Cancer Causes and Control **13**: 91–100, 2002.

Hypothesis: Does ochratoxin A cause testicular cancer?

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Received 7 June 2001; revised version accepted 5 September 2001

Key words: diet, epidemiology, mycotoxin, ochratoxin A, testicular cancer.

Abstract

Little is known about the etiology of testicular cancer, which is the most common cancer among young men. Epidemiologic data point to a carcinogenic exposure in early life or *in utero*, but the nature of the exposure is unknown. We hypothesize that the mycotoxin, ochratoxin A, is a cause of testicular cancer. Ochratoxin A is a naturally occurring contaminant of cereals, pigmeat, and other foods and is a known genotoxic carcinogen in animals. The major features of the descriptive epidemiology of testicular cancer (a high incidence in northern Europe, increasing incidence over time, and associations with high socioeconomic status, and with poor semen quality) are all associated with exposure to ochratoxin A. Exposure of animals to ochratoxin A via the diet or via *in utero* transfer induces adducts in testicular DNA. We hypothesize that consumption of foods contaminated with ochratoxin A during pregnancy and/or childhood induces lesions in testicular DNA and that puberty promotes these lesions to testicular cancer. We tested the ochratoxin A hypothesis using ecologic data on the per-capita consumption of cereals, coffee, and pigmeat, the principal dietary sources of ochratoxin A. Incidence rates for testicular cancer in 20 countries were significantly correlated with the per-capita consumption of coffee and pigmeat ($r = 0.49$ and 0.54, $p = 0.03$ and 0.01). The ochratoxin A hypothesis offers a coherent explanation for much of the descriptive epidemiology of testicular cancer and suggests new avenues for analytic research.

Introduction

Testicular cancer is the most common cancer among Caucasian men aged 15–34 (see refs 1 and 2 for reviews of the epidemiology). The descriptive epidemiology of testicular cancer shows several unusual features. First, unlike most other solid tumors, which increase as a function of age, the incidence of testicular cancer peaks at about 25–34 years of age and then declines. Testicular cancer is uncommon in boys under the age of 10 and in men over the age of 60, accounting for approximately 1% and 8% of testicular cancers, respectively. Approximately 96% of testicular tumors in men aged 10–59 are testicular germ cell cancers and are classified histologically into two groups, seminomas and non-seminomas (also called teratomas). The observation that the incidences of seminomas and non-seminomas have increased in parallel suggests that the two types of germ cell cancer have the same cause [3].

Testicular cancer shows marked geographic variation, with incidence rates higher in northern than in central or southern Europe [4]. A 10-fold geographic variation in incidence was found in Europe in 1980, with the highest rates in Denmark and East Germany, with age-standardized rates of 7.8 and 5.9 per 10^5 per year (respectively), and the lowest in Lithuania and Finland (0.9 and 1.3 per 10^5). The incidence of testicular cancer has been increasing in many Caucasian populations by approximately 2.3% each year since the 1940s. The epidemic increase has been especially marked in young men, with incidence rates doubling every 15–25 years [5].

The peak occurrence of testicular cancer among young men, and its rarity among elderly men, suggests that the critical exposures for testicular cancer occur in early life or *in utero* [6]. Cryptorchidism (i.e., undescended testes) is an established risk factor for testicular cancer, but cryptorchidism accounts for no more than 10% of cases [7]. Increased risks for testicular cancer have been found

among men with testicular atrophy and among men with poor semen quality, the latter determined prospectively [8, 9]. This suggests that testicular cancer and testicular dysgenesis may share a common etiology [10, 11]. The high rates of testicular cancer among countries that are relatively affluent, and the consistent association of testicular cancer with individuals of higher social class [12–14], imply that testicular is caused by some aspect of modern life or behavior [15, 16].

