

MORPHINE DOES NOT PROMOTE ESOPHAGEAL CARCINOGENESIS IN RATS EXPOSED TO DIETHYLNITROSAMINE

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ABSTRACT – Background - The high incidence of esophageal cancer in the north of Iran has been associated to the consumption of opium and exposure to nitrosamines. Diethylnitrosamine has an established potential of producing experimental cancer in the esophagus and liver. **Aim** - To evaluate by histopathology the effect of oral administration of morphine and diethylnitrosamine during 23 weeks on the hepatic and esophageal carcinogenesis on 176 rats. **Methods** - We divided the rats into the following groups: *Morph*: morphine; *Den*: diethylnitrosamine; *Den+morph*: Den and morphine in the same solution; *Den/morph*: Den and morphine in different solutions and days. **Results** - Morphine did not promote neoplasias. The highest neoplastic incidents were found: a) in the esophagus, *Den* in relation to *Den/morph* and *Den+morph* (71.1%, 55.8%, and 50.0%); b) in the liver, *Den* and *Den/morph* in relation to *Den+morph* (73.8%, 81.4%, and 40.9%); c) higher incident of hepatic neoplasia than esophageal in *Den/morph* (81.4% and 55.8%). Different doses of diethylnitrosamine were ingested among the groups *Den*, *Den/morph*, and *Den+morph*, respectively 2.9, 2.8, and 2.3 mg/kg/day. **Conclusions** - These results show that the morphine did not promote esophageal carcinogenesis and may have stimulated the hepatic metabolism of the first pass of the carcinogen.

HEADINGS – Esophageal neoplasms. Carcinoma, squamous cell. Morphine. Diethylnitrosamine. Rats.

INTRODUCTION

Esophageal cancer is among the 10 most frequent in the world and if we consider the social impact on the endemic regions, the problem becomes a severe scourge for those populations. An intimate correlation has been seen between sickness and regional habits, meaning the way people live⁽⁴³⁾, and it has been shown that the origin of esophageal cancer is associated with different factors in each region^(16, 26).

The high incidence of esophageal cancer has been associated with the consumption of opium habit in high-incidence regions in the north of Iran^(15, 19, 24) and with the exposure to nitrosamines in China, mainly through their diet^(8, 21, 22, 41).

The reasons for the association between opium and cancer of the esophagus are not known, but trials show that a dose of morphine sulfate, the main alkaloid of opium, caused an alkylation increase in the DNA in the esophagus and a decrease in the liver in rats that received diethylnitrosamine (DEN) after the morphine. DEN, a nitrosamine, is considered one of the substances with the highest potential for producing cancer in the esophagus and liver of rats and mice^(20, 31), and the alkylation of the DNA constitutes a known alteration precursory to the carcinogenesis provoked by DEN in these organs.

This way the morphine would cause a lowering of the hepatic carcinogenesis and an increase of the esophageal by influencing the hepatic metabolism of DEN. Once suggested that the observations made in rats could be imputed to man⁽³¹⁾, these would be possible metabolic evidences, in an acute trial, for the association between opium and cancer of the esophagus in rats and therefore, by extension, in humans.

In the present study, we offered DEN in the rat's drinking water for 3 days during each one of the 23 weeks, and compared with the groups that consumed morphine simultaneously or not with DEN. We evaluated the effect of the chronic administration of morphine and DEN on the hepatic and esophageal carcinogenesis.

METHODS

We purchased the DEN from SIGMA (St. Louis, MO, USA): N-0756, density – 0.95 g/mL, and the morphine sulfate from CRISTÁLIA (Itapira, SP, Brazil): DCB 0856.03-7, density - 10 mg/mL. We obtained the 176 Wistar rats, ranging from 185-215 g, from Bioterio of the State Foundation of Health Protection and Research in Rio Grande do Sul, Porto Alegre, RS, Brazil. Water,

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food (Nuvilab CR1, based on criteria from the National Research Council and National Institute of Health – USA), and the diluted substances were changed 3 times a week at which time we measured the quantities ingested of each solution. All of the animals received human care and the protocols were approved by the Scientific Commission and the Health Research and Ethics Commission of GPPG-HCPA (the Research and Post-Graduate Group of “Hospital de Clínicas” of Porto Alegre, RS, Brazil).

