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实验动物胃粘膜癌前病变的模型建立、发生机理及其逆转治疗的研究*

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提 要 本文首次报告:(1)分别采用含0.03%雷尼替丁(R)的标准粉末状饲料、50 μg/ml 甲基硝基亚硝基胍(MNNG)溶液及两者联合(MNNG+R)喂饲大鼠共20周,于实验第43周末处死动物进行观察。MNNG+R组大鼠腺胃粘膜肠化生诱发率及平均肠化腺体数(100.0%,696.2±173.6)显著地高于MNNG组(86.5%,330.6±145.6)或R组(22.5%,75.2±30.9)($P<0.01$,$P<0.05$);(2)将维甲酸(RA)和丁酸钠(SB)分别应用于大鼠实验性腺胃粘膜癌前病变的逆转治疗。RA与SB治疗组大鼠腺胃粘膜肠化生(72.0%,60.0%)、中重度异型增生(24.0%,20.0%)及胃癌(0%,0%)发生率均显著地低于未加治疗的对照组($P<0.01$,$P<0.05$)。RA与SB治疗组大鼠幽门腺及胃底腺平均肠化腺体数(130.2±93.9、51.5±39.1,70.3±46.8、39.8±29.6)亦显著地低于未加治疗的对照组($P<0.01$);(3)将40只大鼠带蒂胃壁瓣分别移植到十二指肠、空肠及结肠,于术后第3、6、9、12个月时分批处死动物。组织学及SEM、TEM观察证实,胃粘膜由胃内环境进入肠道环境后,发生了广泛肠化生。结果表明:MNNG+R是建立大鼠实验性腺胃粘膜肠化模型的一种较为理想的方法;维甲酸和丁酸钠对大鼠实验性腺胃粘膜癌前病变有部分逆转治疗作用;胃粘膜肠化生可能是一种pH相关性变化,文中提出了胃粘膜肠化生发生的可能机理。

关键词 胃粘膜;肠化生;癌前状态;维甲酸;丁酸钠;逆转治疗*;大鼠
中图法分类号 R730.21;R363

胃粘膜上皮异型增生和胃粘膜肠化生是内镜活检及手术切除标本上最常见的病理变化。许多研究表明,异型增生是重要的胃癌前病变,肠化生与胃癌特别是肠型胃癌的发生可能亦有密切关系[1,2]。为进一步明确胃粘膜肠化生的发生机理,探讨胃粘膜癌前病变防治方法,本研究工作分为三部分:(1)建立大鼠的实验性腺胃粘膜癌前病变模型;(2)应用已建立的动物模型,分别观察维甲酸和丁酸钠对大鼠实验性腺胃粘膜癌前病变的逆转作用,为其进一步应用于人类胃癌前病变的治疗提供依据;(3)通过大鼠带蒂胃壁瓣肠移植后,胃粘膜的组织学变化及电镜观察,探讨胃粘膜肠化生的发生机理。

1 材料与方法

1.1 实验动物胃粘膜癌前病变模型的建立

健康雄性Wistar鼠160只,体重120~140g,随机分成4组:雷尼替丁(R)组,MNNG组,MNNG+R组和正常对照(NC)组,每组各40只动物。R组大鼠进食含0.03%雷尼替丁的标准粉末状饲料,MNNG组大鼠每日自由饮用浓度为50 μg/ml的MNNG(Fluka产品)溶液,MNNG+R组大鼠每日自由饮用浓度为50 μg/ml MNNG溶液、食用含0.03%雷尼替丁的标准粉末状饲料,时间均为20周,处理完毕后各组动物均改饲自来水与标准颗粒状饲料。NC组大鼠不作特殊处理。于实验第43周末处死全部动物进行观察。

