

EXPERIMENTAL MODEL OF GASTRIC CARCINOGENESIS WITH N-METHYL-N-NITROSOUREA FOR F344 RATS AND C3H MICES IS VALID FOR WISTAR RATS?

O modelo experimental de carcinogênese gástrica induzido por N-methyl-N-nitrosourea em ratos F344 e camundongos C3H é válido para os ratos Wistar?

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ABSTRACT - Background: The N-methyl-N-nitrosourea (MNU) is a direct acting carcinogen, inducing tumors in several species in a variety of organs, including stomach of rats. Treatment of MNU in the drinking water for 25-42 weeks selectively induced glandular gastric carcinoma in F344 rats and C3H mice. **Aim:** To establish an experimental model for selective MNU induction of glandular stomach cancer in Wistar rats. **Methods:** A total of 48 males eight-week-old Wistar rats were used in the present study. MNU (Sigma-Aldrich) was dissolved in DMSO and provided as the drinking water ad libitum for a period ranging from 16 to 70 weeks. After 16 weeks, four rats were randomly selected and killed. After every six weeks four animals were killed until 70 weeks. **Results:** Survival rate was higher than 90%. It had the induction of two adenocarcinomas, one squamous cell carcinoma and one sarcoma. The incidence of gastric adenocarcinoma was 4.5% (0.5 to 15). **Conclusions:** The experimental model of gastric carcinogenesis in Wistar rats, using MNU dissolved in water, showed not practice viability in this study due to the low rate of gastric adenocarcinoma.

HEADINGS – Wistar Rats. Tumor markers, biological. Stomach neoplasms.

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RESUMO – Introdução: O N-metil-N-nitrosourea (MNU) tem ação cancerígena direta, induzindo tumores em várias espécies em uma variedade de órgãos, incluindo o estômago de ratos. Tratamento do MNU na água de beber por 25-42 semanas, seletivamente, induz carcinoma gástrico glandular de ratos F344 e camundongos C3H. **Objetivo:** Estabelecer um modelo experimental para indução seletiva de câncer no estômago glandular de ratos Wistar com MNU. **Métodos:** Um total de 48 ratos Wistar machos com oito semanas, foram utilizados no presente estudo. MNU (Sigma-Aldrich) foi dissolvido em DMSO e liberada água potável ad libitum por um período variando de 16 a 70 semanas. Após 16 semanas, quatro ratos foram selecionados aleatoriamente e mortos. Depois, de seis em seis semanas, quatro animais também foram mortos até 70 semanas. **Resultados:** A taxa de sobrevivência foi superior a 90%. Ocorreu a indução de dois adenocarcinomas, um carcinoma espinocelular e um sarcoma. A incidência de adenocarcinoma gástrico foi de 4,5% (0,5 a 15). **Conclusões:** O modelo experimental de carcinogênese gástrica em ratos Wistar, utilizando MNU dissolvido na água, não mostrou viabilidade prática neste estudo, devido à baixa taxa de adenocarcinoma gástrico que ocorreu.

DESCRITORES - Ratos Wistar. Marcadores biológicos de tumor. Neoplasias gástricas.

INTRODUCTION

Since the first report of experimental production of adenocarcinomas of the glandular stomach in rats with N-methyl-N'-nitro-N-nitrosoguanidina (MNNG)¹⁶, many other mammals have been shown to be susceptible to this carcinogen, including hamsters, ferrets and dogs^{2,3}. The agent N-methyl-N-nitrosourea (MNU) is a direct acting carcinogen, inducing tumors in several species in a variety of organs, including the central nervous system, stomach, intestine, Kidney, and Skin^{1,4,9,10}.

The mouse and rat are particularly useful species for carcinogenesis studies because of the availability of a number of transgenic, mutant,

and chimeric strains. Treatment of MNU in the drinking water for 25-42 weeks selectively induced glandular gastric carcinoma in F344 rats and C3H mice^{5,8,11,13,14,18,21}. These animal models have been widely used not only for investigating the pathogenesis of gastric carcinogenesis but also for identifying possible tumor promoters and chemopreventive agents^{11,14}.

The aims of the present study were establishing an experimental model for selective MNU induction of glandular stomach cancer in Wistar rats and describe the time to get a level of depth tumor (T).

