Implications of fructose in liver tumours of the Egyptian toad

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دور الفركتوز في إحداث أورام الكبد في الضفدعة المصرية

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خلاصة: تلعب العوامل البيئية دوراً مهماً في تسبيب كثير من أنواع السرطان. ولقد أدى اكتشاف هذه الحقيقة إلى قدر كبير من الاهتمام بدور الغذاء في إحداث السرطان. ومن المعروف جيداً أن بعض شركات إنتاج المربات والعصائر تستعمل الفركتوز بدلاً من الغلوكوز أو السكروز لتحلية منتجاتها. وتثبت هذه الدراسة أن الفركتوز يعجل حدوث سرطانات الكبد في الضفادع المصرية التي سبق حقنها بمركب 12,7 - دايميثيل بنز (a) - أنثراسين.

ABSTRACT Environmental factors play an important role in the etiology of several types of cancer; this discovery has led to a great deal of interest in the role of diet in cancer etiology. It is well known that some factories which produce jams and juices use fructose rather than glucose or sucrose to sweeten their products. This study demonstrates that fructose insignificantly enhances the incidence of liver tumours in Egyptian toads previously injected with 7,12-dimethylbenz (a)-anthracene.

Implication du fructose dans les tumeurs du foie chez le crapaud égyptien

RESUME Les facteurs environnementaux jouent un rôle important dans l'étiologie de divers types de cancer; suite à cette découverte, on a porté un grand intérêt au rôle de l'alimentation dans l'étiologie du cancer. Il est bien connu que certaines firmes qui produisent des confitures et des jus de fruits utilisent le fructose plutôt que le glucose ou le sucrose pour édulcorer leurs produits. Cette étude a démontré que le fructose accroît de manière non significative l'incidence des tumeurs hépatiques chez les crapauds égyptiens ayant reçu antérieurement une injection de 7,12-Diméthylbenzanthracène.

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Introduction

Epidemiologic studies have demonstrated the influence of environment and lifestyle on the development of certain forms of cancer. The observation that the cause of several types of cancer may be mainly environmental in origin has led to a great deal of interest in the role of diet in cancer etiology. It has been suggested that deviations in carbohydrate metabolism and related alterations in enzyme histochemical patterns might be a pathogenetic principle of hepatocarcinogenesis [*1*]. If the assumption that deviations in carbohydrate metabolism play a pathogenetic role in the process of hepatocarcinogenesis is valid, an enhancement of these metabolic alterations will promote the development of hepatic tumours.

It has been shown that fructose enhances hepatic glycogen content and the expression of several enzymes of carbohydrate metabolism [*2*] which are also regularly over-expressed during chemical hepatocarcinogenesis in rodents [*3*]. The effects of fructose on hepatocarinogenesis are also of interest since human food contains considerable amounts of fructose.

Toads have been used as advantageous models with which to study the development of tumours [*4*], co-carcinogens [*5*] and vitamins [*6*]. It is worth mentioning that similarities between amphibian and human tumours have been reported [*7*].

The present study was undertaken to determine whether fructose, which is widely used in Egypt, has any promoting effects on liver tumours in Egyptian toads previously injected with the carcinogen 7,12-dimethylbenz (a)-anthracene (DMBA).

Materials and methods

Sexually mature male and female toads, *Bufo regularis*, were used. The average weight per experimental animal was 40 g. The experimental animals were collected by a regular supplier from El-Nozha district, Alexandria, Egypt. Toads were maintained in glass tanks at a temperature of 20–22 °C and were fed equal meals of earth worms once per week. The experimental animals were divided into four groups (50 toads/group) and treated as follows.

- Group A toads were each injected with 1 mg of DMBA into the dorsal lymph sac, twice per week for 12 weeks. DMBA was purchased from Sigma Chemical Company, St. Louis, MO, USA.
- Group B toads were injected with DMBA at the same dose level and given D-fructose orally twice per week for 12 weeks at a dose level of 0.5 g/toad as described for Group A toads [*8*]. Fructose products were purchased from Sigma Chemical Company, St. Louis, MO, USA.
- Group C toads were given D-fructose alone at the same dose level for the same period.
- Group D toads were injected with 0.1 ml of olive oil per toad, twice per week for 12 weeks and were used as a control group.

After 12 weeks of treatment, the toads in all groups were killed and all organs examined grossly. Tumours appeared in the livers of some toads. For histological evaluation, the livers were fixed in Bouin em bedded in paraffin and section (4 μm) stained with haematoxylin and eosin.

Statistical analysis using the chi-squared (χ^2) test was performed to determine the level of significant difference between tumour incidence in toads treated

with DMBA when compared to toads treated with DMBA and fructose.

Results

Liver tumours were found in group A toads which received 1 mg of DMBA/toad, twice per week for 12 weeks. Tumour incidence was 30% in contrast with zero incidence in the control group D which received only olive oil (Table 1). The liver tumours were diagnosed as hepatocellular carcinoma.

Toads treated with DMBA and fructose showed a higher incidence of liver tumours with 18 out of 50 toads (36%) bearing liver tumours. Neither tumour growth nor neoplastic changes were detected after 12 weeks in the livers of toads which were given fructose alone or olive oil (Table 1).

Mortality increased in toads treated with DMBA due to its toxicity compared with those treated with fructose alone (Table 1).

According to the χ^2 test, the increase in tumour incidence was insignificant ($P > 0.05$) between toads treated with both DMBA and fructose when compared with tumour incidence in toads treated with DMBA alone. This suggests that fructose insignificantly increased tumour incidence induced by DMBA.

Discussion

The present investigation demonstrated that fructose insignificantly enhances the incidence of toad liver tumours previously treated with DMBA. Similarly, Enzmann et al. [*8*] found that orally administered fructose enhanced the development of atypical acinar cell nodules (AACN) in the pancreas of rats pretreated with N-nitrosomorpholine. It is well known that AACN are prestages of pancreatic acinar cell carcinomas [*9*]. The mechanisms resulting in the enhancement of hepatocarcinogenesis through the administration of fructose are not well understood at present. Several studies in humans [*1,10*] and in rodents [*11*] indicate that deviations in carbohydrate metabolism, especially glycogen metabolism, are closely associated with hepatocarcinogenesis. Although the oral administration of fructose predominantly affects carbohydrate metabolism, alterations in lipid metabolism must also be taken into account. An increase in serum lipids and serum cholesterol caused by oral application of fructose or sucrose has been reported [*2*]. Moreover, the influence of fat intake on carcinogenesis in humans [*12*] and on chemically induced carcinogenesis in rodents and toads has been demonstrated [*5,13*]. We cannot exclude the possibility that oral fructose introduces a moderately

Table 1 **Effect of fructose on DMBA-induced hepatocarcinogenesis in toads**

Group	Treatment	Dose per week	No. of toads	No. of dead toads	No. of toads bearing liver tumours	Tumour incidence (%)
A	DMBA	1 mg	50	5	15	30
B	DMBA+Fructose	1 mg + 0.5 g	50	4	18	36
C	Fructose	0.5 g	50	1	0	0
D	Olive oil	0.1 ml	50	0	0	0

higher total caloric intake than the normal toad consumes. There is some evidence that total caloric intake rather than fat intake is crucial for tumour promotion in humans and laboratory animals [*14,15*], however, further work is required to clarify this question.

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