抑制作用. Ket 是否对 Neu 蛋白激酶 C (PKC)有直接抑制作用有待研究.

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甘草酸差向异构体对大鼠肝损害的治疗作用

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Therapeutic effects of epimeric glycyrrhizic acids on hepatic injury in rats

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ABSTRACT The effects of the epimeric glycyrrhizic acids (GA), 18α-form and 18β-form, on *D*-galactosamine (Gal)-induced acute liver injury and fulminating hepatic failure (FHF) in rats were studied. In rats of acute liver injury, extensive liver parenchymal cell damage was observed by the elevation of alanine aminotransferase (ALT) activity and confirmed by significant histopathological changes 24 and 48 h after ip Gal 450 mg · kg^{-1}. Moreover, marked elevation in the liver putrescine levels occurred along with that of serum ALT. The spermidine and spermine levels did not alter significantly. GA 18α-form 300 mg · kg^{-1} ip suppressed the elevation of serum ALT and liver putrescine levels, and improved all the histopathologic features. On the other hand, GA 18β-form 300 mg · kg^{-1}, which exhibited inhibitory effects 24 h after ip Gal, showed no action 48 h after ip Gal. The ALT levels in the serum from GA 18α-form, 18β-form, *vs* control groups after 24 h were 70 ± 24 ($P<0.01$) and 78 ± 42 ($P<0.01$) *vs* 155 ± 57, and after 48 h were 74 ± 25 ($P<0.01$) and 258 ± 99 ($P>0.05$) *vs* 293 ± 110. The putrescine contents (nmol · g^{-1}) in the liver from GA 18α-form, 18β-form, *vs* control after 24 h were 34 ± 9 ($P<0.01$) and 51 ± 12 ($P<0.01$) *vs* 139 ± 29, and after 48 h were 16 ± 3 ($P<0.01$) and 150 ± 11 ($P>0.05$) *vs* 156 ± 23.

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In rats of FHF, observed 120 h after iv GA 18α-form (500 mg · kg^{-1}) and 18β-form (500 mg · kg^{-1}), the survival (13 / 29) increased ($P<0.01$) in 18α-form-treated rats vs that of control (2 / 29), but not in 18β-form-treated rats (3 / 29). These results demonstrate that the inhibitory action of GA 18α-form on Gal-induced hepatic injury is superior to that of 18β-form.

KEY WORDS glycyrrhizic acid; galactosamine; putrescine; stereoisomers; liver diseases; liver function tests

提要 甘草酸差向异构 α 体能明显阻止 *D*-氨基半乳糖(Gal) 450 mg · kg^{-1} 中毒 24、48 h 后引起的大鼠血清 ALT 活力增高、使肝组织腐胺水平下降，肝组织病变减轻；提高 Gal 1500 mg · kg^{-1} 引起的暴发性肝衰竭(FHF)大鼠存活率. 而 β 体不能改善 Gal 中毒 48 h 后大鼠的病情，对 FHF 大鼠也无疗效. 提示，α 体抗 Gal 肝损害作用优于 β 体.

关键词 甘草酸；氨基半乳糖；腐胺；立体异构体；肝病；肝功能试验

18*H*-甘草次酸差向异构体(18α-glycyrrhetinic acid, α-GTA; 18β-glycyrrhetinic acid, β-GTA)的生物活性差异很大，α-GTA 的抗炎作用比 β-GTA 大得多，而抗毒作用则不如后者[1]. 但对 18α-甘草酸(18α-glycyrrhizic acid, α-GA)的抗肝损害作用，尚未见报道. 本文比较了两个甘草酸异构体对 *D*-氨基半乳糖致大鼠急性肝损害及暴发性肝功能衰竭(fulminating hepatic failure, FHF)的治疗效果.

MATERIALS

Wistar ♂大白鼠，急性肝损害选用体重 135 ± 6 g; FHF 选用体重 238 ± 12 g. 在实验前禁食 20 h，自由饮水，实验期间喂 10% 葡萄糖水.

