Inhibition of nicotine-DNA adduct formation by polyphenolic compounds in vitro

CHENG Yan, WANG Hai-Fang, SUN Hong-Fang, LI Hong-Li

(Department of Chemical Biology, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871)

Abstract Nicotine [3-(1-methyl-2-pyrrolidinyl)-pyridine], a major alkaloid in tobacco products, has proven to be a potential genotoxic compound. Some polyphenolic compounds can suppress the DNA adduction, and hence act as the potential inhibitors of carcinogenesis. In this study, the inhibitory effects of three polyphenolic compounds, curcumin (diferuloylmethane), resveratrol (trans-3, 5, 4′-trihydroxystilbene) and tea polyphenols, on the nicotine-DNA adduction have been investigated in vitro using radiolabelled nicotine and liquid scintillation counting (LSC) technique. Also, the inhibition mechanism of these chemopreventive agents in regard to the activity of the biotransformation enzymes, including cytochrome P450 (CYP450), cytochrome b_5 (CYb_5) and glutathione S-transferase (GST), has been studied. The results demonstrated that these three polyphenols induced marked dose-dependent decrease in nicotine-DNA adducts as compared with the controls. The elimination rate of adducts reached above 46% at the highest dose for all the three agents with 51.6% for resveratrol. Correspondingly, three polyphenols all suppressed CYP450 and CYb_5, whereas curcumin and resveratrol induced GST. We may arrive at a point that the three polyphenols are beneficial to prevent the nicotine adduct formation, and thus may be used to block the potential carcinogenesis induced by nicotine.

Keywords Nicotine, DNA adducts, Liquid scintillation counting, Inhibition, Polyphenols, Biotransformation enzymes

CLC numbers O657.4, R818.03

1 Introduction

Nicotine [3-(1-methyl-2-pyrrolidinyl)-pyridine], an active alkaloid in tobacco, is generally accepted to be a dominant factor for tobacco addiction. It was implied that nicotine in large dose could increase mutation frequency and sister chromatid exchange (SCE) in cellular experiments.[1] Since 1993, our group has found in accelerator mass spectrometry (AMS) studies that nicotine could form adducts with liver DNA, lung DNA, histone H1/H3, hemoglobin and albumin respectively in mice with a positive dose-dependent relationship.[2-4] We have therefore concluded that nicotine is a potential carcinogen per se. In 2000, Turteltaub reported that nicotine-DNA/protein adducts in sperm of adult smokers and placenta and umbilical cords of newborn children of female smokers were detected by AMS.[5] A non-linear dose response of nicotine-protein binding resulted.

Generally, most chemical carcinogens and mutagens can attack the nucleophilic sites in nucleic acids to form covalent adducts.[6] The formation of DNA adduct is considered an initial and critical step in chemical carcinogenesis.[7] Therefore, the inhibition of DNA adduct formation may play an important role in cancer chemoprevention, which is aimed at inhibiting, suppressing or reversing the onset of carcinogenesis at a premalignant stage.[8] Natural polyphenolic compounds were one group of the effective chemopreventive agents.[9]

We have studied the inhibitory effects of some dietary constituents on the nicotine-DNA adducts in vivo using AMS, and all of the agents studied showed a dose-dependent inhibitory effect on DNA adduction.[10] In this article, an in vitro system was employed to study the inhibitory efficacy of three poly-

Supported by the National Natural Science Foundation of China (Grant No. 19935020).

Received date: 2003-08-25

phenols at three different doses on nicotine induced DNA adduction using liquid scintillation counting (LSC) technique. The three polyphenol agents are curcumin (diferuloylmethane), resveratrol (trans-3, 5, 4′-trihydroxystilbene) and tea polyphenols, all of which are dietary constituents and have previously proven to be chemopreventives against carcinogenesis in different organ targets induced by various environmental toxicants.[11-13] The detoxification activities of these chemopreventive agents have probably resulted from the reduction of phase I enzymes (e.g., cytochrome P450 (CYP450) and cytochrome b_5 (CYb_5)) and/or the induction of phase II enzymes (e.g., glutathione S-transferase (GST)).[14, 15]

2 Experimental

2.1 Materials

L-[N-$^{14}CH_3$]-nicotine (2.035×10^9 Bq/mmol) was purchased from American Radiolabelled Chemical Inc.. $NADP^+$, glucose-6-phosphate dehydrogenase, and D-glucose-6-phosphate disodium salt dihydrate from Sigma Chemical Co.. Tea polyphenols (98%) were extracted following the method described by Agarwal *et al*.[16] All other chemicals were of analytical grade or the highest grade available.

