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Specialist Commentary

Chemopreventive effect of oltipraz on AFB_1-induced hepatocarcinogenesis in tree shrew model

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INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the major cancers in the world with a mortality of more than 250 000 cases yearly. More than 137 000 cases of HCC were diagnosed each year in China, which acount approximately for more than 40 percent of the total number in the world. HCC has become the second major cause of death for cancer in China since 1990, and its annual mortality is expected to be 21.2 cases per 100 000 population in the year 2000. Even though progresses have been achieved for HCC diagnosis and treat ment, its 5-year mortality is still higher than 95 percent[1-3].

The prevalence of HCC is quite different among different areas around the world[4,5]. It is considerably high in South-East Asia and sub-Saharan Africa, particularly in some southern and eastern regions inside China such as Fusui County in Guangxi Zhuang Autonomous Region and Qidong City in Jiangsu Province[6-9]. The standardized incidence of HCC in these high-risk regions may exceed 100 cases per 100000 of population[10]. The obvious difference in geographic distribution of HCC indicates that there must be environmental factors for its pathogenesis.

Aflatoxin B_1 (AFB_1), which is produced by some strains of *Aspergillus flavus*, is a potent hepatotoxigen and hepatocarcinogen[11,12], and is considered as a major cause of HCC in some regions[13-18]. It has also been postulated that chronic infection with hepatitis B virus (HBV) in combination with exposure to AFB_1 in the diet may contribute to the extraordinary high risk of human HCC in some areas. Actually, two case-control studies in Shanghai have demo nstrated a strong interaction between HBV and AFB_1 for risk of HCC[14,15]. A similar chemical-viral interaction has been observed in Taiwan[18-20]. The synergism between virus and mycotoxic carcinogen for the development of human HCC suggests that reduction in both risk factors may bring important public health consequences.

The concept of chemoprevention of cancer is over 40 years old and a number of works have been done in this field[21,22]. Looking for effective and safe reagents against AFB_1 and/or other HCC related risk factors is one of the most important chemoprote ctive strategies for HCC[23-26]. Green tea was identified years ago as one of the effective chemopreventive reagents against HCC through a series of animal experiments as well as a clinical trial[27,28]. Recently oltipraz, another preventive agent which was previously described as a potent inhibitor of AFB_1 induced hepatocarcinogenesis in rat[29-33], has been shown to inhibit the bioactivation of aflatoxin and enhance its detoxification in a clinical trial[34-37] as well as in human hepatocytes in primary culture[38]. Meanwhile, a universal vaccination program against HBV that started a decade ago now results in lower rates of HCC in children[39]. An experimental model to test the synergistic effect of these two agents and their prevention, therefore, is needed.

RESEARCH ON TREE SHREW MODEL OF HEPATOCARCINOGENESIS

Tree shrew (*Tupaia spp.*) is a kind of small, squirrel-like mammals. Formerly it was considered to belong to the Primate order; currently it is classified into a separate order Scandentia and is supposed to be more closely related to human being than rodents[40,41]. They have been used in biomedical researches since as early as the 1960s. Many researches have been done on its visual and nervous systems. In 1976, however, Reddy *et al*[42] successfully induced liver cancer in tree shrew by AFB_1. Yan *et al*[43,44] reported that tree shrews can be infected with HBV and they successfully used this HBV-infected tree shrew model for liver cancer

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research. Recently Walter *et al*[45] reported their *in vivo* and *in vitro* study results from tree shrews infected with HBV. Yan and Li reported a significantly higher incidence of HCC in tree shrews both infected with human HBV and exposed to AFB_1 than with either agent alone[46,47]. Thus, this tree shrew model appears to closely mirror the most common causative factors of human HCC in some prevalent regions. Furthermore, with the exception of the chimpanzee, tree shrew is the only known animal that can be infected with human HBV. Therefore, the application of tree shrew in research related to liver cancer and hepatitis is receiving increasing attentions and a number of works have been published[48-52].

Because of the difficulties in raising tree shrews artificially, most of the tree shrews used so far for research in China are captured individually from Yunnan Province. The drawback of using tree shrews captured in the wild for animal experiments is that their age, health status and reproductive history are unknown. In an attempt to avoid this drawback, we have conducted a preliminary experiment on rearing tree shrews and a promising result was obtained[53].

RESEARCH ON THE PREVENTIVE EFFECT OF OLTIPRAZ IN TREE SHREW

In order to study the preventive effect of oltipraz on AFB_1 by animal models other than rodents, a short-term experiment was conducted on tree shrews.

Male and female adult tree shrews (*Tupaia belangeri chinensis*) were purchased from the Kunming Institute of Zoology (Yunnan Province, P.R. China). Their body weights ranged from 100g to 160g. Upon arrival, 1mL blood was collected from each animal and tested for HBV markers (HBsAg, anti-HBsAg, anti-HBcAg) and ALT. The tree shrews that were negative for these markers of HBV infection and that had ALT value below 55 units were divided into 4 groups with 6 or 7 animals for each group. Group A: normal control; group B: AFB_1 alone; group C: AFB_1 + oltipraz daily; group D: AFB_1+oltipraz weekly. All the tree shrews were allowed 1 week to acclimatize to the facilities prior to the experiment. They were housed in separate, suspended, stainless steel wire cages under controlled environmental conditions with a 12-hour light/dark photop eriod. They had free access to tap water and a natural ingredient diet.