A further clue to the etiology of testicular cancer is the significant reduction in risk that occurred for Danish, Norwegian and Swedish men born during World War II and that increased among men born thereafter [3, 17]. This suggests that the cause of testicular cancer may somehow be related to the goods and provisions that were reduced in these countries during the occupation of Denmark and Norway by the German military forces. Several factors have been proposed as potential risk factors for testicular cancer, including *in utero* exposure to endogenous and pharmacologic estrogens [18, 19], environmental xenoestrogens [20], endogenous androgens [21], testicular trauma [22], and early exposure to viruses [23]. However, none of these factors appears to be convincingly correlated with the geographic distribution or temporal trends in the incidence of testicular cancer, and none has attained conclusive epidemiologic support [24, 25].

We hypothesize that ochratoxin A (OTA), a mycotoxin that is a widespread contaminant of human food, is a cause of testicular cancer [26]. The purposes of this study are to: (a) describe the OTA hypothesis for testicular cancer; (b) review evidence that OTA is a plausible testicular carcinogen; (c) test the OTA hypothesis using ecologic data; and (d) propose new areas for analytic research. Before describing the OTA hypothesis, we briefly review some toxicological properties of OTA.

Toxicological properties of ochratoxin A

Ochratoxins, of which OTA is the most prevalent, are mycotoxins that result from the growth of the molds *Aspergillus ochraceus, Aspergillus ostianus*, and *Penicillium verrucosum* on grain that is stored at temperatures of 15 °C and 15–19% humidity [27]. (For a review of factors influencing OTA production see ref. 28; for reviews of the toxicology of OTA see refs. 29–31.) OTA consists of a dihydroisocoumarin moity coupled through its 7-carboxyl group by an amide bond to L-β-phenylalanine. In addition to grains, OTA occurs in plant products such as coffee, nuts, and spices. High levels of OTA are found in some animal-derived food, especially pork products, via "carry-over" – the result of feeding moldy fodder to non-ruminant animals [32]. The meat of ruminants, *e.g.* cows, contains little OTA because OTA is cleaved by protozoan and bacterial enzymes that are present in the rumen [33]. However, OTA occurs in the milk of cows that have been exposed to large quantities of OTA [34]. In 1994 a cooperative European effort was undertaken to determine exposure of Europeans to OTA (Scientific Co-operation on Questions Relation to Food, SCOOP). SCOOP determined that exposure to OTA is widespread, with > 50% of blood samples from Germany and Denmark testing positive [35]. In 1997 the Codex Committee on Food Additives and Contaminants of the World Health Organization recommended a maximum residue level of 5 μg OTA per kg for grain products that are to be used for human consumption. This same value was recommended by a Scandinavian health commission, and assumes a tolerable daily intake (TDI) of 5 ng OTA/kg body weight [36].

Until recently few surveillance data for OTA were available in the US and Canada [37]. However, a recent survey of cereal grains and coffee imported into the US found OTA in 11/103 (10.7%) samples of barley, 56/383 (14.6%) samples of wheat, 9/19 (47.4%) samples of green coffee, and 9/13 (69.2%) samples of roasted coffee (detection limits > 0.03 ng/g) [38]. Frohlich *et al.* analyzed stored grain, swine blood, and human blood in Canada. Thirty-four percent of 164 stored grain samples had OTA levels between 0.5 and 2.0 mg/kg. Thirty-six percent of 1588 samples of blood from slaughterhouse pigs were positive for OTA, with a mean concentration of 12.3 ng/ml. Forty percent of 159 blood samples from Canadians had detectable OTA; 11% had concentrations greater than 0.5 ng/ml. The levels of OTA in the blood of Canadians are comparable to those observed among Europeans [39]. These data confirm that OTA is present in the blood of North Americans, and indicate that possible points of entry of OTA into the human food chain are contaminated grain, coffee, and pork [40].