1.2 实验动物胃粘膜癌前病变的逆转治疗研究

维甲酸和丁酸钠逆转治疗研究分别采用上述MNNG+R组及MNNG组的胃癌前病变模型建立方法。每种药物观察均设5组动物。第Ⅰ组动物于实验第35周处死,用于了解逆转治疗前胃粘膜癌前病变的诱发率;第Ⅱ组为未加治疗的对照组;第Ⅲ组为治疗组;第Ⅳ组用于观察治疗药物本身对胃粘膜的作用;第Ⅴ组为正常对照组。于实验第36周开始,第Ⅱ、Ⅳ组动物按体重喂维甲酸(40 mg/kg)或丁酸钠(150 mg/kg),每日喂服1次,共8周。第Ⅰ~Ⅴ组大鼠于实验第43周末处死。

1.3 大鼠带蒂胃壁瓣肠移植

健康雄性Wistar鼠[illegible]只,体重180~200 g,分成3组:(1)带蒂胃壁瓣肠移植组40只。常规打开腹腔,于大鼠腺胃前壁正中部取一大小约1.5cm×1cm梭形胃壁瓣,保留胃小弯侧血管2~4条,7/0号线缝合胃壁。分别在十二指肠中部、空肠末端及中段结肠纵行切开肠管,将梭形胃壁瓣粘膜面向肠腔进行类似侧-侧吻合,7/0号线连续缝合,关闭腹腔。术后1周内有4只大鼠死于肠梗阻。有效手术动物数共36只,其中带蒂胃壁瓣十二指肠、空肠、结肠移植各12只。(2)手术对照组20只。带蒂胃壁瓣切口处原位缝合,以了解手术本身对胃粘膜的影响。(3)正常对照组20只。分别于术后第3、6、9、12个月时处死大鼠进行组织学、透射电镜(TEM)及扫描电镜(SEM)观察。手术组每次处死3只,手术对照组及正常对照组每次处死5只。

1.4 胃粘膜肠化生腺体计量

模型及逆转治疗研究组动物,每例胃标本沿长轴剪成8块宽约2mm的条状物,分别进行石蜡包埋、切片,每例胃标本的8个组织块各取1张切片,行AB(pH2.5)/PAS粘液组化染色后,

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于光学显微镜下进行肠化腺体计量。此8张组织片上肠化腺体总数代表该例胃标本的肠化腺体总数。

1.5 病理诊断与分级

按1978年全国胃癌研究协作组制订的标准。

1.6 统计学处理

计数资料用 χ^2 检验，计量资料用 t 检验。

2 结果

2.1 实验动物胃粘膜癌前病变模型的建立

2.1.1 各组动物胃粘膜癌前病变的发生率：MNNG 组大鼠在第6、11、19周各死亡1只，MNNG＋R 组大鼠在第8、15、20、27周时各死亡1只。各组动物腺胃粘膜肠化生、中重度异型增生及胃癌的发生率分别为：R组22.5%(9/40)、0%、0%；MNNG组86.5%(32/37)、45.9%(17/37)、13.5%(5/37)；MNNG＋R 组100.0%(36/36)、47.2%(17/36)、16.7%(6/36)；NC 组7.5%(3/40)、0%、0%。经统计学处理，MNNG＋R 组大鼠腺胃粘膜肠化生发生率显著地高于 MNNG 组（$P<0.05$）和 R 组（$P<0.01$）；MNNG＋R 组与 MNNG 组大鼠腺胃粘膜中重度异型增生及胃癌的发生率无统计学差异（$P>0.05$）。

2.1.2 胃粘膜肠化生腺体的计量分析(表1)

表1 不同组大鼠胃粘膜肠化生腺体的计量分析（$\bar{x}\pm s$）

Tab 1 The quantitative analysis of gastric mucosal metaplastic glands in different group of rats（$\bar{x}\pm s$）

