

# 绿茶及几种茶酚的体外抗致突变和 抗脂质过氧化作用

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## 内容提要

本文以鼠伤寒沙门氏菌致突变试验研究了安徽歙县一级绿茶的抗致突变作用。绿茶水浸液可抑制甲基脲和亚硝酸钠的反应, 阻断亚硝胺类致癌物的生成, 并抑制已生成的亚硝基甲基脲的致突变性; 可抑制苯并(a)芘及黄曲霉毒素B<sub>1</sub>经大鼠肝S9活化的致突变性; 显著地抑制过氧化羟基异丙苯及四氯化碳诱变的大鼠肝微粒体脂质过氧化作用。本文还报告了D-儿茶素等茶酚对亚硝化反应、致突作用及脂质过氧化的影响。

目前, 癌症的化学预防已成为癌症研究的一个重要方向<sup>[1]</sup>, 已经发现了化学类别不同的60余种防癌物, 按作用机理可分为防止前体生成致癌物的化合物、阻断剂、阻遏剂和/或抗促进剂。在这些防癌物中, 仅有少数几种可试用于人群。我们正致力于开发天然存在的抗致突变物和抗致癌物, 本文报告了我国歙县绿茶的体外抗致突变作用和抗脂质过氧化作用。

## 材料和方法

1. 主要试剂 <sup>3</sup>H-苯并(a)芘(47.0 ci/mmol, New England Nuclear), 黄曲霉毒素B<sub>1</sub>和鞣花酸(Aldrich Chemical Co.), 甲基脲(Fluka AG), NADPH和橙皮素(Sigma Chemical Co), 过氧化羟基异丙苯(上海白鹤化工厂), 硫代巴比妥酸、芦丁、绿原酸、没食子酸、亚硝酸钠、四氯化碳(北京化工厂)、D-儿茶素(ICN Pharmaceutical Inc.)。其它试剂均为分析纯级。

2. 绿茶水浸出液的制备: 取绿茶(Camellia Sinensis O. Ktze, 安徽歙县一级绿茶) 2g, 加沸水20ml, 于沸水浴提取2min, 过滤后即为绿茶10%水浸出液。

3. 鼠伤寒沙门氏菌回变试验, 菌种TA98和TA100由Ames教授提供, 菌种鉴定合格, 肝S9由Aroclor 1254诱导的大鼠制备, 以标准平皿掺入法<sup>[2]</sup>测试受试物对甲基脲的亚硝化作用及对苯并(a)芘和黄曲霉毒素B<sub>1</sub>致突变作用的影响。甲基脲的亚硝化作用根据Stich法<sup>[3]</sup>改进, 反应混合物体积为2.0ml, 含0.068M柠檬酸/0.064M磷酸氢二钠缓冲液(pH3.5)、甲基脲25mM及亚硝酸钠100mM, 于室温下反应30min, 加入7.5%碳酸氢钠0.7ml及10×PBS(pH7.4) 0.3ml, 混匀后取0.5ml/皿的剂量以TA100菌株进行致突变试验。

4. 苯并(a)芘羟化酶活性测定: 按Van Contfort等法<sup>[4]</sup>以<sup>3</sup>H-苯并(a)芘为底物测定受试物对3-甲基胆蒽诱导的大鼠肝微粒体苯并(a)芘羟化酶活性的影响, 苯并(a)

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芘代谢产物的放射活性以液闪法(国产FJ2100型液闪仪)测定。

5. 过氧化羟基异丙苯(CumOOH)脂质过氧化作用: 反应混合液终体积1.0ml, 含50mM Tris-HCl缓冲液(pH7.4)、大鼠肝微粒体约1mg蛋白质及0.1mM CumOOH, 于37℃保温20min, 以硫代巴比妥酸法<sup>[5]</sup>显色, 定量丙二醛的生成。

6. 四氯化碳脂质过氧化作用: 反应混合液终体积1.0ml, 含50mM Tris-HCl缓冲液(pH7.4)、大鼠肝微粒体约1mg蛋白质及0.4μmol NADPH, 加入40%四氯化碳乙醇溶液, 于37℃反应20min, 以硫代巴比妥酸法<sup>[5]</sup>显色, 定量丙二醛的生成。