METHODS

Experimental design

A total of 48 males eight-week-old Wistar rats were housed in plastic cages (three rats/cage) on hard ward chips in air-conditioned room with a 12 h light/12 h dark cycle. Rats were allowed two weeks for acclimation before starting of experiments. MNU (Sigma-Aldrich) was dissolved in dimethyl sulfoxide (DMSO) and the resulting stock solution was stored in a cool, and dark place. The solution was diluted to 200 ppm with tap water just before using (three times a week), and provided as the drinking water ad libitum from light-shielded bottles to prevent photolysis for 16 to 70 weeks. Regular chow pellets (Nuvilab CR-1 – Nuvital S/A) were available ad libitum. At 16 weeks, four rats were randomly selected and killed, from each six weeks four animals were killed until 70 weeks. The study protocol was approved by the Research Ethics Committee of the Research and Postgraduate Group of Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil.

Exclusion criteria

Animals that survived less than 16 weeks were excluded in the effective numbers. Necropsies were performed on all animals which died.

Histopathological analysis

The excised stomachs were fixed in buffered formalin, cut into about eight strips, and routinely processed for embedding in paraffin. Tissue sections were stained with HE for histopathological assessment of lesion development. Neoplastic lesions were classified as adenocarcinomas, squamous carcinoma and sarcomas.

Statistical analysis

The confidence interval for proportions using the Binomial Method was applied for calculated of gastric adenocarcinomas incidence. Student's *t* test was performed for means of quantitative variables.

RESULTS

A total of 48 rats were used. Four animals died before the week 16 and were undergone to autopsy. These deaths were caused by pneumonia, and they were excluded from the effective numbers. Survival rates were higher than 90%. The average initial body weight was 346 ± 33 g. The weekly body weight gain during follow-up was lower in rats that developed cancer than those who did not ($2.1\text{g} \times 3.2\text{g}$, $p=0.07$). A total of 44 animals was killed from weeks 16 to 70. Characteristics of the effective number of rats are summarized in Table 1.

TABLE 1 - Characteristics of the effective number of rats

Effective Number of rats	Initial body weight (g)	Final body weight (g)	Final age
44	346 ± 33	469 ± 59	50 ± 14

Gross and microscopic findings

From weeks 52 to 70, diffuse inflammatory infiltration in the gastric mucosa was found (Figure 1). Intestinal metaplasia was not found in the stomach of any rat in this experiment. At 64 weeks, one extensive lesion in the anterior wall of the stomach was found and histopathological analysis revealed to be a sarcoma. At 70 weeks, one adrenal tumor was found. In the forestomach, only one squamous carcinoma was found at week 22. At week 52 and 70, two well differentiated adenocarcinomas were observed in the glandular stomach. The tumors invaded the muscularis proper superficially (T2a) (Figure 2). The incidence of gastric adenocarcinoma was 4.5% (0.5 to 15). Histopathological findings for the stomach are summarized in Table 2.

TABLE 2 - Histopathological findings for the stomach

Week	Inflammatory infiltrate	Adenocarcinoma	Squamous carcinoma	Sarcoma
22	0	0	1	0
52	2	1	0	0
58	3	0	0	0
64	1	0	0	1
70	0	1	0	0

DISCUSSION

It was not able to confirm that MNU in drinking water can induce a high incidence of carcinomas in the Wistar rat glandular stomach. Only two adenocarcinomas developed in the animals receiving 200 ppm MNU. Earlier studies indicate that cancer of the glandular stomach can be produced in F344 rats and C3H mice by the administration of MNU, a

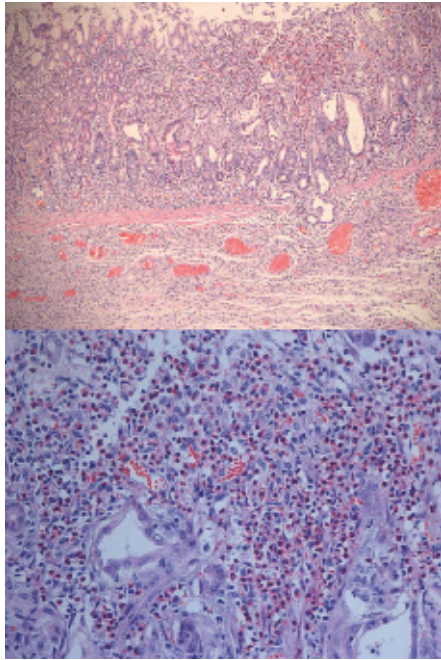


FIGURE 1 - Inflammatory infiltration in the gastric mucosa. HE 100X (A), HE 400X (B)