In a Comparative Study of Multiple Groups, we divided 176 rats into groups with 44 animals and they ingested: *Morph*: morphine; *Den*: DEN; *Den+morph*: DEN + morphine in the same solution; *Den/morph*: DEN and morphine in different solutions and days. The rats ingested morphine solution during 4 days a week in the *Den/morph* group and 3 in the other groups, while the DEN was ingested during 3 days a week in the respective groups. We used the estimated doses of 5 mg/kg/day for morphine and DEN. The animals were weighed in the beginning, at 3 months, and before euthanasia, which occurred at 161 days (23 weeks). We dried esophagi and livers and immediately placed them in 10% buffered formalin until analysis.

We examined the esophagus in its entire extension since it wraps around itself (like a jellyroll) and removed three samples to represent the liver. Pathologists that were unaware of the group of origin of the specimen, examined the sections in hematoxylin-eosin (HE) under a common light microscope. We considered always the lesion of higher grade of alteration of each piece.

We classified the esophagi as⁽³²⁾: 1) normal histology, 2) hyperplasia, 3) esophagitis, 4) papilloma, 5) low-grade dysplasia, 6) high-grade dysplasia (including carcinoma in situ), and 7) invasive carcinoma: of the mucosa, of the muscularis of mucosa, and of the sub-mucosa (Figure 1A and 1B). We considered malignant neoplasias the high-grade dysplasia and invasive carcinoma, and called them neoplastic esophageal lesions or esophageal CA.

We classified the livers as⁽⁵⁾ 1) normal histology, 2) focus of clear cells, 3) neoplastic nodules, and 4) hepatocellular carcinoma (Figure 1C and 1D). We considered malignant neoplasias the focus of clear cells, the neoplastic nodules, and the hepatocellular carcinoma, and called them neoplastic hepatic lesions or hepatic CA.

The quantitative variables was analysed by ANOVA with a criterion of classification and the differences localized by the Tukey Test. Among the categorical variables, we compared the groups by chi-square with the differences located among the groups by the post-hoc procedure proposed by Zar. The significance level adopted was $\alpha = 0.05$.

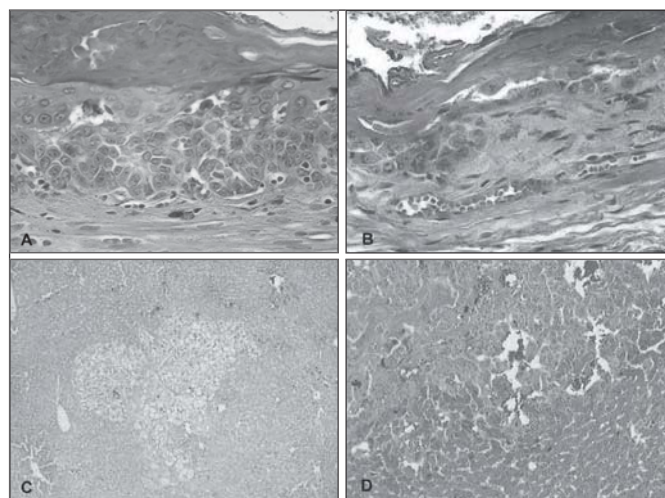


FIGURE 1. (A) HE 200x, high-grade dysplasia in esophagus of animal in the *Den+morph* group, dysplastic cells affecting more than half of the squamous epithelium, without invading the base membrane; (B) HE 200x, invasive epidermoid carcinoma that affects the muscularis of mucosa in the esophagus of animal in the *Den+morph* group; (C) HE 40x, focus of clear cells in liver of animal in the *Den* group; (D) HE 100x, hepatocellular carcinoma in animal of the *Den* group

RESULTS

We did not observe changes in conduct of the rats subordinate to the ingestion of morphine. There were two non-programmed deaths at 31 and 74 days of the trial, both of them in the *Den* group. Histopathologic analysis was not carried out in 13 esophageal specimens and in 3 hepatic: 2 due to non-programmed deaths and the others due to lack of material for the analysis. Therefore, we submitted 163 esophaguses and 173 livers to the histological exam.

