

绿茶对甲基硝基亚硝基胍诱发LACA小鼠 肺癌及癌前病变的预防作用*

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内容摘要 实验先后两批,共取LACA小鼠390只。每批随机分为正对照、绿茶、复合及空白对照4组。正对照组和复合组每5天于小鼠尾静脉注射MNNG250 μ g一次,共7次,总量1.75mg;绿茶组和复合组每日用5%绿茶末普通饲料长期喂养,正对照组和空白对照组长期喂饲普通饲料。两批实验分别经96.4周及106周结束。结果绿茶不仅使肺发病率从79.75%下降到13.59%,而且改变了肺肿瘤良、恶性及病理类型构成比,恶性肿瘤和癌前病变减少,对MNNG诱发小鼠肺癌及癌前病变可产生不同程度的预防作用。实验结果重复性好。

关键词 绿茶 甲基硝基亚硝基胍 LACA小鼠 肺癌及癌前病变 诱癌作用 预防作用

“茶叶与防癌”近年来引起了国内外有关学者的重视,1989年为绿茶与防癌举行了第一届国际学术研讨会〔1〕。N-亚硝基化合物可能与人类癌症发生密切相关〔2〕,而绿茶能抑制亚硝胺诱发的某些动物的消化道癌〔1〕。为了进一步对绿茶的防癌作用提供实验依据,作者首次报道用绿茶(green tea,GT)长期喂饲LACA小鼠,观察对甲基硝基亚硝基胍(MNNG)诱发肺癌及癌前病变的预防作用,现报道如下。

材料和方法

1. 绿茶 采用春茶(烘青),四川省兴文县建新茶场出产。

2. MNNG 本校化学实验室合成〔3〕。

3. 动物、分批及分组 实验先后分两批,共取7周龄LACA小鼠(从北京中医研究院实验动物中心引入,我所自行繁殖)390只,体重21~25g。每批随机分为正对照(MNNG)、绿茶(GT)、复合(MNNG+GT)及空白对照(C)4个组。每组动物平均体重22.6g,雌

雄各半。

4. 方法 MNNG,用蒸馏水配制。正对照组及复合组尾静脉注射MNNG,每5天1次,每次0.2ml含MNNG250 μ g,共7次,总量1.75mg;绿茶组及复合组每日用5%绿茶末普通饲料长期喂饲;正对照组及空白对照组长期喂饲普通饲料。各组每鼠每天平均摄食饲料10g及自由饮自来水,终生饲养。每月称体重1次。实验过程中动物自死或濒死处死后,常规病理解剖,重点观察肺脏病变。所有动物双肺经福尔马林液初固定后,在体视显微镜下观察肺表面并切成薄片观察,记录肿瘤数目。全部组织再按常规石蜡制片,光镜观察。

结 果

1. 肺发病率及发癌率 实验各组小鼠肺发病率及发癌率见表1。

由表1可见两批分别或合计的复合组与正对照组间有非常显著性差异(χ^2 检验, $P < 0.001$)。

2. 肺肿瘤数目 结果见表2。

肉眼及体视显微镜下,有肺肿瘤的每只动物肿瘤数范围,在复合组为1~14个,在正对

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表1. LACA 小鼠肺发病率及发癌率
Table 1. Incidence of lung tumor and lung cancer in LACA mice

Group	1st batch				2nd batch				Total			
	No. of mice		Incidence(%)		No. of mice		Incidence(%)		No. of mice		Incidence(%)	
	Beg	Eff	Tumor	Cancer	Beg	Eff	Tumor	Cancer	Beg	Eff	Tumor	Cancer
MNNG ^a	40	37	27/37 (72.97)	26/37 (70.27)	50	42	40/42 (95.24)	37/42 (88.10)	90	79	67/79 (84.81)	63/79 (79.75)
MNNG + GT ^b	50	40	7/40 (17.50)	5/40 (12.50)	70	63	11/63 (17.46)	9/63 (14.28)	120	103	18/103 (17.48)	14/103 (13.59)
GT	50	47	0/47 (0)	0/47 (0)	50	50	0/50 (0)	0/50 (0)	100	97	0/97 (0)	0/97 (0)
C	30	30	0/30 (0)	0/30 (0)	50	45	3/45 (6.67)*	3/45 (6.67)*	80	75	3/75 (4.00)*	3/75 (4.00)*
Total	170	154			220	200			390	354		
χ^2 - test a vs b,	P<0.001		P<0.001		P<0.001		P<0.001		P<0.001		P<0.001	