Group	Treatment	Effective No.	Pylorus	Fundus	Pylorus and fundus
Ⅰ	Rantidine	40	48.2± 23.4	28.2± 18.5	75.2± 30.9
Ⅱ	MNNG	37	221.4±189.3	113.8±101.8	330.6±145.6
Ⅲ	MNNG+R	36	459.4±231.8△	253.0±115.3△	696.2±173.6△
Ⅳ	Untreated	40	5.0± 17.9	0	5.0± 17.9

△：*vs* group Ⅱ or group Ⅰ，$P<0.01$

2.2 大鼠实验性腺胃粘膜癌前病变的逆转治疗研究

2.2.1 不同组大鼠腺胃粘膜病变的发生率(表2)

表2 不同组大鼠腺胃粘膜病变的发生率

Tab 2 The incidences of glandular gastric mucosal lesions in different group of rats

	Group	No.	Metaplasia (%)	Dysplasta※ (%)	Gastric cancer
RA group	Ⅰ	10	9 (90.0)	3 (30.0)	0 (0.0)
	Ⅱ	25	25 (100.0)	13 (52.0)	4 (16.0)
	Ⅲ	25	18 (72.0)△	6 (24.0)△△	0 (0.0)△△
	Ⅳ	25	0 (0.0)	0 (0.0)	0 (0.0)
	Ⅴ	40	3 (7.5)	0 (0.0)	0 (0.0)
SB group	Ⅰ	20	16 (80.0)	5 (25.0)	0 (0.0)
	Ⅱ	25	22 (88.0)	12 (48.0)	4 (16.0)
	Ⅲ	25	15 (60.0)△△	5 (20.0)△△	0 (0.0)△△
	Ⅳ	25	0 (0.0)	0 (0.0)	0 (0.0)
	Ⅴ	40	3 (7.5)	0 (0.0)	0 (0.0)

※：moderate and severe dysplasia；RA：Retinoic acid，SB：Sodium butyrate

vs group Ⅱ，△：$P<0.01$，△△：$P<0.05$；*vs* group Ⅰ，$P>0.05$

2.2.2 胃粘膜肠化生腺体的计量分析(表3)