The morphine ingested in isolated form (*Morph*) did not induce significant carcinogenesis in any of the organs analyzed (Table 1).

The incidence of neoplastic esophageal lesions was larger in the *Den* group (71.1%) in relation to the other groups ($P < 0.001$). This incidence was similar between the two groups that ingested DEN and morphine (*Den+morph* 50.0% and *Den/morph* 55.8%) (Table 1).

The incidence of neoplastic hepatic lesions was less in the group that ingested DEN and morphine simultaneously in the same solution (*Den+morph* 40.9%) than in the others that

TABLE 1. Neoplastic incidences and doses of the substances broken down by treatment group

	<i>Morph</i>	<i>Den</i>	<i>Den+morph</i>	<i>Den/morph</i>	<i>P</i>
Esophageal CA	n = 40 0 (0.0) ^a	n = 38 27 (71.1) ^c	n = 42 21 (50.0) ^b	n = 43 24 (55.8) ^b	< 0.001
Hepatic CA	n = 44 1 (2.3) ^a	n = 42 31 (73.8) ^c	n = 44 18 (40.9) ^b	n = 43 35 (81.4) ^c	< 0.001
DEN dose	-	2.9 ± 0.3 ^a	2.3 ± 0.1 ^b	2.8 ± 0.1 ^c	< 0.001
Morphine dose	2.5 ± 0.2 ^a	-	2.3 ± 0.1 ^b	2.4 ± 0.1 ^a	< 0.001

Data is expressed in numbers (percentages) of positive cases or as an average ± standard deviation of mg/kg/day. Distinct letters-index represent statistically significant differences among the groups. *Morph* group: ingested morphine; *Den*: diethylnitrosamine (DEN); *Den+morph*: DEN and morphine in the same solution; *Den/morph*: DEN and morphine in different solutions and days. Esophageal CA: lesions considered pre-malignant (high-level dysplasias) and malignant (epidermoid carcinomas) in the histopathologic exam. Hepatic CA: lesions considered pre-malignant (focus of clear cells, dysplastic nodules), and malignant (hepatocellular carcinomas) in the histopathologic exam.
^{a-c}: Solution not provided for ingestions to the respective groups

ingested the carcinogen (*Den/morph* 81.4% and *Den* 73.8%) ($P < 0.001$) (Table 1).

The possible effect of the morphine on the metabolism of first hepatic pass of the DEN may be observed in Table 1. The *Den* and *Den+morph* presented a similar incidence of esophageal and hepatic lesions while the *Den/morph* presented a higher percentage of hepatic lesions than esophageal ones ($P = 0.013$).

The esophageal histology was normal for the vast majority of the animals of the *Morph* group (95.0%), in contrast with the groups that ingested DEN, which were normal in only 2.6% of the *Den*, 16.7% of the *Den+morph*, and 14.0% of the *Den/morph*. The *Den* group caused a greater incidence of alterations, with a tendency to lesions of greater epithelial destructuring than the groups treated also with morphine (*Den+morph* and *Den/morph*) (Table 2).

Almost all of the animals of the *Morph* group presented a normal hepatic histology (97.7%), which did not occur with the groups that ingested DEN. In these, the histology was normal in 26.2% of the *Den*, 59.1% of the *Den+morph*, and 18.2% of the *Den/morph*. Though the *Den/morph* group represented greater incidence of changes, the three cases of hepatocellular carcinoma occurred in the *Den* group (Table 3).