Several lines of evidence suggest that OTA may be carcinogenic to humans. First, OTA causes a nephropathy affecting pigs in Scandinavian countries that closely resembles a fatal kidney disease in humans, Balkan endemic nephropathy (BEN) [41, 42]. BEN is a chronic, non-inflammatory nephropathy endemic to populations in Bulgaria, Romania, and the former Yugoslavia [43]. Approximately one-third of patients dying with BEN have papillomas and/or carcinomas of the renal pelvis, ureter, or bladder [44]. OTA is strongly suspected as the cause of BEN and its associated urinary

tract tumors [45]. Second, OTA is immunotoxic, genotoxic, and carcinogenic in many species. For example, dietary feeding of OTA induces renal adenomas and carcinomas in male mice and rats [46]. In light of these data, in 1993 the International Agency for Research on Cancer (IARC) concluded that OTA is possibly carcinogenic to humans (Group 2B). More recent data demonstrate that, in pregnant mice and rats, OTA crosses the placenta and accumulates in fetal organs where it induces DNA adducts in target tissues. Thus, OTA is a transplacental genotoxic carcinogen [47, 48].

The ochratoxin A hypothesis for testicular cancer

The carcinogen responsible for testicular cancer should meet the following five criteria: (a) a geographic distribution high in northern Europe and lower in central and southern Europe; (b) a high prevalence in Denmark; and (c) an increasing prevalence over time. Additionally, the factor should be: (d) associated with higher socioeconomic status, and (e) a cause of poor semen quality.

We suggest that OTA satisfies these criteria. First, the increased risk in northern Europe is intelligible because OTA contamination of food and feedstuffs is especially high in temperate areas of northern Europe [49]. Areas at high risk for mycotoxin contamination of grain are climatic regions with long periods of $>65\%$ humidity, rain and fog, especially during harvest time. Denmark, Sweden, the British Isles, Germany's coastal areas, and the Danube lowlands exemplify these conditions [50]. High incidences of OTA in human blood have been detected in northern Europe [51]. The highest mean value for OTA in blood, 1.8 ng/ml, was reported for Denmark (144 plasma samples, 49% positive) [35].

Secondly, any etiologic hypothesis for testicular cancer must explain the unusually high incidence of testicular cancer in Denmark (7.8 per 10^5/year) compared to other Nordic countries, especially Finland (1.3 per 10^5/year). These differences are unlikely to be due to genetic differences between Danes and other Nordic populations and do not appear to be attributable to differences in cancer registration. We hypothesize that this difference reflects higher consumption of OTA in Denmark. After cereals, pigmeat contains the highest levels of OTA, with values in pig blood, sausage and liver approaching 8 μg/kg [52]. Data from food balance sheets indicate that the consumption of pigmeat in Denmark is among the highest in the world and is more than twice that in Finland (see below). Cereals are the most important source of OTA in Denmark, and the cereal that is most often contaminated, and which contains the highest levels of OTA, is rye.* It is intriguing that the quantity of rye in the official, nationally recommended infant diets of Denmark and Finland is almost five times higher in Denmark. According to the nationally recommended diets for 9-month-old infants, the cereal protein content of the Danish infant diet contains 74% rye [53], *vs* 16% rye for the Finnish infant diet [54]. These differences in nationally recommended diets are consistent with the view that marked differences in the incidence of testicular cancer between neighboring countries reflect the influence of national, rather than individual, practices [5].

Thirdly, the increasing incidence of testicular cancer over time in developed countries is consistent with increases in the consumption of OTA-contaminated foods, *e.g.* pigmeat. Over the years 1961–1994 "meat" consumption overall in developed countries grew at an average rate of 1.9% per year. Much of this is pigmeat [55]. For example, in Sweden and Denmark, 50% and 68% of the meat consumed from 1970 to 1980 was pigmeat [56]. In France pork consumption more than doubled in the time period 1950–1985 [57]. Similar trends have been observed in other European countries. The magnitude of these increases is similar to those noted for incidence rates of testicular cancer. The reduced risk for testicular cancer for the cohorts of Danish, Norwegian and Swedish men born during World War II is also consistent with a greatly reduced supply of pork and other OTA-contaminated food during the German military occupations of Denmark and Norway [58].