7. 以牛血清白蛋白作标准, Folin-酚法测定蛋白质浓度。上述酶学和化学测定都作双份测定, 变异系数不超过15%。

### 结 果

1. 绿茶水浸出液及有关多酚类的抗致突变作用

10%绿茶水浸出液剂量达100μl/皿(相当于10mg绿茶/皿)对TA98和TA100菌株均未发现直接或间接(经S9活化)的致突变性。

在甲基脲亚硝化反应之前和之后加入绿茶水浸出液均可抑制甲基脲亚硝化产物对TA100菌株的致突变性, 而且反应前加入绿茶水浸液的抑制作用(EC<sub>50</sub>为7mg绿茶/ml)强于反应后加入的抑制作用(图1)。可见绿茶水浸出液具有抑制甲基脲亚硝化反应及灭活已生成的亚硝基甲基脲致突变性的双重作用。D-儿茶素、芦丁、橙皮素、绿原酸、没食子酸和鞣花酸均有抑制亚硝化反应的作用。儿茶素、橙皮素、绿原酸和没食子酸可与亚硝酸钠反应, 发生颜色改变或沉淀。并且, 儿茶素、芦丁、橙皮素、绿原酸及鞣花酸还可抑制已生成的亚硝基甲基脲致突变性(表1)。

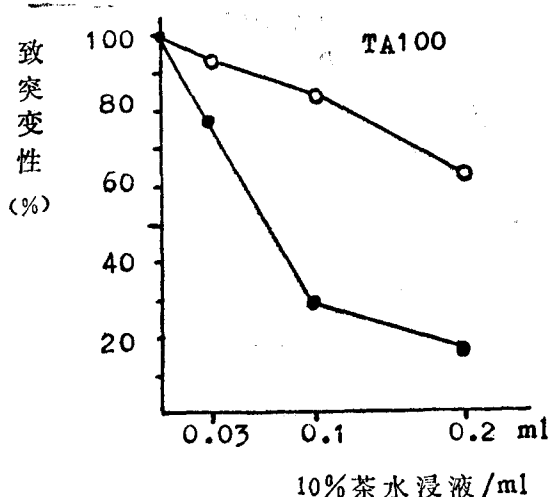


图1 甲基脲亚硝化反应前(●)和反应后(○)加入绿茶水浸液对致突变性的影响。TA100溶剂对照为147±19.7/皿, 未加茶水浸液的对照为801±50.6/皿

表1 茶多酚类对甲基脲亚硝化产物致突变性的影响\*

茶多酚 (浓度)	致突变活性为对照的%	
	受试物于亚硝化作用 前加入	受试物于亚硝化作用 后加入
D-儿茶素 (1mg/ml)	45.7	60.3
芦丁 (1mg/ml)	47.9	44.9
橙皮素 (1mg/ml)	31.3	67.6
绿原酸 (1mg/ml)	51.2	84.4
没食子酸 (1mg/ml)	34.8	90.4
鞣花酸 (0.2mg/ml)	46.1	79.4

\* TA100菌株, 未加茶多酚的对照值为801±50.6回变菌落/皿, 溶剂(二甲亚砜)对照为147±19.7回变菌落。

绿茶水浸出液及上述六种茶多酚可抑制苯并(a)芘及黄曲霉毒素B<sub>1</sub>经肝S9活化的致突变性, EC<sub>50</sub>为2—3mg绿茶/ml(图2和图3)。体外试验表明, 绿茶水浸出液及上述六种茶多酚均可抑制大鼠肝微粒体苯并(a)芘羟化酶活性, 但在较高浓度时抑制一般也不超过50%(图4)。