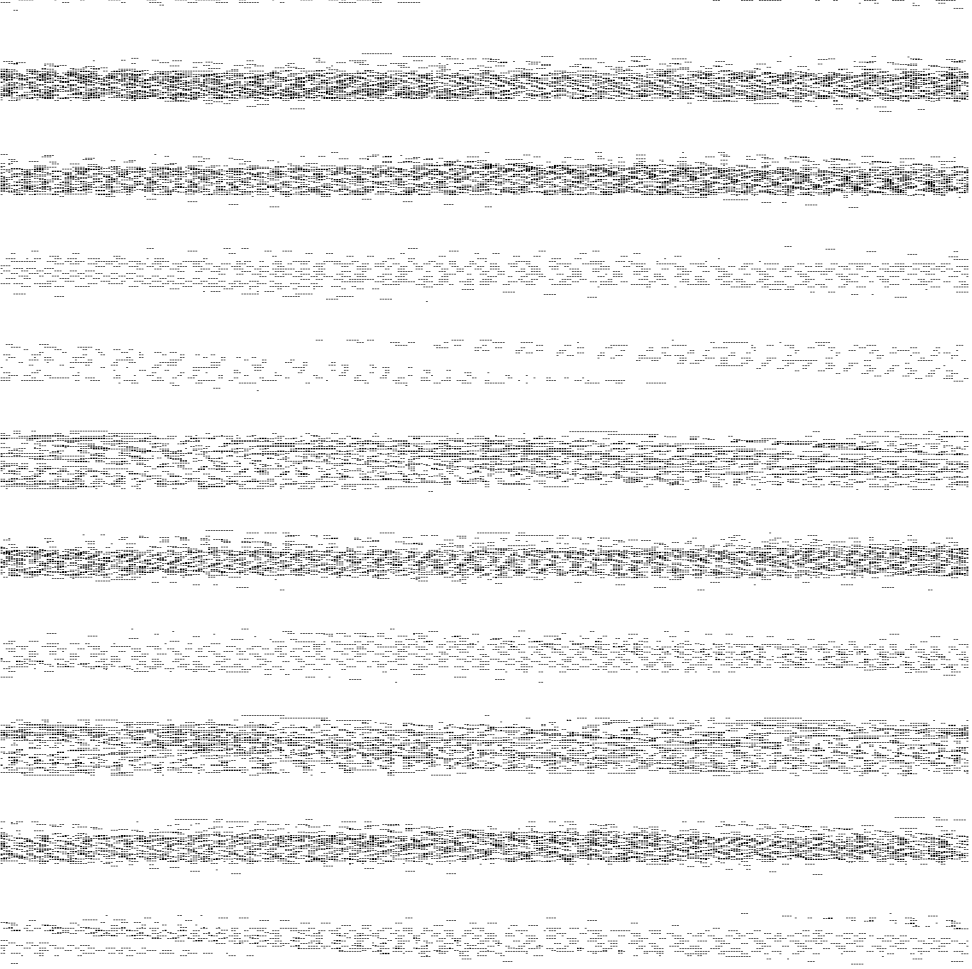
表3 胃粘膜肠化生腺体和计量分析

Tab 3 The quantitative analysis of metaplastic glands of gastric mucosa

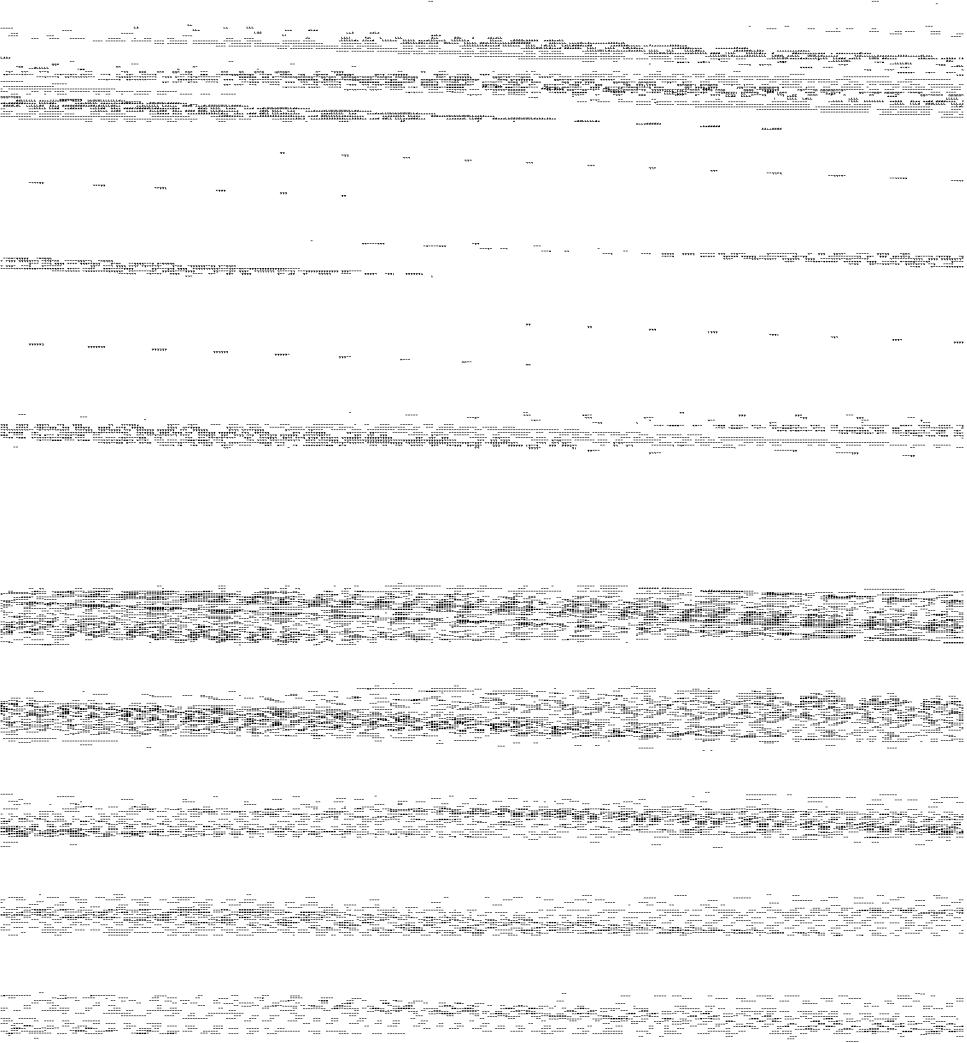
	Group	No.	Pylorus	Fundus	Pylorus and fundus
RA group	Ⅰ	10	396.2±202.2	221.4± 98.0	612.3±250.5
	Ⅱ	25	442.4±230.0	247.4±112.0	689.5±271.0
	Ⅲ	25	130.2± 93.9△	51.5± 39.1△	146.6±105.1△
	Ⅳ	25	0	0	0
	Ⅴ	40	5.0± 17.9	0	5.0± 17.9
SB group	Ⅰ	20	216.3± 98.4	130.7± 59.2	342.2±129.6
	Ⅱ	25	241.4±113.9	146.4± 66.3	382.9±138.5
	Ⅲ	25	70.3± 46.8△	39.8± 29.6△	106.7± 40.3△
	Ⅳ	25	0	0	0
	Ⅴ	40	5.0± 17.9	0	5.0± 17.9