Fourthly, the association of testicular cancer with individuals of higher socioeconomic status may reflect the higher pigmeat content of the diets of more affluent women and/or the association of higher social class with breastfeeding [59]. High OTA consumption by women during pregnancy and/or lactation would lead to high fetal and/or neonatal exposure to OTA *via* transfer of OTA in serum and/or breast milk [60]. Breast milk is frequently contaminated with OTA. For example, OTA was detected in 23/40 (58%) of breast milk samples in Sweden (range 10–40 ng/L) and in 38/115 (33%) of

*Most cereal consumption in Denmark is consumed in the form of rye bread. Ninety-four percent of 1671 Danish children aged 11–15 surveyed in the 1980s reported eating rye bread daily (Due P, Holstein BE, Ito H, Groth MV (1991) Diet and health behavior of Danish children aged 11–15 years. *Ugeskr Laeger*, **153**: 984–988). Estimates of daily OTA intake for adults with a high intake of rye bread (baked from conventionally grown grain during an average harvest) are 7.5 ng/kg body weight per day. OTA values for "ecologically" grown rye bread are 29.9 ng/kg body weight per day. These values readily exceed the Nordic TDI value for OTA of 5 ng/kg body weight per day (Jørgensen K, Rasmussen G, Thorup I (1996) Ochratoxin A in Danish cereals 1986–1992 and daily intake by the Danish population. *Food Add Contam* **13**: 95–104, and erratum, p. 476.)

breast milk samples in Norway (range 10–130 ng/L). Twenty-six percent of the Norwegian breast milk samples contained >40 ng/L OTA, which would cause a daily intake of OTA from milk exceeding the Nordic TDI. Conversely, no OTA was detected in any of 20 samples of infant formula (detection limit, 10 ng/L) [61, 62].

Data on breastfeeding demonstrate consistently that, in developed countries, women of higher education and social class breastfeed at higher rates and for longer periods of time than do women with lower education (see ref. 63 for a comprehensive review of factors related to breastfeeding). For example, in a study of 249 randomly selected healthy Danish infants, 79% of mothers with 12 years of education or greater were still breastfeeding at 6 months after delivery *vs* 29% of mothers with 9 years of education or less ($p < 0.001$) [64]. Differences in breastfeeding by social class could explain the epidemiologic observation that the risk of testicular cancer is more closely associated with the social class of the mother (and particularly with her level of education) than with the social class of the father [65].

Finally, the association of testicular cancer with testicular atrophy and low semen quality is understandable because OTA is a testicular toxin. Evidence in support of this statement includes the following: culturing of interstitial cells from testes of adult gerbils in the presence of OTA caused a dose-dependent inhibition of testosterone secretion [66], and intratesticular injection of 4–5 mg/kg OTA to rats caused cytolysis of the seminiferous epithelium [67]. Additionally, boars fed OTA at five and ten times the human TDI showed significant reductions of sperm motility and longevity that persisted after OTA was withdrawn [68]. Testicular function is impaired by OTA even at very low doses. For example, OTA administered at 2 ppm/day to rats by gastric intubation caused dramatic increases in testicular γ-glutamyltransferase, an enzyme associated with impaired spermatogenesis [69]. Similarly, OTA administered orally to Swiss mice at a level equivalent to the human dietary concentration of 1 μg/kg bodyweight/day caused a significant increase in defective sperm and a significant decrease in sperm count, from 36×10^4 to 16×10^4 sperm/ml [70]. Lastly, male weanling rats fed diets naturally contaminated with traces of OTA developed testicular hypoplasia of the germinal epithelium and produced no mature spermatozoa [71]. These data demonstrate that OTA exerts toxic effects on the testis during early life, and that even low doses of OTA impair sperm quality.

Tests of the ochratoxin A hypothesis

The OTA hypothesis predicts that the geographic pattern of testicular cancer should be correlated with the geographic pattern of exposure to OTA. However, because different countries have used different methodologies for measuring OTA in food and in serum, this correlation cannot be tested directly. Consequently, we used data on the per-capita consumption of food items that are known to be contaminated with OTA as a surrogate measure for exposure to OTA.

Materials and methods

We examined the Pearson product–moment correlation coefficients (r values) between the incidence of testicular cancer in 20 countries and the per-capita consumption of cereals, coffee, and pigmeat, the principal dietary sources of OTA. Weighted averages for testicular cancer incidence per country were calculated from *Cancer Incidence in Five Continents* [72]. Data on per-capita food consumption per country for 1980 for cereals and pigmeat were obtained from the United Nations *Food Balance Sheets* [73]. Data on coffee consumption for 1957–1965 were obtained from published sources [74].