D-氨基半乳糖盐酸盐 (*D*-galactosamine hydrochloride, Gal) 98% GLC 测定，mp 182–185 ° (dec); 18α-甘草酸单铵盐 (18α-glycyrrhizic acid monoammonium salt dihydrate, α-GA, α 体) 97% GLC 测定，mp 212 ° (dec); 18β-甘草酸单铵盐 (18β-glycyrrhizic acid monoammonium salt dihydrate, β-GA, β 体) 99% HPLC 测定，mp 216 ° (dec). Gal, α-GA, β-GA 均由本院实验室制备. 以上试药使用时以灭菌生理盐水配制.

METHODS AND RESULTS

α 体与 β 体对急性肝损害的影响 大白鼠 76 只，均分 4 组. ①正常动物给药组、②肝损害对照组、③ α 体给药组与④ β 体给药组. 除 ①外，3 组动物同时 ip 10% Gal 450 mg · kg^{-1}[1,2]给药剂量根据 α 体小鼠 ig LD_{50} 为 6.46 g · kg^{-1} (95%可信限为 4.42–9.44 g · kg^{-1})，β 体小鼠 ig LD_{50} 为 4.32 g · kg^{-1} (95%可信限为 3.37–5.53 g · kg^{-1})，以及参考文献[3]剂量，于中毒 2 h、24 h、48 h 后分别 ip α 体与 β 体 300 mg · kg^{-1}; 第一组 ip 同剂量 α 体，对照组 ip 同体积生理盐水，然后于 24 h、48 h 从尾静脉取血，分离血清用金氏改良法测定 alanine aminotransferase (ALT)，按 Malloy-Evelyn 法测定胆红质含量，反向血凝法检测胎甲球蛋白(AFP)及蛋白电泳分析 γ-球蛋白. 同时取肝组织两份，一份作病理组织观察，另一份用气相色谱法[4]测定多胺(腐胺、亚精胺及精胺)及比色法[5]测定糖原含量. 统计学方法：实验结果求均数与标准差，显著性检验用非配对 *t* 检验(unpaired *t* test)，病理组织采用等级分类法(ordered classification method)计算 *t* 值.

结果表明，由 Gal 所引起的急性肝损害大鼠血清 ALT 在中毒后 24 h、48 h，α 体组均明显低于对照组($P<0.01$)，β 体组于中毒后 24 h，与对照组比较，也有明显差异($P<0.01$)，中毒后 48 h，与对照组无显著性差异(Fig 1).

α 体组于 Gal 中毒后 24 h、48 h 的血清胆红质 mg · dl^{-1} 分别为 0.60 ± 0.38, 0.22 ± 0.04，对照组相应为 1.95 ± 0.37, 1.57 ± 0.69，两组间均有显著性差异($P<0.01$); β 体组于 Gal 中

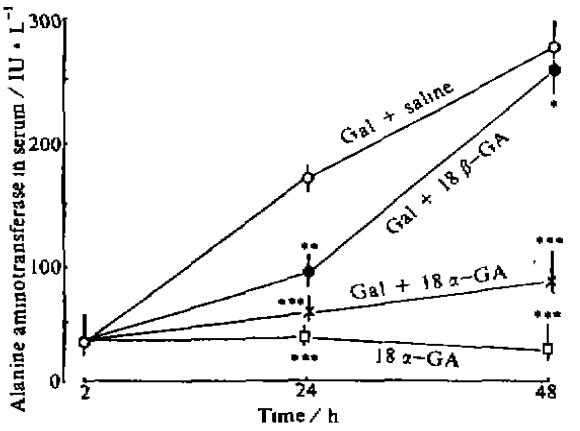

Fig 1. Effects of ip epimeric glycyrrhizic acids 300 mg · kg^{-1} on serum alanine aminotransferase of rats given galactosamine 450 mg · kg^{-1} ip. n=9–10. $\bar{x}\pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control group (Gal plus saline).

毒后 24 h、48 h，分别为 1.45 ± 0.62, 1.50 ± 0.87, 与对照组比较，无显著差异($P>0.05$).

两给药组于中毒后 24 h，血清 AFP 的检出率均明显高于对照组；中毒后 48 h，α 体组仍保持较高检出率，而 β 体组与对照组无明显差异(Tab 1).