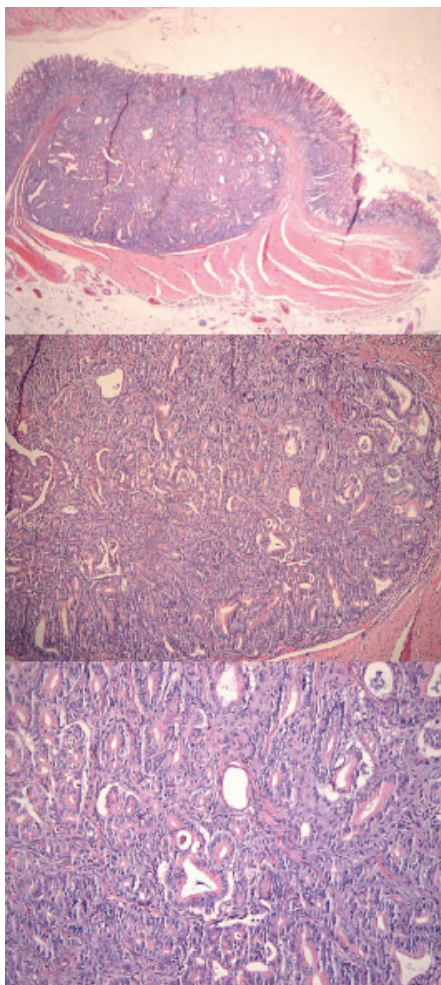


FIGURE 2 - Gastric adenocarcinoma (T2a).HE 40X (A), HE 100X (B) e HE 400X (C)

widely used mutagen. When MNU is administered in a drinking water for 25-42 weeks, selectively induced glandular gastric carcinoma in F344 rats and C3H mouse^{5,8,11,13,14,18,21}.

Maekava, et al.⁸ examined the effects of MNU in the drinking water at 100 ppm in F344 rats. After 42 weeks, 18% of rats had adenocarcinoma gastric. Tatematsu, et al.¹⁸ induced glandular stomach cancer in C3H mice with MNU in the drinking water at 120 ppm for 30 weeks and had 40% of adenocarcinomas after 54 weeks.¹² Hirota used MNU at 400 ppm in F344 rats for 25 weeks and had 100% of invasive adenocarcinomas.⁹ It was used here an intermediate concentration of MNU (200 ppm), because studies with lower concentrations showed a low incidence of adenocarcinoma, and higher concentrations have developed advanced tumors. The aim was to obtain early lesions in order to describe the time to get a level of depth tumor (T).

Lee⁷ examined the effects of concomitant administration of dimethylitaconate (DMI) and showed a significant increase of adenocarcinoma incidence, which was 95% compared with 12% MNU alone group ($p < 0.005$). There is evidence that sodium chloride administration enhances the carcinogenic effects of MMNG on the rat stomach¹⁷. Maybe the addition of promoting substance with MNU could have increased our incidence of gastric adenocarcinoma.

Tatsuta, et al.¹⁹ examined the effects of a very-low-protein on the incidence of gastric cancer induced by MNNU in Wistar rats. Administration p.o. of a very-low-protein diet resulted in a significant increase in the incidence of gastric cancers. The mechanism of this diet is not known, but at least three possible ways may be considered.

One is immunomodulation by dietary protein. Dietary components may influence the development of neoplasias by effects on the host immune mechanisms. In particular, the level and type of fat have been shown to modulate immune responsiveness^{12,20}. Another possible mechanism is an effect on the secretions of various hormones, including growth hormone, thyroid hormones, gastrin, and somatostatin^{6,15}. Growth hormone and thyroid hormones are closely related to the growth of gastrointestinal mucosa. A third possibility, is an increase in activity of the sympathetic nervous system. Diet influences the activity of the sympathetic nervous system in experimental animals.

There is evidence of neural involvement in the control of cell proliferation²². In this study, it was used a normal protein diet.