Results

The Pearson r values for per-capita consumption of cereals, coffee, and pigmeat are shown in Table 1. Correlations between barley, rye, wheat, and maize were not significant. Significant correlations were observed for the per-capita consumption of coffee and pigmeat ($r = 0.49$ and 0.54, $p = 0.03$ and 0.01, respectively).

Discussion

These data have several limitations, including those common to food balance sheets and to ecologic data. For example, although food balance sheets provide an estimate of the per-capita consumption of basic food ingredients, *e.g.* rye, variability in food consumption by subgroups within the population, *e.g.* pregnant women and infants, is unknown. Furthermore, food balance

Table 1. Correlation between the incidence of testicular cancer by country and per-capita consumption of commodities potentially contaminated with ochratoxin A. The N for each comparison reflects the number of paired observations available for study.

Variables	*N*	*r*	*p*-Value
Barley	19	0.30	0.22
Rye	15	0.31	0.26
Wheat	20	−0.17	0.45
Maize	19	−0.39	0.09
Coffee	19	0.49	0.03
Pigmeat	20	0.54	0.01

sheets do not provide information about processed products that contain several basic ingredients, *e.g.* rye bread [75]. These factors may have limited our ability to detect a significant correlation between grain consumption and testicular cancer.

We used per-capita consumption of OTA-containing foods as a surrogate measure for exposure to OTA. This approach assumes that differential exposure to OTA results from *differences in the level of consumption* of OTA-contaminated foods rather than from *differences in the level of OTA contamination* of foods. This assumption is conservative and is likely to bias correlations between OTA exposure and testicular cancer toward the null. Despite this, we observed significant correlations between the per-capita consumption of coffee and pigmeat in 20 countries and the incidence of testicular cancer (Figures 1 and 2). Correlations between these commodities and testicular cancer incidence rates have not been reported previously. Although it is possible that these correlations are due to chance, the fact that these commodities were selected *a priori*, and that only a small number of statistical tests were made, suggests that they are not merely the fortuitous result of multiple comparisons.

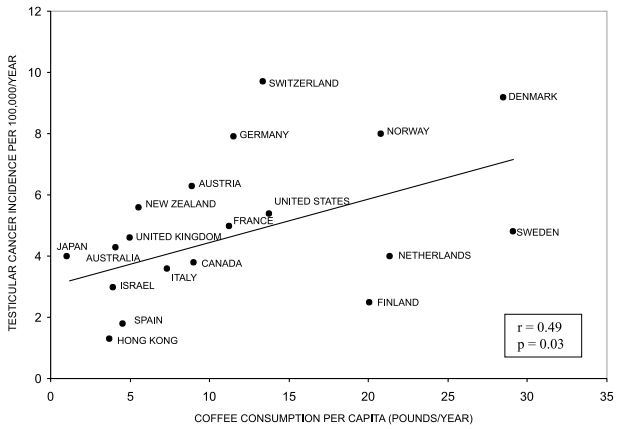

Fig. 1. Scatterplot of the incidence of testicular cancer in 19 countries *vs* the per-capita consumption of coffee.

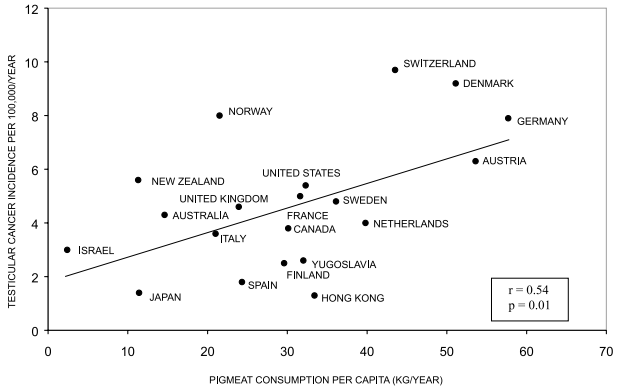

Fig. 2. Scatterplot of the incidence of testicular cancer in 20 countries *vs* the per-capita consumption of pigmeat.