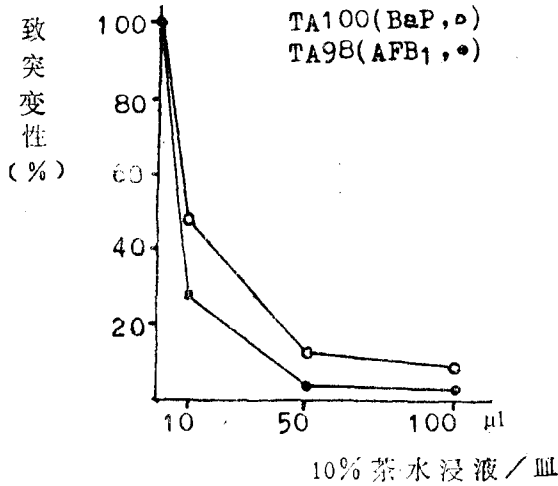


图2 绿水浸液对苯并(a)芘(BaP)和黄曲霉毒素B<sub>1</sub>(AFB<sub>1</sub>)致突变性的影响。溶剂(二甲基亚砜)对照; TA100 151±4.2/皿, TA98 53±7.9/皿, BaP对照: 10μg, 2980±282.3/皿; AFB<sub>1</sub>对照: 0.5μg, 1529±26.6/皿

## 2. 绿水浸出液及有关多酚类的抗脂质过氧化作用

绿水浸出液对过氧化羟基异丙苯引起

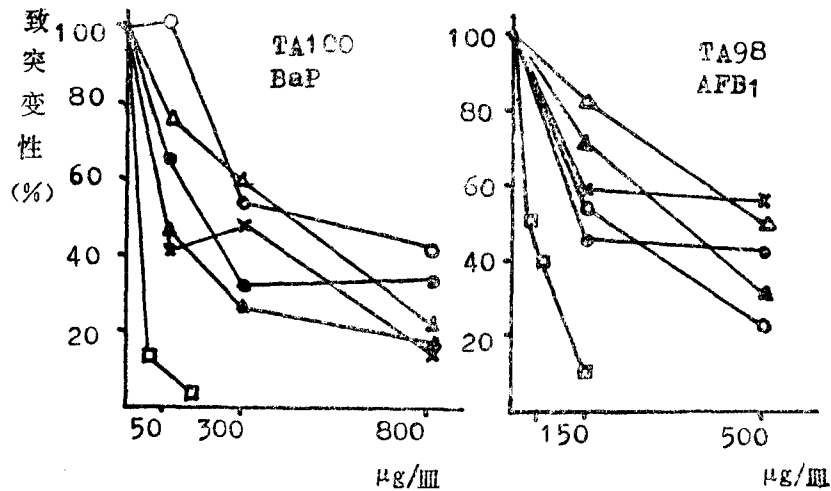


图3 茶多酚对苯并(a)芘(BaP)和黄曲霉毒素B<sub>1</sub>(AFB<sub>1</sub>)致突变性的影响。○为D-儿茶素, ●为芦丁, △为橙皮素, ▲为没食子酸, ×为绿原酸, □为鞣花酸

## 讨 论

茶叶是我国传统的饮料, 十六世纪传播到国外, 也成为世界重要饮料之一。近年来,

的肝微粒体直接脂质过氧化作用及四氯化碳经代谢活化引起的间接脂质过氧化作用均有非常显著的抑制作用(图5), 并且EC<sub>50</sub>分别为10μg绿茶/ml和5μg绿茶/ml, 远低于抗致突变作用的EC<sub>50</sub>。上述六种茶多酚也都具有一定的抗脂质过氧化作用(表2)。

表2 茶多酚类的抗脂质过氧化作用

茶多酚(浓度)	丙二醛的生成量为对照的%*	
	过氧化羟基异丙苯	四氯化碳
D-儿茶素 50μg/ml	25.9	19.5
5μg/ml	76.9	72.1
芦丁 50μg/ml	76.2	60.1
5μg/ml	85.2	107.1
橙皮素 50μg/ml	18.7	19.8
5μg/ml	78.9	56.2
绿原酸 50μg/ml	80.7	49.0
5μg/ml	94.6	84.1
没食子酸 50μg/ml	49.0	88.0
5μg/ml	66.6	92.9
鞣花酸 50μg/ml	43.9	55.2
5μg/ml	66.7	75.3

