncocíticas, pequenas ou acidófilas, espongioses e pelioses) e lesões neoplásicas (colangiomas e adenomas) contendo hepatócitos atípicos de permeio, não identificados no grupo 3. **Conclusão:** No modelo de carcinogênese colorretal, lesões hepáticas pré-neoplásicas e neoplásicas aparecem e evoluem na proporção do tempo e dose de exposição ao azoximetano.*

***Descritores:** Neoplasias do colo. Neoplasias colorretais. Marcadores biológicos de tumor. Azoximetano. Fígado gorduroso.*

REFERENCES

1. Brasil. Ministério da Saúde. Instituto Nacional de Câncer (INCa). Estimativa 2010: incidência de câncer no Brasil [Internet]. Rio de Janeiro: INCa, 2009. [citado 20 ago 2012]. 98p. Disponível em: http://www.inca.gov.br/estimativa/2010/estimativa20091201.pdf
2. Horner MJ, Ries LAG, Krapcho M, Neymar N, Aminou R, Howlader N, et al. Cancer statistics review 1975-2009 (Vintage 2009 population) [Internet]. Bethesda (MD): National Cancer Institute; 2009 [Update 2012 Aug 20]. Disponível em: hppt://seer.cancer.gov/scr/1975_2006/
3. Santos Jr JCM. Câncer ano-reto-cólico: aspectos atuais II - câncer colorretal – fatores de riscos e prevenção. Rev Bras Coloproctol. 2007;27(4):459-73.
4. Castro LS, Corrêa JHS, editores. Tratamento Cirúrgico do câncer gastrointestinal. Rio de Janeiro: Leonaldson dos Santos Castro; 2005.
5. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell. 1990;61(5):759-67.
6. Bird RP. Role of aberrant crypt foci in understanding the pathogenesis of colon cancer. Cancer Lett. 1995;93(1):55-71.
7. Bird RP. Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen: preliminary findings. Cancer Lett. 1987;37(2):147-51.
8. Roncucci L, Pedroni M, Vaccina F, Benatti P, Marzona L, De Pol A. Aberrant crypt foci in colorectal carcinogenesis. Cell and crypt dynamics. Cell Prolif. 2000;33(1):1-18.
9. Bird RP, Good CK. The significance of aberrant crypt foci in understanding the pathogenesis of colon cancer. Toxicol Lett. 2000;112-113:395-402.
10. Matsuda Y, Toda M, Kato T, Kuribayashi K, Kakimi K. Fulminant liver failure triggered by therapeutic antibody treatment in a mouse model. Int J Oncol. 2006;29(5):1119-25.
11. Bémeur C, Desjardins P, Butterworth RF. Antioxidant and anti-inflammatory effects of mild hypothermia in the attenuation of liver injury due to azoxymethane toxicity in the mouse. Metab Brain Dis. 2010;25(1):23-9.
12. Bannasch P, Enzmann H, Klimek F, Weber E, Zerban H. Significance of sequential cellular changes inside and outside foci of altered hepatocytes during hepatocarcinogenesis. Toxicol Pathol. 1989;17(4 Pt 1):617-28.
13. Bannasch P, Zerban H. Predictive value of hepatic preneoplastic lesions as indicators of carcinogenic response. IARC Sci Publ. 1992;(116):389-427.
14. Bannasch P. Hormonal and hormone-like effects eliciting hepatocarcinogenesis. Folia Histochem Cytobiol. 2001;39 Suppl 2:28-9.
15. Nozaki T, Fujihara H, Watanabe M, Tsutsumi M, Nakamoto K, Kusuoka O, et al. Parp-1 deficiency implicated in colon and liver

tumorigenesis induced by azoxymethane. Cancer Sci. 2003;94(6):497-500.
16. Nishihara T, Baba M, Matsuda M, Inoue M, Nishizawa Y, Fukuhara A, et al. Adiponectin deficiency enhances colorectal carcinogenesis and liver tumor formation induced by azoxymethane in mice. World J Gastroenterol. 2008;14(42):6473-80.
17. Stopera SA, Davie JR, Bird RP. Colonic aberrant crypt foci are associated with increased expression of c-fos: the possible role of modified c-fos expression in preneoplastic lesions in colon cancer. Carcinogenesis. 1992;13(4):573-8.
18. Caderni G, Femia AP, Giannini A, Favuzza A, Luceri C, Salvadori M, et al. Identification of mucin-depleted foci in the unsectioned colon of azoxymethane-treated rats: correlation with carcinogenesis. Cancer Res. 2003;63(10):2388-92.
19. Fiala ES. Investigations into the metabolism and mode of action of the colon carcinogens 1,2-dimethylhydrazine and azoxymethane. Cancer. 1977;40(5 Suppl):2436-45.
20. Bannasch P, Nehrbass D, Kopp-Schneider A. Significance of hepatic preneoplasia for cancer chemoprevention. IARC Sci Publ. 2001;154:223-40.
21. Bannasch P, Haertel T, Su Q. Significance of hepatic preneoplasia in risk identification and early detection of neoplasia. Toxicol Pathol. 2003;31(1):134-9.
22. Bannasch P, Zerban H. Tumor of the liver. In: Turosov VS, Mohr U, editors. Pathology of tumor in laboratory animals. Lyons: IARC; 1990. p. 199-240.
23. Dragan YP, Campbell HA, Xu XH, Pitot HC. Quantitative stereological studies of a 'selection' protocol of hepatocarcinogenesis following initiation in neonatal male and female rats. Carcinogenesis. 1997;18(1):149-58.
24. Ito N, Imaida K, Asamoto M, Shirai T. Early detection of carcinogenic substances and modifiers in rats. Mutat Res. 2000;462(2-3):209-17.
25. Gaglianone MC, Silva Netto CR, Sala MA, Lopes RA, Campos GM, Petenusci SO. Estudo morfológico do fígado de ratos durante o envelhecimento. Rev Esc Farm Odontol Alfenas. 1991;(13):91-100.

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