Chemical analyses of the OTA content of coffees from different European countries indicate that consumption of four cups of coffee per day (approximately 24 g of roasted and ground coffee or 8 g of instant coffee) contributes an average of 19 and 10 ng OTA [76]. Coffee consumption contributes less to overall OTA exposure than do cereals or pigmeat [77]. However, coffee consumption during pregnancy and/or lactation could increase overall OTA burden in serum and/or breast milk, especially in regions where coffee consumption is traditionally high, *e.g.* Scandinavia. Temporal trends in coffee consumption, at least in the United States, show relatively steady rates of coffee consumption during the 1950s to 1980 [78]. Thus, coffee consumption does not show the temporal pattern required to explain the epidemic increase in testicular cancer. Conversely, the steady increase in pigmeat consumption in developed countries, approximately 2% per year, is consistent with reported increases in the incidence of testicular cancer (2.3% per year). In order for these changes to be important etiologically, the increases in pigmeat consumption would have to precede the increases in testicular cancer by a period of at least 15 years.

It is well known that correlations observed at the level of the group provide no information about associations at the level of individuals [79]. However, these data suggest that future analytic studies of testicular cancer at the individual level should inquire about consumption of OTA-contaminated foodstuffs, *e.g.* pigmeat and coffee, particularly during pregnancy and lactation.

Biologic plausibility of the ochratoxin A hypothesis

OTA is a known genotoxic carcinogen in animals and is a likely cause of urinary tract cancers in humans. Thus, it is conceivable that OTA may also cause other human cancers. How could OTA act as a carcinogen in testis? **We hypothesize that consumption of OTA-contaminated foods during pregnancy or childhood induces lesions in testicular DNA and that testicular growth at puberty promotes these lesions to testicular cancer.** Data in support of this hypothesis include the following:

1. *Animals exposed to OTA show OTA in testis*. Several groups have studied the tissue distribution of radioactively labeled OTA after its administration to animals. Fuchs *et al.* [80] administered ^{14}C-labeled OTA at 200 ng/g body weight into the tail vein of mice.

The mice were sacrificed at various intervals and subjected to whole-body autoradiography. *Radioactive OTA was detectable in testis within 5 min of injection*, and detectable levels persisted at 24 h [80]. Similarly, Galtier *et al.* administered a single oral or intravenous dose (2.5 mg/kg) of ^{14}C-labeled OTA to adult rats. OTA was detected in testis at every time measured, 1, 6, and 48 h. Concentrations of OTA in testis at 6 and 48 h were comparable to levels in kidney, the "classical" target organ for OTA toxicity in males [81].

2. *OTA is transferred to the fetus by transplacental transfer*. *In utero* transfer of OTA has been reported in experimental studies in mice [82], rats [83], in a sow [84], and in humans [85]. Zimmerli and Dick studied 10 pairs of maternal (venous) and umbilical cord serum (at delivery) of human mothers and infants in Switzerland [86]. OTA concentrations were twice as high in fetal as in maternal serum, suggesting active placental transport of OTA in humans.
3. *OTA is transmitted to the newborn by lactational transfer*. There is extensive transfer of OTA to infants via lactation. For example, transfer of OTA from blood to milk occurred in lactating rabbits fed a diet naturally contaminated with OTA. There was a linear relationship between the OTA content of the milk and the plasma of the sucklings [87]. Cross-fostering studies indicate that lactation is a more important source of OTA than placental transfer. Rat pups exposed to OTA via milk only had OTA levels in blood and kidney 4–5 times higher than pups exposed to OTA via placenta only. Pups accumulate high levels of OTA; by 14 days of age OTA levels in the blood and kidneys of rat pups were 4 and 6 times higher than in their dams [88].
4. *OTA causes adducts in testicular DNA*. Gharbi *et al.* [69] administered OTA by gastric intubation to adult male rats at a dose of 289 μg/kg every 48 h for up to 8 weeks. This dose corresponds to a contamination of 2 ppm/day in food. After 3 weeks of exposure, 17.5 ($\pm$1.0) ng/g OTA was detected in testis. *DNA adducts were detected in testicular DNA*. These adducts were similar chromatographically to adducts observed in the liver and kidneys after feeding OTA to rats [89]. This finding is important because OTA is an established cause of liver and kidney tumors in rodents [90, 91].
5. *DNA adducts predict later tumor development*. Several studies have shown a strong correlation between the quantity of DNA adduct formation and tumorigenicity [92, 93]. For example, the incidence of OTA-induced DNA adducts in the kidney was significantly correlated with the frequency of tumors in the kidney [94]. This suggests that DNA adducts in the testis may be precursors of tumors in the testis.