\* 过氧化羟基异丙苯的对照值为3.72nmol丙二醛硫代巴比妥酸结合物/mg微粒体蛋白, 四氯化碳的对照值为1.46nmol/mg

国外有些学者报告饮茶习惯与食道癌死亡率呈正相关<sup>[6]</sup>。Kapadia等报告<sup>[7]</sup>, 大鼠皮下注射茶叶的水提取物无致癌作用, 但含鞣质的组份可诱发注射部位的恶性纤维组织细胞

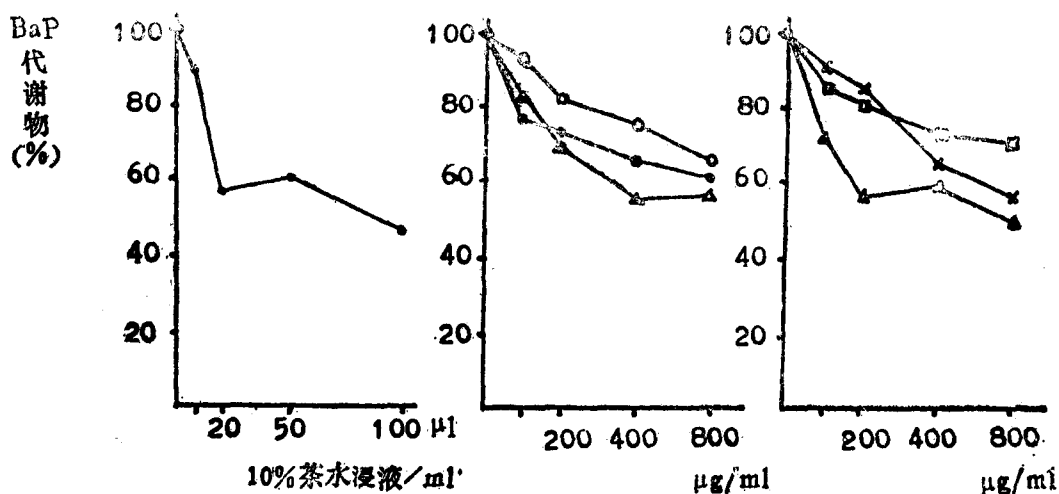


图4 绿茶水浸液(左)及茶多酚(中、右)对苯并(a)芘(BaP)羟化酶活性的影响。茶多酚的标号同图3。

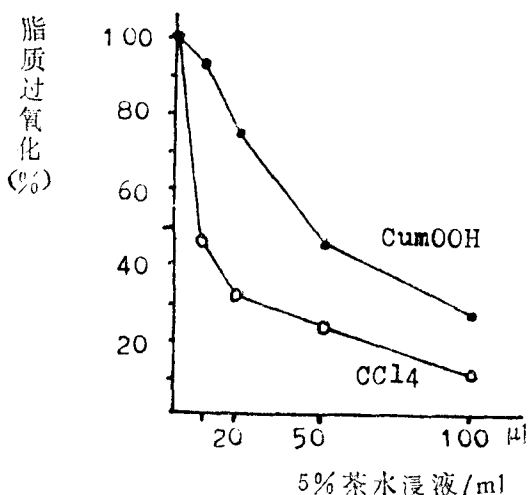


图5 绿茶水浸液对过氧化羟基异丙苯和四氯化碳诱发的大鼠肝微粒体脂质过氧化作用的影响

瘤。Nagao等<sup>[8]</sup>利用Ames试验发现日本红茶、绿茶和烤茶在一定剂量时均有致突变性。Uyeta等报告<sup>[9]</sup>日本茶叶水提取物的水解产物有显著的致突变性,主要的致突变成分是堪菲醇、杨梅黄酮及槲皮酮。但也有报告<sup>[10]</sup>,在动物实验中茶叶具有抗致癌作用。由于我国向来以绿茶为主要饮用品种,与红茶不同,绿茶是非发酵茶,基本上不含茶鞣质,而且美洲、非洲的有些“茶树”与我国茶树是不同属的植物,因此在评价茶叶在人类肿瘤发生中的地位时,必须重视茶叶的品种及加工