The dose ingested was based on the quantity of weekly solution ingested, on the animal's weight, and on the concentration of the substances in the liquid offered the animals. The average dose ingested was inferior to the estimate of 5 mg/kg/day in all the groups.

Among the groups exposed to the carcinogen, they all ingested different doses of DEN among themselves (*Den*: 2.9 mg/kg/day, *Den+morph*: 2.3 mg/kg/day, *Den/morph*: 2.8 mg/kg/day) ($P < 0.001$). From the groups that ingested morphine (*Morph*: 2.5 mg/kg/day; *Den+morph*: 2.3 mg/kg/day; and *Den/morph*: 2.4 mg/kg/day), the *Den+morph* group ingested less morphine than the other groups ($P < 0.001$) (Table 1).

DISCUSSION

The nitrate compounds are presented in the diet or in the environment in doses much below those necessary to develop cancer, and for this reason the biggest concern is with chronic exposure. The carcinogenic nitrates are present in the diet (mainly in food preservatives, colorings, and flavorings), in tobacco and alcoholic beverages (beer, whisky, and liquors), through occupational exposure (rubber, pesticide, cosmetic, and leather tanning industries), and in personal and domestic hygiene products (cosmetics, shampoos, and detergents)^(9, 13, 20, 36). Their hepatotoxic and carcinogenic potential has been established both in humans as well as in animals^(6, 27, 35) and described that the nitrosamines produce the alkylation of the DNA in human tissues *in vitro*⁽²⁰⁾. Based on these studies, it has been suggested that nitrosamines could be related to cancer of the esophagus in man^(2, 14, 21, 22, 23).

The discovery that nitrosamines present a well-defined organotropism allowed the development of study models of cancer in various organs. Many authors used the model of esophageal and hepatic carcinogenesis induced by DEN in mice and rats and showed various levels of neoplastic incidence depending on the dose and time of duration of their studies^(4, 17, 18, 29, 30, 32, 33, 35). The knowledge that the N-nitroso compounds show similar biological activity in animal and human tissues would suggest that the observations made in rats could be extrapolated to man^(2, 31).

The incidence of cancer of the esophagus is influenced by different factors in the various endemic areas of the world. In Europe and United States it is related with the exposure to alcohol and tobacco, associated or not^(7, 40), in the south of Brazil with the consumption of hot matte tea⁽¹¹⁾, and in the north of Iran it has been associated by epidemiologists with the smoking of opium and the ingestion of opium pipe residues (sukhteh)⁽¹⁹⁾.

TABLE 2. Relative frequency of the results from the esophageal microscopy broken down by treatment groups

	<i>Morph</i> (n = 40)	<i>Den</i> (n = 38)	<i>Den+morph</i> (n = 42)	<i>Den/morph</i> (n = 43)	P
Normal	38 (95.0)	1 (2.6)	7 (16.7)	6 (14.0)	< 0.001
Hyperplasia	0 (0.0)	2 (5.3)	4 (9.5)	1 (2.3)	0.166
Esophagitis	0 (0.0)	2 (5.3)	4 (9.5)	3 (7.0)	0.283
Papilloma	1 (2.5)	0 (0.0)	1 (2.4)	0 (0.0)	0.572
Low-grade dysplasia	1 (2.5)	6 (15.8)	5 (11.9)	9 (20.9)	0.084
High-grade dysplasia	0 (0.0)	19 (50.1)	19 (45.2)	18 (41.9)	< 0.001
Invasive carcinoma	0 (0.0)	8 (21.0)	2 (4.8)	6 (14.0)	< 0.001
Mucous	0 (0.0)	6 (15.8)	2 (4.8)	4 (9.3)	0.051
Muscle of mucous	0 (0.0)	1 (2.6)	0 (0.0)	2 (4.7)	0.314
Sub-mucous	0 (0.0)	1 (2.6)	0 (0.0)	0 (0.0)	0.346

Data is expressed in numbers (percentage) of cases. *Morph* Group: ingested morphine; *Den*: diethylnitrosamine (DEN); *Den+morph*: DEN and morphine in the same solution; *Den/morph*: DEN and morphine in different solutions and days