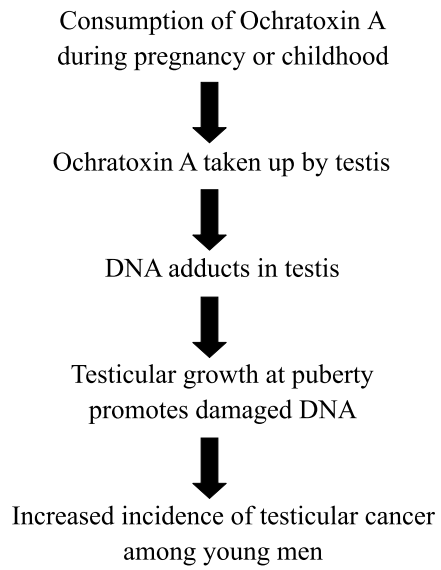

Fig. 3. Proposed biologic model of the ochratoxin A hypothesis for testicular cancer.

The OTA hypothesis for testicular cancer is summarized schematically in Figure 3.

Relationships of OTA to the age distribution of testicular cancer, to cryptorchidism, and to infertility

We have suggested that OTA satisfies many of the epidemiologic and biologic requirements of a testicular carcinogen. The OTA hypothesis also raises several questions, *e.g.*, concerning the unusual age distribution of testicular cancer, and the possible roles of OTA in the etiologies of carcinoma *in situ* of the testis, in cryptorchidism, and in male infertility.

It is generally accepted that the precursor lesions to most testicular germ cell tumors are atypical, intratubular germ cells called carcinoma *in situ* (CIS) of the testis (also termed "intratubular germ-cell neoplasia") [95]. The etiology of CIS is unknown. We propose that OTA induces lesions in the DNA of testicular germ cells and causes the malignant transformation of some of these cells into CIS cells.

The OTA hypothesis can explain the rarity of testicular cancer at birth (since CIS cells must undergo promotion in order to become tumors) and the peak incidence of testicular cancer in young adulthood. However, since dietary exposure to OTA may be

lifelong, why are testicular cancers rare during middle and old age? There are at least two possible explanations. First, susceptibility of the testis to OTA-induced damage may be greater during early life. In many rodent species the risk of cancer increases if exposure to a carcinogen occurs *in utero* or in infancy rather than in adult life [96]. Alternately, although OTA may induce DNA lesions in adult testis, lesions that occur in adulthood will not be subjected to the promotional influence of puberty and thus will not be promoted to invasive cancer.

Because cryptorchidism is the only known cause of testicular cancer, the question arises whether cryptorchidism is also caused by OTA. Cortes *et al*. have shown that cryptorchidism in humans is associated with malformations and dysplasias of the kidneys, ureters, and T10 to S5 of the spine [97]. They propose that cryptorchidism is part of a syndrome of defects of the caudal embryonic axis termed "caudal dysgenesis" (also known as caudal dysplasia) [98, 99]. It is noteworthy that OTA can cause caudal dysgenesis in an avian model [100]. This suggests that the association between cryptorchidism and testicular cancer may occur because OTA causes both conditions. Conversely, epidemiologic data argue against a shared cause between cryptorchidism and testicular cancer. For example, although testicular cancer is associated with higher socioeconomic status, cryptorchidism shows the reverse pattern [65]. Additionally, whereas rates for testicular cancer have increased markedly over time and show strong geographic determinants, incidence rates for cryptorchidism have not increased consistently and do not vary greatly geographically [101]. This suggests that OTA is unlikely to be an important cause of cryptorchidism. However, there may be disease heterogeneity within cryptorchidism. Thus, some cases of cryptorchidism could be caused by OTA. Finally, the correlation between the increasing incidence of testicular cancer and declining male fertility worldwide [10] suggests that OTA may contribute to declining male fertility.