Beg = Beginning Eff = Effective

^a 2 cases of malignant lymphoma, 1 case of adenoma with malignant change

表2. 肉眼、体视显微镜及光学显微镜下肺肿瘤数目
Table 2. Number of tumors by naked eye and under stereomicroscope, and under light microscope

Group	Batch	No. of mice	No. of tumors by naked eye and under stereomicroscope (mean)			No. of tumors under light microscope (mean)
			Left lung	Right lung	Total	
MNNG ^a	1st	37	69(1.86)	135(3.65)	204 (5.51)	87(2.35)
	2nd	42	233(5.55)	397(9.45)	630(15.00)	210(5.00)
	Total	79	302(3.82)	532(6.73)	834(10.56)	297(3.76)
MNNG + GT ^b	1st	40	8(0.20)	7(0.18)	15 (0.38)	16(0.40)
	2nd	63	23(0.36)	28(0.44)	51 (0.81)	37(0.59)
	Total	103	31(0.30)	35(0.34)	66 (0.64)	53(0.51)

No. of tumors by gross and under stereomicroscope, 1st batch, b:a=1:14.5; 2nd batch, b:a=1:18.5; Total, b:a=1:16.5 No. of tumors under light microscope, 1st batch, b:a=1:5.88; 2nd batch, b:a=1:8.47; Total, b:a=1:7.37

照组为1~43个, 绝大多数动物肺肿瘤数右肺多于左肺。两批合计, 复合组平均每例肺肿瘤数仅为正对照组的1/16。复合组与正对照组间的肿瘤均数在各批中及两批合计中均有非常显著性差异 (t检验, $P<0.001$)。

光镜下观察肺肿瘤数目, 两批合计, 复合组平均每例也仅为正对照组的1/7。复合组与正对照组间的肿瘤均数相差的显著性测验, 两批分别或合计均有非常显著性差异 (t检验, $P<0.001$)。

3. 肺良、恶性肿瘤的构成 小鼠肺肿瘤

良、恶性构成比的比较, 结果见表3。

由表3可见复合组与正对照组间在各批中及两批合计中均有非常显著性差异 (χ^2 检验, $P<0.005$)。复合组使免于患肺肿瘤与肿瘤的鼠数百分率(从正对照组的15.19%和20.25%)升高(到82.52%和86.41%)。

4. 肺各类腺瘤、腺癌分类构成 MNNG 诱发的肺肿瘤中, 以呼吸道上皮性肿瘤最多, 达91.49%, 主要类型为腺瘤和腺癌, 其肿瘤数目及病理组织学分类构成比的比较, 结果见表4。

由表4可见在两批合计中复合组与正对照

表3. 肺良、恶性肿瘤构成比的比较

Table 3. In comparison with constituent ratio on the benign and malignant lung tumor

Batch	Group	Constituent ratio (%)				
		Tumor-free	Malignant	Benign·Malignant	Benign	Total
1st	MNNG ^a	10(27.03)	25(67.57)	1(2.70)	1(2.70)	37(100)
	MNNG+GT ^b	33(82.50)	4(10.00)	1(2.50)	2(5.00)	40(100)
	Total	43	29	2	3	77
2nd	MNNG ^c	2 (4.76)	36(85.71)	1(2.38)	3(7.14)	42(100)
	MNNG+GT ^d	52(82.54)	7(11.11)	2(3.17)	2(3.17)	63(100)
	Total	54	43	3	5	105
1st+2nd	MNNG ^e	12(15.19)	61(77.22)	2(2.53)	4(5.06)	79(100)
	MNNG+GT ^f	85(82.52)	11(10.68)	3(2.91)	4(3.88)	103(100)
	Total	97	72	5	8	182