中毒后 24 h，两给药组的血清 γ-球蛋白均低于对照组，有显著性差异(Tab 1).

Tab 1. Effects of epimeric glycyrrhizic acids ip 300 mg · kg^{-1} on serum γ-globulin and α-fetoprotein of rats after galactosamine ip 450 mg · kg^{-1}. $\bar{x}\pm s$, $^{*}P>0.05$, $^{***}P<0.01$ *vs* control group (Gal plus saline). The "ridit" analysis was employed to establish the significance when all sign (−, +, ++, +++) were compared. Each "mean^{+}" represents the mean of serum AFP positive for rats.

Group	Control	Normal	18α-GA	18β-GA
n	13	9	13	13
γ-Globulin/%	24 ± 4	16 ± 4**	14 ± 3**	16 ± 4***
α-Fetoprotein / 1 : 10				
24 h −	10	9	2	3
+	3	0	5	6
++	0	0	6	4
+++	0	0	0	0
Mean	0.2^{-}	0^{+*}	1.3^{-***}	1.1^{+***}
48 h −	11	9	4	10
+	2	0	4	3
++	0	0	5	0
+++	0	0	0	0
Mean	0.2^{-}	0^{+*}	1.1^{-***}	0.2^{+*}

中毒后 24 h，两给药组肝组织腐胺(putrescine, Put)含量均明显低于对照组，中毒后 48 h，α 体组仍保持低水平，β 体组显著增高至对照组水平，亚精胺(spermidine, Spd)和精胺(spermine, Spm)改变不大(Tab 2).

Tab 2. Effects of epimeric glycyrrhizic acids ip 300 mg · kg^{-1} to rats on polyamine and glycogen contents / g wet liver after galactosamine. n=9 ♂ rats. $\bar{x}\pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control group (Gal plus saline).

Group	Time after galactosamine / h	Putrescine / nmol · g^{-1}	Spermidine / nmol · g^{-1}	Sperimine / nmol · g^{-1}	Glycogen / mg · g^{-1}
Control	24	139.3 ± 29.2	1 066 ± 112	896 ± 39	0.51 ± 0.04
	48	156.2 ± 22.7	986 ± 88	768 ± 44	0.28 ± 0.06
Normal	24	9.5 ± 3.2**	1 154 ± 92*	1 015 ± 107*	42.10 ± 6.25**
	48	8.5 ± 2.7**	1 261 ± 132*	988 ± 39*	52.42 ± 5.13***
18α-GA	24	33.5 ± 9.2**	1 205 ± 115*	919 ± 64*	16.92 ± 1.80**
	48	16.2 ± 3.0***	1 192 ± 106*	1 041 ± 55*	21.51 ± 3.64**
18β-GA	24	51.4 ± 11.8***	987 ± 75*	1 065 ± 69*	11.22 ± 1.54***
	48	149.6 ± 11.4*	821 ± 76*	890 ± 81*	0.66 ± 0.08**

中毒后 24 h，两给药组均有对抗 Gal 降低肝内糖原的作用，48 h β体组肝糖原含量下降至对照组水平(Tab 2).

组织学观察表明，由 Gal 所引起的空泡变性，嗜酸性变、肝细胞坏死及炎症细胞浸润等，α体组均见明显减轻．中毒后 48 h，几乎 80%大鼠肝组织恢复接近正常水平；β体组只有在中毒 24 h，上述病理改变有明显减轻，而中毒 48 h 其肝组织损害反而加重，与对照组无明显差异(Tab 3, Fig 2, Plate 1).

α体与β体对 FHF 的影响 大鼠 87 只，均分 3 组．3 组同时 ip 10% Gal 1500 mg · kg^{-1} [6]，两给药组于注射 Gal 后 2、24、48 h 分别 ip α体、β体 500 mg · kg^{-1} (共给药 3 次)，对照组 ip 同体积生理盐水．然后于 24 和 48 h 从尾静脉取血测定 ALT，并观察 120 h 内三组大鼠死亡数.