Opportunities for future study

The OTA hypothesis makes numerous predictions about place and person. For example, although the precise role of OTA in the etiology of BEN is unclear, high levels of OTA in grain, pork, and in human blood and milk have been found in the Balkans [102]. This implies that an increase in the rate of testicular cancer could occur in young men who were exposed to OTA *in utero* or in infancy. Recent evidence of an epidemic of testicular cancer in Slovenia (Yugoslavia) may be relevant to this prediction [15]. Because people who abstain from pork should have decreased risks of testicular cancer, the OTA hypothesis predicts that the incidence of testicular cancer should be lower among observant Jews and Muslims and among Seventh-day Adventists. There are few published data on the risk of testicular cancer among members of these religious groups.

OTA levels in human serum have a half-life of approximately 36 days [103]. Thus, serum OTA levels could be useful in prospective studies of testicular cancer, but they would not be useful in case–control studies [104]. Rather, case–control studies would require questionnaire data on dietary consumption patterns. The OTA hypothesis predicts that cases who developed testicular cancer would have had diets higher in OTA than controls without testicular cancer. Because breakfast cereals (*e.g*. cornflakes) may be a significant source of OTA, childhood diets should be examined for these products [105]. Additionally, women whose sons developed testicular cancer may have had a higher intake of OTA-containing foods, such as pigmeat, coffee, and grain products during pregnancy and breastfeeding than women whose sons did not develop testicular cancer. Breast milk can contribute heavily to OTA levels in infants and should increase the risk for testicular cancer. Conversely, the use of infant formula should be protective. Additionally, cow's milk may pose a risk for children who consume large quantities of cow's milk [106]. A recent case–control study in East Anglia, UK, found a significantly increased risk for testicular cancer for men who consumed high quantities of milk during adolescence [107]. This finding was not confirmed in a study conducted in Texas, USA [108]. However, the level of OTA in cow's milk would be expected to be higher in East Anglia than in Texas.

If analytic studies confirm an association between OTA and testicular cancer, public health efforts should seek to reduce OTA exposure. Moreover, it may be possible to reduce the genotoxicity of OTA exposure [109]. For example, the quantity of DNA adducts that are induced by OTA in animals can be reduced dramatically by pretreatment of the animals with aspirin and indomethacin. Both drugs inhibit prostaglandin H synthase, a step in the metabolism of OTA to DNA reactive metabolites [110]. Vitamins A, C, and E, which are superoxide anion scavengers, can reduce DNA adduct levels in mouse kidney by as much as 90% [111]. Similarly, the artificial sweetener, aspartame, is a structural analog of OTA and is a potent OTA antagonist [112]. These observations suggest that testicular cancer might be prevented by vitamin and/or aspartame supplements, much as neural tube defects can be prevented by supplements of folic acid [113].

In summary, we propose that exposure to OTA-contaminated food provides a coherent explanation for much of the descriptive epidemiology of testicular cancer. OTA induces adducts in testicular DNA and is a known genotoxic carcinogen in animals. Thus, OTA is a biologically plausible cause of testicular cancer. Future epidemiologic studies of testicular cancer should focus on breastfeeding practices and the consumption of OTA-containing foods such as cereals, pork products, milk, and coffee by mothers and their male children.

Acknowledgements

We thank Abe Sahmoun, PhD, for help in the preparation of this article, and Jim Schlesselman, PhD, for his thoughtful review. Henrik Møller, MD.Sc., provided valuable discussion on the *in press* version of the MS. The Department of Interlibrary loan at Wake Forest University School of Medicine assisted in acquiring many of the publications on OTA.

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