TABLE 3. Relative frequency of the results from the hepatic microscopy broken down by treatment groups

	<i>Morph</i> (n = 44)	<i>Den</i> (n = 42)	<i>Den+morph</i> (n = 44)	<i>Den/morph</i> (n = 43)	P
Normal	43 (97.7)	11 (26.2)	26 (59.1)	8 (18.6)	< 0.001
Focus of clear cells	1 (2.3)	25 (59.5)	8 (18.2)	28 (65.1)	< 0.001
Neoplastic nodules	0 (0.0)	3 (7.1)	10 (22.7)	7 (16.3)	0.005
Hepatocellular carcinoma	0 (0.0)	3 (7.1)	0 (0.0)	0 (0.0)	0.023

Data is expressed in numbers (percentage) of cases. *Morph* Group: ingested morphine; *Den*: diethylnitrosamine (DEN); *Den+morph*: DEN and morphine in the same solution; *Den/morph*: DEN and morphine in different solutions and days

Experiments show that ethanol alters the pharmacokinetics of the nitrosamines^(1, 37, 38, 39). It causes higher exposure of nitrosamines to the esophageal tissue and capable of inducing cancer of the esophagus in animals^(1, 3, 37, 38, 39). RIBEIRO-PINTO and SWANN⁽³¹⁾ showed that morphine, opium's main alkaloid, also changes the pharmacokinetics and distribution of DEN in a way similar to ethanol. This reinforces the hypothesis that these two substances have a common base in their influence on esophageal carcinogenesis: their effects on pharmacokinetics from the nitrosamines to which man is exposed.

The dose of 5 mg/kg/day of DEN used in this study during 3 days a week was based on the studies of RUBIO et al⁽³³⁾ and RIBEIRO-PINTO and SWANN⁽³¹⁾. The first author administered DEN in the concentration of 0.04 mL/L of drinking water (7 mg/kg/day) during 3 days a week, which promoted esophageal tumors after periods of 4 and 6 months in mice (1 and 3 tumors/cm of esophageal mucous, respectively), with low mortality. RIBEIRO-PINTO and SWANN⁽³¹⁾ demonstrated the alkylation of the hepatic and esophageal DNA with 3 mg/kg/dose of DEN. Other authors confirm the carcinogenic power of these doses: GIBEL⁽¹⁷⁾ provoked esophageal cancer in 30% and 56% of the rats with the respective doses of 2.5 and 10 mg/kg/day, and SCHMÄHL et al⁽³⁵⁾ provoked hepatocellular carcinomas in 92% of the rats of his trial in a period of 138 ± 10 days using doses between 5 and 7.5 mg/kg/day of DEN, ingested daily.

In relation to morphine, it has been demonstrated that a single dose of 5 mg/kg increased the alkylation of the esophageal DNA of rats by 90% and lowered the hepatic by 10% when administrated 45 minutes previously to a single dose of DEN. These results do not change significantly with the increase of the doses of morphine sulfate from 10 and 20 mg/kg⁽³¹⁾. In the present study, we used 5 mg/kg/day of morphine sulfate, which would not cause changes in the activity of the animals and provoke the pharmacokinetic changes referred to above⁽³¹⁾.

The dose ingested by the animals in this study was substantially lower to the dose estimated. Though the esophageal and hepatic carcinogenesis are known to be dependent on the dose of DEN, RUBIO et al.^(33, 34) described that the time elapsed would also be of great importance in the formation of tumors in the esophagus of mice. According to these authors, clones of esophageal cells would be "programmed" for carcinogenesis in early stages of the treatment with DEN, and that a large number of tumors would occur in longer intervals even after only a few doses of DEN. While animals treated for 3 months presented a tumor index (TI) = 0.9, animals treated for 3 months and maintained alive for 4 additional months, with a carcinogen-free diet, presented a TI that was 5 times higher (TI = 4.6)⁽³⁴⁾. In this present study, a percentage of animals affected by esophageal neoplastic lesions higher than that estimated in the beginning of the study was developed in the *Den* group (obtained = 71%; estimated = 30%), despite the doses ingested were only 59% of that expected in this group. Based on this, it can be inferred that both the time elapsed of 23 weeks as well as the doses actually ingested of DEN were enough to provoke the carcinogenic effects expected in the two organs evaluated with practically no mortality.