2.2 In vitro experiments

Phenobarbital induced S_9 components were prepared as described before.[17] Curcumin, resveratrol, and tea polyphenols (10, 100 and 200 μg/mL for each, except for the controls without adding polyphenols) were preincubated respectively at room temperature (20 °C) for 20 min in 2.5 mL Tris-HCl buffer (10 mmol/L, pH 7.4) consisting of calf thymus DNA (1.0 mg/mL), S_9 components (3.0 mg/mL), $MgCl_2$ (2.0 mmol/L), $NADP^+$ (1.0 mmol/L), glucose-6-phosphate dehydrogenase (2 Units/mL), and Na_2 glucose-6-phosphate (5.0 mmol/L). Then nicotine (1.0 mmol/L, 3.7×10^6 Bq/mmol) was added to the above mixture, and incubation continued for 1 h at 37 °C. The reaction was terminated by adding saturated sodium dodecyl solution. DNA was isolated and purified as reported by Gupta.[18] The isolated DNA was dissolved in Tris-HCl buffer and the DNA concentration was determined by UV-spectrophotometer (UV-240, Shimadzu).

2.3 Measurement of radioactivity

1.0 mL of DNA solution was added to a glass scintillation vial and incubated at 70 °C with perchloric acid (40 μL) for 30 min. After cooling 10 mL liquid scintillation cocktail (Ultima Gold^{TM} LLT, Packard BioScience B.V.) was added to the vial for ^{14}C measurement. The samples were measured by a low background Liquid Scintillation Counter (LSC, Tri-Carb 2750 TR/LL Packard Co.). The radioactivity in decay per minute (DPM) was converted to adducts/10^6 nucleotides.

2.4 Assay of biotransformation enzyme activity

The microsomes and the cytosol were prepared from the above reaction mixture prior to addition of nicotine. The activities of CYP450 and CYb_5 were measured through the difference spectra by the method described by Omura and Sato,[19] and the activities of GST were measured according to the method of Habig *et al*,[20] using 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate.

3 Results

3.1 Inhibitory effects on nicotine-DNA adduct formation

Fig.1 shows the inhibitory effects of three polyphenols on the in vitro nicotine-DNA adduct formation. Nicotine induced remarkable DNA adducts (286±13 adducts/10^6 nucleotides) in vitro. The pretreatment with these polyphenols remarkably reduced the adducts level in a dose-dependent manner as compared with the control, with the elimination rate reaching above 46% at the highest dose of the three agents.

Among these chemopreventive agents, resveratrol demonstrated the strongest inhibitory effect, reaching 51.6% at the dose of 200 μg/mL.

3.2 Effects on biotransformation enzymes

Fig.2 and Fig.3 show the effects of three polyphenols on CYP450, CYb_5 and GST activities, respectively. All the three polyphenolic compounds suppressed CYP450 and CYb_5, whereas curcumin and

resveratrol increased GST in a dose-dependent manner.

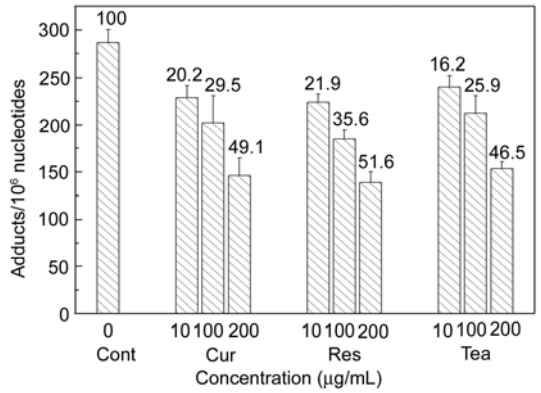

Fig.1 Inhibitory effects of curcumin (Cur), resveratrol (Res) and tea polyphenols (Tea) on nicotine-DNA adduct formation in vitro (Cont: the control containing nicotine and DNA without adding inhibitors). Values represent mean ± SD (n=4). The values at the top of each column indicate the inhibitory effects (% of elimination) compared with the control (as 100%). Inhibitory effect (% of elimination) =[(adducts of control - adducts with inhibitor) / adducts of control] ×100%. Bars of three different concentrations of each agent are all significantly different from the control with $p < 0.05$ using a one-way analysis of variance (ANOVA) followed by *t*-test.