It is well known that MNU is a multipotent carcinogen and the carcinogenic action of MNU varies with the strain of rat used and the route of administration⁸. In addition, it has been demonstrated that the organ specificity of N-nitroso compounds given orally is influenced not only by chemical structure of the test compounds or by the strain of rats used,

but also by the dose level of the test compounds⁸. In this experiment, it was also shown that the organ specificity of MNU was influenced by the strain of rats and/or by the dose level. Further studies are needed to explain this phenomenon.

CONCLUSIONS

The experimental model of carcinogenesis in Wistar rats, using a 200 ppm of MNU dissolved in water, showed not practice viability in this study, due to the low rate of development of adenocarcinomas.

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HISTOPATHOLOGICAL DETERMINATION OF *Helicobacter pylori* IN GASTRIC CANCER

Determinação histopatológica da presença do Helicobacter pylori em câncer gástrico

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ABSTRACT - Background - Etiology of gastric cancer (GC) remains controversial and several factors have implicated in its carcinogenesis process, including *Helicobacter pylori* (Hp) infection. Hp infection's role on GC remains uncertain, with several conflicting studies. **Aim** - To look for any correlation between *H. Pylori* and gastric cancer from gastric specimen after gastrectomy. **Method** - Ninety-one patients with diagnosis of adenocarcinoma of the stomach treated by surgical resection were reviewed. Pathological examination was repeated in all patients to determine the presence of Hp infection, intestinal metaplasia (IM) and confirmation of the histologic type by conventional haematoxylin-eosin staining. Statistical analysis was performed using Chi-square and log-rank tests. **Results** - IM was observed in 81 tumours (89%). Overall, the presence of Hp infection was observed in 46 tumours (50.5%). There was no association between age and Hp status. In the group of patients with early and advanced GC, Hp infection was present in 47.7% and 54% of tumours. Hp infection was present in 40 tumours (49%) in the group of patients with IM. In patients with tumours without IM Hp was present in five (50%) tumours. Proximal tumours had more Hp infection when compared to distal tumours. **Conclusions** - The infection rate had no significant association with histologic type, IM, gender or stage. These results may indicate that participation of Hp infection during GC development cannot be ruled out; however, it is probably not essential during all stages of GC development and the mechanism may be distinct of the chronic gastritis and IM progression. Finally, it is possible that the proposed association is merely coincidental and that there is no actual influence of the bacteria in the carcinogenesis process.

HEADINGS - Stomach Neoplasms. *Helicobacter pylori*.

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RESUMO - Racional - A causa do câncer gástrico (CG) é controversa e tem vários fatores envolvidos no seu processo de carcinogênese, incluindo o *Helicobacter pylori* (Hp) O papel da infecção pelo Hp no CG permanece incerto, com vários estudos controversos. **Objetivo** - Correlacionar a presença da infecção pelo Hp com câncer gástrico, através de exame anatomopatológico convencional do estômago ressecado. **Método** - Noventa e um pacientes tratados por ressecção cirúrgica foram revistos. O exame anatomopatológico foi feito em todos os pacientes para determinar a presença de infecção por Hp, metaplasia intestinal (MI) e confirmação do tipo histológico por hematoxilina-eosina. A análise estatística foi realizada através do qui-quadrado e testes de log-rank. **Resultados** - MI foi observada em 81 tumores (89%). Em geral, a presença de infecção pelo Hp foi observada em 46 casos (50,5%). Não houve associação entre idade e Hp. Nos grupos de pacientes com CG avançado e precoce, a infecção pelo Hp estava presente em 47,7% e 54% dos tumores. A infecção pelo Hp ocorreu em 40 tumores (49%) no grupo de pacientes com MI. Nos com tumores sem MI, Hp estava presente em cinco (50%). Tumores proximais tiveram mais infecção por Hp, quando comparados aos tumores distais. **Conclusões** - A taxa de infecção não teve associação significativa com o tipo histológico, sexo, MI ou estágio de desenvolvimento tumoral. Esses resultados podem indicar que a participação da infecção pelo Hp durante o desenvolvimento do CG não pode ser descartada; no entanto, provavelmente não é essencial em todas as fases e o mecanismo do CG pode ser distinto da gastrite crônica e MI. Finalmente, é possível que a associação proposta é mera coincidência e que não há nenhuma influência real das bactérias no processo de carcinogênese.

DESCRITORES - Neoplasias gástricas. *Helicobacter pylori*