The dose ingested of morphine varied between 2.3 and 2.5 mg/kg/day, which is only 48% of the estimated 5 mg/kg/day. These doses are equivalent to 170 mg/day for man, which are similar to the levels used in analgesics in occidental medicine and making them comparable to this population of chronic users of morphine. On the other hand, the dose is possibly inferior to those reported in the population of addicts in the north of Iran, who received 3 g of opium daily⁽¹⁹⁾. For this reason, it most likely does not apply to this population where the relation between opium and cancer of the esophagus was first described⁽¹²⁾.

With the ethical intention of lowering the unnecessary euthanasia of animals, a control group with eight rats was created, which ingested only water. This group presented a normal macro and microscopic analysis in the present study, the same of hundreds of animals that used the same source of water in other research from this Institution⁽¹⁸⁾. This group was excluded from the analysis since the number of eight animals caused a strong asymmetry in the data, jeopardizing an adequate statistical analysis.

Among the animals exposed only to DEN, 71% presented esophageal neoplastic lesions and 73% hepatic, which are incidents similar to the other authors^(17, 29, 25, 35). Among the groups exposed only to morphine, esophageal neoplasias were not found, but only one pre-neoplastic hepatic lesion (2.3%) was developed in one animal group of *Morph*, a focus of clear cells. BANNASCH et al.⁽⁵⁾ affirm that although these lesions can occur in control animals, the incidence is low, as is the incidence of naturally occurring hepatocellular carcinoma.

There was a higher incidence of esophageal neoplastic lesions in the animals that ingested only the carcinogenic (71.1%) ($P < 0.001$) in relation to the two groups that ingested DEN and morphine (*Den+morph* and *Den/morph*). These two groups presented a neoplastic incidence similar between themselves (50% and 55.8%) (Table 1). Even though the doses of DEN were different among the groups of *Den*, *Den/morph*, and *Den+morph* (2.9, 2.8, and 2.3 mg/kg/day) ($P < 0.001$), a relative correspondence could be seen between them and the incidence of esophageal neoplasias: the group that ingested more carcinogen present a greater incidence of neoplasia. These results suggest that the morphine does not exert an inductive carcinogenic effect on the esophageal mucosa.

The incidence of neoplastic hepatic lesions did not have a correspondence with the dose ingested of the carcinogen. The *Den* and *Den/morph* groups presented incidences similar between themselves and higher than *Den+morph* (73.8%, 81.4%, and 40.9%) ($P < 0.001$), even though the doses of DEN were different among all the groups (2.9, 2.8, and 2.3 mg/kg/day) ($P < 0.001$) (Table 1). Notice that *Den* ingested a significantly larger dose of the carcinogen than *Den/morph*, but suffered from 73.8% of neoplasias in comparison to 81.4% of the other group. Despite the incidence not being significantly different, these results could express some effect of the morphine on the hepatic carcinogenesis. A question still remains: if the doses of carcinogen were similar, would there be a significantly higher incidence of neoplasias in the *Den/morph* group in relation to the *Den* group? The data does not allow us to make conclusions if the morphine would exert an effect on the hepatic carcinogenesis.