The experimental design is presented schematically in Figure 1. Tree shre ws in groups B, C and D were given AFB_1 (400μg/kg b.w./day in liquid milk) daily beginning at one week after the experiment started and continued for 4 weeks. One week before giving AFB_1, tree shrews in group C and D were respectively given oltipraz (0.5mmol/kg b.w.) daily or weekly, by gavage in a saturated solution of sucrose for 5 weeks. Blood samples and 24-hour urine samples were collected once a week from each animal throughout the experiment. At the termination of the 9-week experiment, tree shrews were killed by cervical dislocation. Three blocks of liver tissue were taken from each animal. Serial sections from each block were stained histochemically for r-glutamyl transpeptidase (γ-GT)[54] and HE respectively. The γ-GT positive liver cells were counted with a nest-ruler under microscope. The results were analyzed by the medical statistics analyzing software PEMS that was designed by West China University of Medical Sciences. The levels of aflatoxin-albumin adducts in serum samples were determined by radioimmune assay[35] and the levels of Aflatoxin-N^7-guanine adducts in urine samples were assayed by HPLC[29].

No γ-GT positive liver cell focus, a postulated precancerous marker[54-56] was observed in any liver of the variously treated tree shrews in our study. However, different numbers of γ-GT positive liver cells, which scattered mainly around the portal spaces, were observed in each group. Even though the distribution patterns of these cells were similar among the 4 groups, the number was quite different. Groups B and D had obviously less γ-GT positive cells than groups A and C (Table 1).

γ-GT normally exists in embryonic liver cell in human being and rat. In adult rat, it exists only in some cells around portal spaces[57] but can be re-expressed by mature hepatocytes during the recovery process after liver damage[58]. In this study a number of γ-GT positive hepatocytes presented in periportal regions in the normal control group. On the contrary, the number of γ-GT positive hepatocytes of the same sites was markedly reduced in the AFB_1 treated B group. This phenomenon is fairly consistent with the findings on AFB_1 induced damage in rat, in which the periportal hepatocytes are the major targets of AFB_1. As shown in the same table, the number of γ-GT positive hepatocytes in group C was strikingly similar to the normal control group. This result indicates strongly the preven tive action of oltipraz against AFB_1 toxicity. The apparent ineffectiveness of oltipraz in group D is most possibly due to its inadequate dose[59]. These results might indicate that oltipraz has the preventive was dose-related effect on AFB_1.

Insufficient duration and/or insufficient dosage of AFB_1 treatment may result in that no separate focus of γ-GT positive liv er cell formed at the end of this 9-week experiment[60]. However, the decrease of γ-GT positive hepatocytes in the periportal regions may be an early marker for the damage induced by AFB_1.

The levels of both aflatoxin-albumin adducts in serum samples and aflatoxin-N^7-guanine adducts in urine samples of the tree shrews were also significantly affected by oltipraz. Following daily

exposures to AFB_1, the levels of serum aflatoxin-albumin adducts in group B increased rapidly over 2 weeks to reach a plateau that did not diminish until cessation of AFB_1 exposure. In group C however, oltipraz attenuated the aflatoxin-albumin adducts significantly ($P < 0.05$) with a median reduction of 80%. The mean levels of aflatoxin-N^7-guanine (ng/mg creatinine) in the urine samples collected at week 5 were 6.34 ± 2.04 and 0.47 ± 0.13 in groups B and C respectively. This 93% decrease represented a statistically significant difference ($P<0.05$). These results were reported in detail in another article[61]. The major mechanism of oltipraz's chemopreventive effect is probably through inducing the activities of cytochrome P450 system and phase 2 en zymes such as glutathione transferases, epoxide hydrolase, etc, as reported by Langouet *et al*[38]and Fahey *et al*[62].

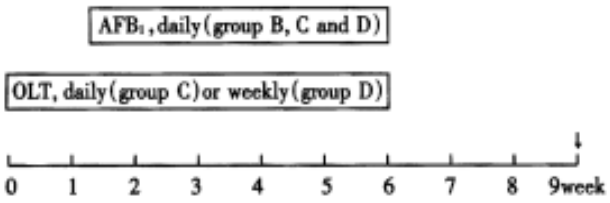

Figure 1 Experimental design for animal treatment.
OLT: oltipraz; ↓: all the animals were sacrificed.

Table 1 The number and size of γ-GT positive hepatocyte-group ($\bar{x} \pm s_{\bar{x}}$)

Group	Treatment	No./cm²	mm²/cm²	mm²/No.
A	Normal control	167.29±50.47	2.90±1.01	0.017±0.002
B	AFB_1	71.92±42.19[a]	1.14±0.69[a]	0.015±0.002
C	AFB_1+OLT daily	167.10±45.94[c,d]	2.73±0.87[c,d]	0.016±0.001
D	AFB_1+OLT weekly	83.66±34.94[a]	1.33±0.86[a]	0.015±0.002[b]

t test: [a]$P < 0.05$, [b]$P < 0.01$, *vs* group A; [c]$P < 0.01$, *vs* group B; [d]$P<0.01$, *vs* group
D. OLT: oltipraz;
No./cm²: the number of γ-GT positive hepatocyte-group per cm² of liver tissue;
mm²/cm²: mm² of γ-GT positive hepatocyte-group per cm² of liver tissue;
mm²/No.: mm² of γ-GT positive hepatocyte-group per each one.

SUMMARY

Tree shrew is phylogenetically more closely related to human being than ro dents. It is susceptible both to HBV infection and AFB_1 intoxication. It is a suitable experimental animal for hepatocarcinogenesis. Attempt for its rearing is promising.

Oltipraz is an effective reagent to protect AFB_1 intoxication. This effect is proved clearly not only by histological examination, but also by reduction of aflatoxin-albumin adducts in serum and aflatoxin-N^7 guanine adducts in urine.

All of these studies mentioned above provide a foundation for further HCC chemop revention study by using tree shrews in the future.

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