vs group Ⅰ or group Ⅱ，△：$P<0.01$

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A study of the establishment of a rat-model of gastric mucosal precancerous lesion and its reverse therapy

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Abstract Ranitidine 0.03% in the diet (R), N-methyl-N'-nitro-N-nitrosoguanidine 50μl/ml added to the drinking ad libitum (MNNG), or the combination of 2 (R+MNNG) were administered to Wistar rats for 20 weeks. The rats subsequently were raisded without R or MNNG for additional 23 weeks. A group of rats without drug administration were under observation for 43 weeks and served as the control.

It was found that the rate of intestinal metaplasia (IM) in the glandular stomach was 22.5% with R, 86.5% with MNNG and 100% with R+MNNG while it was only 7.5% in the control. The rate of IMand the number of metaplastic glands were significantly different between the R+MNNG group and MNNG group and between the R+MNNG group and R group as well.

The therapeutic effects of retinoic acid (RA) and sodium butyrate (SB) on the induced precancerous lesion were assessed. It was found that the rate of IM, moderate and severe dysplasia and gastric cancer and the number of metaplastic glands in the pyloric and the fundic glandular mucosa were significantly lower in RA treated group (72.0%, 24.0%, 0% and 130.2±93.9 and 51.5±39.1 respectively) and SB treated group (60.0%, 20.0%, 0% and 70.3±29.6 respectively) than in the untreated groups.

The mucosal flaps with a pedicle from the stomach wall were transplanted to the duodenum, jejunum and colon respectively and the rats were killed in the 3rd, 6th, 9th and 12th month after transplantation. IM was observed in all the gastric grafts to the intestines photomicroscopically.

On the basis of the findings listed above, it is concluded that the administration of R+MNNG is the method of choice to induce IM of gaatric mucosa in Wistar rats; RA and SB are safe and efficacious therapeutic agents in the reverse therapy for the precancerous lesion of the gastric glandular mucosa in Wistar rats; and IM of gastric mucosa might be a pH-related change. A hypothesis for the mechanism of IM of gastric mucosa is proposed.

Key words gastric mucosa; intestinal metaplasia ; precancerous conditions; tretinoin; sodium butyrate ; reverse therapy ; rat

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