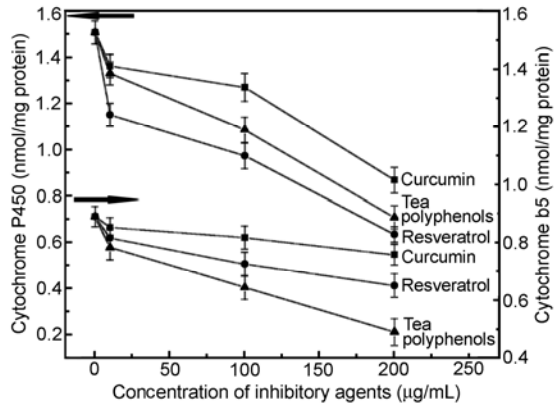

Fig.2 Suppression of CYP450 and CYb_5 by curcumin, resveratrol and tea polyphenols in vitro versus concentration of inhibitory agents. Values represent mean ± SD (*n*=4).

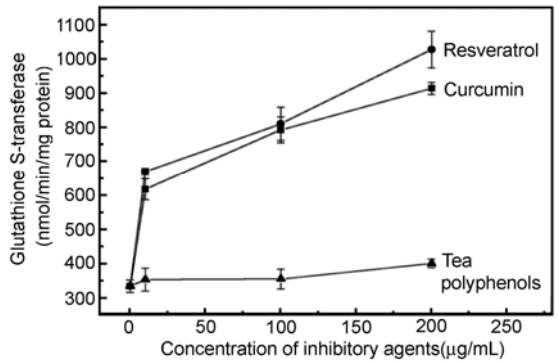

Fig.3 Enhancement of GST by curcumin, resveratrol and tea polyphenols in vitro versus concentration of inhibitory agents. Values represent mean ± SD (*n*=4).

Resveratrol showed the strongest effect in reducing CYP450, whereas curcumin was the weakest inhibitor (Fig.2). Tea polyphenols was more effective than resveratrol, and resveratrol more than curcumin, in suppressing CYb_5 (Fig.2). However, tea polyphenols led to little increase of GST, and resveratrol increased GST more effectively than curcumin (Fig.3).

4 Discussion

In the present paper, the efficacy of three polyphenols in suppressing the nicotine-DNA adduct formation has been investigated in vitro using LSC technique. The three agents exhibited a similar concentration-dependent inhibition profile. The results indicated that these agents were all effective inhibitors for the in vitro nicotine-DNA adduction, and thus might indirectly inhibit the potential carcinogenesis induced by nicotine.

Resveratrol is a polyphenolic phytoalexin in grapes, peanuts and other plants, and possesses a broad spectrum of biological, pharmacological and therapeutic activities,[21] particularly against cancer.[22] Curcumin, a yellow ingredient isolated from turmeric, has been found to possess anticarcinogenic properties.[11] Of the tea polyphenols, the main components, (+)-catechin, (-)-epicatechin, (+)-gallocatechin, (-)-epigallocatechin, (-)-epicatechin gallate, (-)-gallocatechin gallate, (-)-epigallocatechin gallate etc.,[12] are considered responsible for the chemopreventive function. Compared with the control, the inhibition ability of the three polyphenols at three concentrations was: resveratrol>curcumin>tea polyphenols.

In our previous study of the inhibitory effects of some dietary constituents on the nicotine-DNA adducts in vivo,[10] we have found that curcumin and tea polyphenols also inhibited nicotine-DNA adduct formation in a dose-dependent manner like that in vitro. The inhibitory effect in vivo was larger than that in vitro for these two polyphenols.

One important mechanism for the protective effects of many types of chemopreventive agents is the modulation of carcinogen metabolism.[9] Nicotine requires metabolic conversion to DNA reactive intermediates, which is catalyzed by phase I cytochrome system.[23] Inhibition of cytochrome isozymes will effectively block the conversion of procarcinogens

into the ultimate carcinogens, thus inhibit the initiation of carcinogenesis.[14] All three agents can reduce CYP450 and CYb_5 (Fig.2) to block the metabolism of nicotine and decrease the DNA binding with the reactive metabolites, and hence indirectly detoxify the potential nicotine induced carcinogenesis.