The mechanism of changes produced by the morphine is not clear, but the participation of the enzymatic cytochrome P450 system is probable⁽³¹⁾. Though chronic or sub-acute treatment of adult male rats with morphine have lowered the levels of some P450s, elevated doses of morphine between 5 and 20 mg/kg/day administered to rats for 4 or more days induced other P450s such as sub-groups 1A2, 2B1, and 2E1⁽²⁸⁾. The P450 2E1 carries out a substantial part of the hepatic metabolism of the DEN in the rat⁽⁴²⁾. The morphine is not only metabolized by the hepatic P450^(28, 31), but it also acts as an inductive agent of this P450 and could have an influence on the metabolism of the DEN's first hepatic pass, which would stimulate and attenuate the activation of the carcinogen. Morphine, when administered in an isolated, non-continual (3 days a week), and chronic way, could induce weekly the 2E1 sub-group of the hepatic enzyme P450, maintaining it active and free for the moment when the carcinogen is ingested. This way, the DEN would be mostly metabolized in the liver, causing it to be locally active and capable of provoking a high incidence of carcinoma in this organ. If it is mostly metabolized in the liver, there would be less hematic bioavailability for other organs that also have P450 such as the esophagus, and consequently a lower local activation and capacity to form carcinomas in this organ. Doses of DEN known to be carcinogenic such as those offered to the *Den/morph* group could have been utilized by this metabolic mechanism of pre-activation by the morphine and promote a significantly higher percentage of hepatic neoplasias than esophageal, respectively 81.4 and 55.8% (Table 1).

When offered DEN and morphine simultaneously, the induction and utilization of the hepatic P450 2E1 by the morphine could occur, keeping this P450 "occupied" at the time in which the carcinogen does its metabolism of the first pass, thus characterizing a competitive phenomenon between the two substances. Depending on the level of competition at the hepatic level, the hematic

bioavailability of DEN would be unaltered or even increased and consequently an unaltered or increased metabolization and carcinogenesis in the esophagus. This could have been the metabolic phenomenon that occurred in the *Den+morph* group when statistically similar results were seen of 40.9% of hepatic neoplasias and 50% of esophageal neoplasias (Table 1). These results suggest that the morphine could stimulate the hepatic metabolism of DEN in chronic exposure when the substances are ingested in an interspersed way. This hypothesis is based on indirect results of neoplastic incidence in histological results and needs new studies with direct metabolic measurement in order to reach more definite conclusions.

We concluded that morphine did not present an inductive effect on the esophageal carcinogenesis induced by the ingestion of diethylnitrosamine in this experimental model. We suppose that the morphine may have stimulated the hepatic metabolism of the first pass of the carcinogen.

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RESUMO – Racional - A alta incidência de câncer esofágico no norte do Irã foi associada ao consumo de ópio e exposição às nitrosaminas. A dietilnitrosamina possui potencial estabelecido de produzir câncer experimental em esôfago e fígado. **Objetivo** - Avaliar por histopatologia o efeito da administração oral de morfina e de dietilnitrosamina na carcinogênese esofágica e hepática em ratos. **Métodos** - Durante 23 semanas, 176 ratos ingeriram diferentes soluções, sendo divididos em grupos: *Morf*: morfina; *Den*: dietilnitrosamina; *Den+morf*: dietilnitrosamina e morfina numa mesma solução; *Den/morf*: dietilnitrosamina e morfina em diferentes soluções e dias. **Resultados** - *Morf* não promoveu neoplasias. Encontraram-se maiores incidências neoplásicas: a) no esôfago, *Den* em relação à *Den/morf* e *Den+morf* (71,1%, 55,8% e 50,0%); b) no fígado, *Den* e *Den/morf* em relação à *Den+morf* (73,8%, 81,4% e 40,9%); c) maior incidência de neoplasia hepática do que esofágica em *Den/morf* (81,4% e 55,8%). Diferentes doses de dietilnitrosamina foram ingeridas entre os grupos *Den*, *Den/morf* e *Den+morf*, respectivamente 2,9, 2,8 e 2,3 mg/kg/dia. **Conclusões** - A morfina não promoveu a carcinogênese esofágica e pode ter estimulado o metabolismo hepático de primeira passagem do carcinógeno.

DESCRITORES – Neoplasias esofágicas. Carcinoma de células escamosas. Morfina. Dietilnitrosamina. Ratos.

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