方式。

上述体外研究的结果表明,所用绿茶的水浸出液有抑制甲基脲的亚硝化作用和抑制直接致突变物亚硝基甲基脲及间接致突变物苯并(a)芘和黄曲霉毒素B<sub>1</sub>对鼠伤寒沙门氏菌的致突变作用,以及抑制大鼠肝微粒体苯并(a)芘羟化酶的活性。绿茶水浸出液可显著地抑制过氧化羟基异丙苯和四氯化碳诱导的大鼠肝微粒体脂质过氧化作用。而且,在试验的剂量范围内,绿茶水浸出液对鼠伤寒沙门氏菌并无直接和间接致突变作用。目前认为,化学致癌作用的起阶段与体细胞突变有关,而脂质过氧化及自由基生成是促进剂的共同特点。因此,上述结果提示所用绿茶可能具有抗起动的抗促进双重作用。目前我们正在进行绿茶提取物的体内抗致突变作用和抗脂质过氧化作用的研究。

茶叶的化学成分非常复杂,目前已鉴定了约500多种,茶多酚是茶叶的重要成分之一。上述结果表明,D-儿茶素、芦丁、橙皮素、绿原酸、没食子酸和鞣花酸均具有一定的体外阻断亚硝化反应、抗致突变及抗脂质过氧化作用。我们认为需要进一步研究这些茶多酚在绿茶抗致突变及抗脂质过氧化作用

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效的抗氧化剂。维生素E的作用部位是内质网，它可以与在此形成的自由基结合，使之失去活性。另一方面，它又可使不饱和脂肪酸的过氧化物还原，阻止进一步反应<sup>[8]</sup>，从而保护内质网，减轻组织损伤。虽然目前尚未证明脂质过氧化是TNT引起肝损伤的原因，但本实验结果间接证明，在TNT肝损伤过程中，有脂质过氧化反应参与。

GSH、GSH-PX和维生素E是细胞内两个独立的保护系统<sup>[9]</sup>，但本实验未观察到半胱氨酸对TNT肝损伤具有保护作用，提示TNT可能不是通过破坏GSH、GSH-PX参与的保护系统而损伤肝脏的。

### 三、维生素E和半胱氨酸的治疗作用，

大鼠TNT亚慢性中毒造成肝损伤后，再给予维生素E和半胱氨酸，对损伤的恢复情况似乎无明显影响。Dilley等报道<sup>[10]</sup>，短期接触TNT后，造成大鼠血液学方面的改变可以完全恢复。本实验观察到，大鼠TNT亚慢性中毒造成的肝脏损伤，经过4周后，有了一定程度的恢复，但是否能够完全恢复，还有待于进一步观察。

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中的作用，并且系统分离鉴定其它有效成分。

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## ABSTRACTS OF ORIGINAL ARTICLES

Evaluation of genotoxic effect of 16 chemicals using  
the micronucleus assay in vitro      *Gu Zu wei,*

*Wang Yi lan. (Laboratory of Cytotoxicology,  
Shanghai Medical University)*

The genotoxic effect of 16 chemicals has been evaluated using the micronucleus assay in vitro. Xipamide, hydrochlorothiazide, acetone, DMSO, ethyl acetate, dichloromethane, methanol, ethanol, propanol-1, and butanol-1 were not active in inducing micronuclei in V79 cells. The results of air pollutant extracts are dependent on when the pollutant samples were collected. BaP, BrdU, MN-NG, \*, 2-dibromoethane and epichlorohydrin have induced micronuclei at least twice more than the solvent control. An additive joint action in induction of micronuclei by MNNG and BrdU has been revealed.

The criteria of evaluation and the relationship between induction of micronuclei and SCE are discussed. The suitable sample time for micronucleus assay in V79 cells in vitro was suggested.

(Original article On Page 1 )

Studies of toxicokinetics of methyl methacrylate in rabbits

*Shi Tao, et al. (Institute of Pharmacology and Toxicology,  
Academy of Military Medical Sciences, Beijing 100800)*

In this paper the toxicokinetics of methyl methacrylate (MMA) was studied in rabbits and in vitro respectively.