The induction of phase II enzymes, such as GST, is another important detoxification pathway.[14, 15] GST detoxifies the carcinogens by reacting with the potential carcinogenic electrophile intermediates with the -SH group of glutathione to prompt the products excreted easily.[20] Thus, GST inducers are considered potential inhibitors of carcinogenesis. Curcumin and resveratrol can enhance GST (Fig.3) to suppress the metabolism of nicotine and decrease the DNA binding with the reactive metabolites, and hence indirectly detoxify the possible nicotine induced carcinogenesis.

Except for the inhibition of bioactivation of nicotine, inhibitor's direct binding to the electrophilic intermediates of nicotine can also decrease the chance of its binding to DNA, thus probably detoxify the possible nicotine induced carcinogenesis.

In conclusion, our findings suggest that curcumin, resveratrol and tea polyphenols were all effective chemopreventives in inhibiting of the metabolic activation of nicotine to form DNA adducts in vitro, and might indirectly block the events associated with the smoking specific carcinogenesis via suppression of phase I enzymes (CYP450 and CYb_5) and induction of phase II enzyme (GST).

Acknowledgement

We are indebted to Prof. Liu Yuanfang for his guidance.

References

1 Trivedi A H, Dave B J, Adhvaryu S G. Cancer Lett, 1990, **54**(1-2): 89-94

2 Li X S, Wang H F, Shi J Y *et al.* Radiocarbon, 1996, **38**(2): 347-353

3 Wu X H, Wang H F, Liu Y F *et al.* Radiocarbon, 1997, **39**(2): 293-297

4 Wang Y, Wang H F, Sun H F *et al.* Chem J Chinese U (in Chinese), 2002, **23**: 809-812

5 Turteltaub K. Tissue distribution and macromolecular binding of ^{14}C-nicotine at doses equivalent to passive smoke in mice, Conference: International society for the study of xenobiotic metabolism-accelerator mass spectrometry, Indianapolis, Indiana, USA, 2000

6 Wogan G N, Gorelick N J. Environ Health Perspect, 1985, **62**: 5-18

7 Warren A J, Shields P G. P Soc Exp Biol Med, 1997, **216**(2): 172-180

8 Sporn M B. Cancer Res, 1976, **36**(7): 2699-2702

9 Surh Y J. Muta Res —Fund Mol M, 1999, **428**(1-2): 305-327

10 Cheng Y, Li H L, Wang H F *et al.* Food Chem Toxicol, 2003, **41**(7): 1045-1050

11 Singletary K, MacDonald C, Wallig M *et al.* Cancer Lett, 1996, **103**(2): 137-141

12 Inagake M, Yamane T, Kitao Y *et al.* Jpn J Cancer Res, 1995, **86**(11): 1106-1111

13 Joshi S S, Kuszynski C A, Bagchi M *et al.* Toxicology, 2000, **155**(1-3): 83-90

14 Offord E A, Macé K, Avanti O *et al.* Cancer Lett, 1997, **114**(1-2): 275-281

15 Smith W A, Gupta R C. Carcinogenesis, 1996, **17**(6): 1285-1290

16 Agarwal R, Katiyar S K, Zaidi S I A *et al.* Cancer Res, 1992, **52**(13): 3582-3588

17 Bartsch H, Malaveille C, Barbin A *et al.* Arch Toxicol, 1979, **41**: 249-277

18 Gupta R C. Proc Natl Acad Sci USA, 1984, **81**: 6943-6947

19 Omura T, Sato R. J Biol Chem, 1964, **239**(7): 2370-2378

20 Habig W H, Pabst M J, Jakoby W B. J Biol Chem, 1974, **249**(22): 7130-7139

21 Lin J K, Tsai S H. Proc Natl Sci Counc ROC(B), 1999, **23**(3): 99-106

22 Savouret J F, Quesne M. Biomed Pharmacother, 2002, **56**(2): 84-87

23 Oscarson M. Drug Metab Dispos, 2001, **29**(2): 91-95