χ^2 -test: a vs b, $P<0.005$; c vs b, $P<0.005$; e vs f, $P<0.005$

表4. 各类肺肿瘤的构成比的比较

Table 4. In comparison with constituent ratio on the kinds of lung tumor

Batch	Group	Constituent ratio (%)				Total
		Adenoma	Malignant adenoma	Adenoma with malignant change	Adenocarcinoma	
1st+2nd	MNNG ^a	5 (1.79)	41(14.70)	228(81.72)	5 (1.79)	279(100)
	MNNG·GT ^b	10(18.87)	3 (5.66)	40(75.47)	0	53(100)
	Total	15	44	268	5	332

χ^2 -test: a vs b, $P<0.005$

组间有非常显著性差异 (χ^2 检验, $P<0.005$)。

5. 肺腺瘤样增生及异型增生 各种致癌物诱发肺癌过程中, 除腺瘤外, 腺瘤样增生及异型增生也是重要的癌前病变^[4], MNNG 诱发肺癌过程中同样也有出现, 见表 5。

在两批合计中, 腺瘤样增生的发生率复合

组低于正对照组, 两组间有非常显著性差异 (χ^2 检验, $P<0.005$)。每只小鼠腺瘤样增生灶平均数, 复合组为正对照组的1/7。

在两批合计中, 异型增生发生率在正对照组为6.33%, 复合组未发现异型增生灶, 两组间有显著性差异 (χ^2 检验, $P<0.025$)。

表5. 肺腺瘤样增生及异型增生发生率和每鼠平均病灶数

Table 5. Incidence and mean number of pulmonary adenomatoid hyperplasia and atypia

Group	Batch	Effective mice	Adenomatoid hyperplasia		Atypia	
			Mice(%)	No. of foci (mean)	Mice(%)	No. of foci (mean)
MNNG	1st	37	6(16.22)	11(0.30)	2(5.40)*	5(0.14)
	2nd	42	13(30.95)	37(0.88)	3(7.14)	5(0.12)
	Total	79	19(24.05) ^a	48(0.61)	5(6.33)	10(0.13) ^d
MNNG+GT	1st	40	1 (2.50)	5(0.13)	0	0
	2nd	63	4 (6.35)	4(0.06)	0	0
	Total	103	5 (4.85) ^b	9(0.09)	0	0 ^d

* 1 case with 3 foci, 1 case with 2 foci and 3 foci of adenomatoid hyperplasia
 $P<0.005$; c vs d, $P<0.025$

χ^2 -test: a vs b,

讨 论

为观察绿茶对 MNNG 诱发肺癌及癌前病变的预防作用, 所以两批实验分别经 96.4 周及 106 周结束。从复合组与正对照组比较可见绿茶有以下作用: ①能显著降低小鼠肺发瘤率和发瘤率(如发瘤率正对照组为 79.75%, 复合组为 13.59%, 降低 66.16%), 使复合组免于患肺肿瘤鼠数百分率升高(为 67.33%, 正对照组为 15.19%, 复合组为 82.52%); ②能显著减少小鼠肺肿瘤发生的数目, 复合组每只小鼠平均患肺肿瘤结的数目仅为正对照组的 1/7~1/16; ③能改变肺肿瘤良、恶性的构成比和肺腺瘤、腺癌的病理类型构成比, 恶性腺瘤和腺癌恶变数减少, 而良性腺瘤相对增加, 引起了质的变化, 说明绿茶对 MNNG 诱发 LACA 小鼠肺癌具有不同程度的防癌作用。

此外, 肺腺瘤样增生和异型增生两类癌前病变是肺癌发生的基础, 如果这些病灶数目增加, 意味着发生肺癌的可能性也增加, 反之则减少。绿茶使复合组每只小鼠平均肺腺瘤样增生灶和异型增生灶的数目较正对照组非常明显或明显减少, 说明绿茶对 MNNG 诱发小鼠肺癌前病变同样有预防作用。