The toxicant concentration-time curves of methyl methacrylate (150 mg/kg) given intravenously in rabbits could exactly fit the one-compartment open model. MMA were not detected in blood after percutaneous application at dose of 600 mg/kg and subcutaneous injection in rabbits.

MMA was eliminated according to a first-order process in whole blood, plasma, erythrocyte suspension, homogenate of brain, heart, liver, lung, kidney, small intestine and muscle in vitro. The top rate of elimination of it had been found in blood and liver homogenate. We had observed that the elimination of MMA was undertook most rapidly in supernatant liquid of liver. The rate of elimination of MMA could be decreased by TOCP in vivo or vitro. Our results suggested that esterase might be the major enzyme which took part in the metabolism of MMA.

The toxicokinetics parameters of MMA were presented.

(Original article on page 5 )

Inhibition of Green Tea and Tea Phenolics on Mutagenesis  
and Lipid Peroxidation in vitro      *Zhou Zongcan, et al.*

*School of Public Health, Beijing Medical University*

One kind of Chinese green tea (*Camellia Sinensis* O.Ktze) was examined for its inhibitory effect in vitro on nitrosation, mutagenesis and lipid peroxidation. In Ames test, tea aqueous extract (TAE) prevented the formation of mutagenic compound formed by nitrosation of methyluracil

(EC<sub>50</sub>, 7 mg tea/ml) and inhibited the mutagenicity of the formed nitrosomethylurea. TAE inhibited the mutagenicity of benzo(a)pyrene and aflatoxin B<sub>1</sub> (EC<sub>50</sub>, 2-3 mg tea/ml) and suppressed the activity of benzo(a)pyrene hydroxylase. TAE inhibited cumene hydroperoxide and carbon tetrachloride-induced lipid peroxidation of rat liver microsomes in vitro (EC<sub>50</sub>, 10 and 5 μg tea/ml, respectively). The effects of D-catechin, rutin, hesperetin, chlorogenic acid, gallic acid and ellagic acid on nitrosation, mutagenesis and lipid peroxidation were reported.

(Original article on page 10 )

### The Evaluation of the Role of Metabolic Enzymes in the Toxicity Induced by Four Different Kinds of Dust

*He Xiwen, et al. (Institute of Occupational Medicine  
Chinese Academy of Preventive Medicine)*

Four kinds of different compositions dust were used to compare their effects on the activity of microsomal cytochrome P-450 (P-450), arylhydrocarbon hydroxylase (AHH) and glutathione-S-transferase (GST) in liver and lung of rats.

The results showed that P-450 activity of liver was not changed after the single intratracheal injection to four different kinds of dust, but it was markedly inhibited after multijection of the mixed dust of Yunnan tin mine. AHH activity of lung was decreased after single or multijection of As<sub>2</sub>O<sub>3</sub>, on the contrary, the activity was markedly induced after multijection of Fe<sub>2</sub>O<sub>3</sub>. It is supposed: (1) The intensities of effect in sequence were As<sub>2</sub>O<sub>3</sub>, Fe<sub>2</sub>O<sub>3</sub>, the mixed dust of Yunnan tin mine and the mixed dust of Hunnan mine. (2) The induction of AHH activity in lung after multijection to Fe<sub>2</sub>O<sub>3</sub> might play an important role in promoting lung cancer. (3) The inhibition of P-450 activity may be considered as the result of the combination effect of these two (As and Fe) carcinogenic metals.

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### The Effects of Cadmium, Mercury and Lead in vitro on Hepatic Microsomal Mixed Function Oxidase and Lipid Peroxidation

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In this paper, the authors studied the effects of cadmium, mercury and lead in vitro on hepatic microsomal mixed function oxidase and lipid peroxidation. The results showed that these metals could inhibit the activity of hepatic microsomal aniline hydroxylase, decrease the concentration of hepatic microsomal cytochrome P-450 and increase the concentration of inactive form of the hemoprotein, P-420; besides, they could enhance hepatic microsomal lipid peroxidation. There were marked concentration-dependent responses in these microsomal reactions. Thus, these data suggested that cadmium, mercury and lead were capable of impairing hepatic microsomal mixed function oxidase in vitro by stimulating membrane lipid peroxidation.

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