实验表明，由 Gal 引起的 FHF 大鼠，各组于中毒后 24 h，血清 ALT 明显增高；48 h 绝大多数动物出现肝昏迷，严重出血、瘫痪及抽搐等症状，大鼠死亡时间集中在 49–72 h 间．α体组的中毒症状要比对照组轻得多，血清 ALT 也显著低于对照组，存活率明显提高．β体组 48 h 血清 ALT、死亡率与对照组无显著差异(Tab 4).

DISCUSSION

本文根据临床甘草酸剂量与疗效基本呈正相关[7]，采用 300 mg · kg^{-1} 及 500 mg · kg^{-1} α、β体治疗大鼠急性肝损害及 FHF，结果显示α体能明显抑制 Gal 450 mg · kg^{-1} 24、48 h 所引起的肝功能改变，提高 Gal 引起 FHF

Tab 3. Pathological findings in rat liver 48 h after ip epimeric glycyrrhizic acids 300 mg · kg^{-1}. Galactosamine were ip at a dose of 450 mg · kg^{-1}. *P* value *vs* control group (Gal plus saline).

		Degree of change (number of livers)				*P* value
		Severe	Moderate	Mild	None	*vs* control
Cytoplasmic vacuolization	Control	8	2	1	0	
	α–GA	0	5	2	4	<0.01
	β–GA	6	3	2	0	>0.05
Acidophilic degeneration	Control	4	6	1	0	
	α–GA	1	3	4	3	<0.01
	β–GA	3	5	2	1	<0.05
Councilman bodies	Control	7	2	2	0	
	α–GA	0	3	4	4	<0.01
	β–GA	5	1	3	2	<0.05
Necrosis	Control	8	2	1	0	
	α–GA	1	1	2	7	<0.01
	β–GA	6	4	1	0	>0.05
Inflammatory cell infiltration	Control	3	7	1	0	
	α–GA	0	2	3	6	<0.01
	β–GA	2	5	4	0	<0.05
Hemorrhagic change	Control	0	6	2	3	
	α–GA	0	0	2	9	<0.01
	β–GA	0	4	5	2	>0.05

Tab 4. Effects of 18α- and 18β-glycyrrhizic acids ip 500 mg · kg^{-1} on activities of serum alanine aminotransferase and survivals in rats with galactosamine (1500 mg · kg^{-1})-induced 5-d fulminant hepatic failure. $n=29$, $\bar{x} \pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control group (Gal plus saline).

	Control	18α-GA	18β-GA
Alanine aminotransferase			
24 h	1 482 ± 788	625 ± 199**	1 150 ± 650**
48 h	1 671 ± 821	991 ± 256**	1 596 ± 793*
Survivals	2	13	3

大鼠的存活率，同剂量的β体仅对中毒后 24 h 有效，中毒后 48 h 无治疗效果，也不能增加 FHF 大鼠的存活数.

α体对正常大鼠的 ALT 活力无明显影响，说明其降 ALT 的作用并非是直接抑制 ALT 活力所致；血清 AFP 检出率增高，表明肝细胞再生活跃[13]；血清 γ-球蛋白的下降，反映了肝组织间质炎症减轻，具有非特异性抑制炎症反应作用；肝组织 Put 含量下降，反映肝细胞恢复加速[8]. 病理组织学证明，α体具有明显抑制或减轻肝细胞的变性、坏死及炎性浸润，说明生化上的反映与病理组织的改善相一致. α体明显提高 FHF 大鼠存活率，可能与抑制肝细胞坏死，促进肝细胞再生有关. 由此可见，甘草酸两差向异构体虽都具有抗肝损害作用，但α体明显优于β体.

根据构象分析表明[9,10]，18β-H 与 C_{30} 上的羧基存在同一平面，18α-H 不在同一平面，由于位阻效应，后者的亲脂性大于前者，在体内易与受体蛋白结合. 此外，α体分子结构中 D / E-环为反式构型(*trans*-conformation)与泼尼松龙(prednisolone)相似，易与类固醇激素的靶细胞受体结合，故其抗炎作用大于β体(D / E-环为 *cis*-conformation). 因此α体可能通过控制炎症而发挥抗肝损害作用，而β体不能抑制坏死性剂量 Gal 48 h 的肝损害，可能与其低抗炎作用有关. 至于α体的抗肝损害作用与抗炎作用是否一致，有待研究.

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