茶叶是世界上三大无酒精饮料之一。茶叶有抗致突变及抗染色体损伤, 其提取物能抑制细胞恶性转化、癌变等机制的研究已有重大进

展。绿茶中抗氧化 GTA 的主要成分是茶多酚, 在其中起关键作用的是儿茶素^[1,5]。茶叶能阻断人体 N-亚硝基化合物的内源性合成, 特别是大剂量茶叶能抑制其合成^[2], 具有降低人体对强致癌物 N-亚硝基化物接触的作用^[7], 因而提示茶叶可能有助于人类癌症的病因预防^[5]。茶叶水是人们乐于接受的一种无酒精饮料。为防癌, 有人提出每天饮用较大剂量(约 5g) 绿茶茶叶冲泡绿茶水的建议^[7]值得提倡。

* * *

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Preventive Effect of Green Tea on MNNG-induced Lung Cancers and Precancerous Lesions in LACA Mice

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Abstract Three hundred and ninety LACA mice of seven weeks old were used in 2 batches (96.4 wks and 106 wks) for studying the preventive effect of green

tea on MNNG-induced lung cancers and precancerous lesions. These mice (within each batch) were randomly allocated to four groups, namely, positive control

(MNNG), green tea (GT), complex (MNNG+GT), and blank control (C) group. In MNNG group, MNNG 250 μ g was injected intravenously every five days for seven times in each mouse, the total dosage of MNNG was 1.75mg. In GT group, according to W/W, 5% GT dust was well mixed into 95% common diet for long-term breeding. In complex group, MNNG was given as that in MNNG group and the mice were reared as those in GT group. The mice in MNNG group and in C group were all reared by common diet. The mean amount of daily intake of feed was 10g. The number of effective animals was 354.

The results of experiments showed different degrees of preventive effect of green tea on MNNG-induced lung cancers and precancerous lesions in LACA mice. Green tea exerted an effect on the number

of induced cancers and precancerous lesions, causing a drop of the cancerous rate from 79.75% to 13.59% and the number of lung tumor down to 1/7-1/16 that of the MNNG group, i.e. down to less than one tumor nodule per mouse. It also altered the proportional rate of the kinds and pathological types of tumor, causing an increase of tumor-free and cancer-free animals by 67.33% (from 15.19% up to 82.52%) and 66.16% (from 20.25% up to 86.41%) respectively, and denoting a decrease in malignancy (adenoma with malignant change, and malignant adenoma) from 96.42% to 81.13%, there was no adenocarcinoma in the complex group. In addition, green tea decreased the adenomatoid hyperplasia and dispelled the atypia of lung. The results of experiments showed good duplication and absence of adverse reactions to green tea.

Key words Green tea N-methyl-N'-nitro-N-nitroso-guanidine (MNNG)
LACA mice Lung cancer and precancerous lesion Carcinogenesis Preventive effect

36 The Effectiveness of Suppositories in Ulcerative Proctitis and Sigmoiditis—A Randomized Double-Blind Controlled Trial in 70 Cases

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Suppositories of hydrocortisone, furazolidon, disodium cromoglycate and placebo were used in 70 cases of ulcerative proctitis and sigmoiditis. Each patient was treated with two pieces of the suppository per night for 4 weeks in a randomized, double-blind controlled trial. The activity of the disease was assessed by reformed Williams' disease activity index (DAI).

Using ^{99m}Tc -labelled hydrocortisone, the authors measured the extent of spread by scintiscanning and the blood concentration of drug given via different routes by HPLC to find out the differences between the routes. The results showed an effective rate of 94.4% and a complete clinical remission rate of 83.3% for the group of hydrocortisone, which were significantly different from those for the groups on placebo and others. No side effects were found in the investigation. The drug could spread up to sigmoid with a high concentration locally. Suggesting that hydrocortisone suppository is an effective, safe and well-tolerated topical therapy for proctitis and sigmoiditis.