arrhythmias induced by hypothalamic electric stimulation. *Acta Pharmacol Sin* 1989; **10** : 147–50.

6 Meerson FZ, Shabunina EV, Belkina LM, Pshennikova MG. Prevention of rhythm disturbances in acute ischemia and reperfusion of the heart with GABA–accumulating factor. *Kardiologia* 1987; **27** : 87–9.

外源性 γ-氨基丁酸对实验性心律失常的作用

王来仪、孟娟如、吴 弢
(北京心肺血管医学中心，北京 100029，中国)
李荣芷、何云庆、张启博
(北京医科大学药学院，北京 100083，中国)

提要 本实验证明 GABA 10 mg · kg^{-1} iv 使乌头碱诱发的 VT 由对照组的 6/10 减少至 0/10 ($P<0.05$)，VT 持续时间由 25.4 ± 2.8 min 缩短到 14.5 ± 9.8 min ($P<0.01$)。冠状动脉结扎诱发的 VF 由 4/5 减少到 0/5 ($P<0.01$)，哇巴因诱发 VT 和 VF 的阈剂量显著增加。以上作用呈剂量依赖性并与普鲁卡因胺 10 或 5 mg · kg^{-1} iv 作用相同。提示 GABA iv 可预防 VT 和 VF。

关键词 γ-氨基丁酸；心律失常；乌头碱；哇巴因；缺血

BIBLID: ISSN 0253–9756 中国药理学报 *Acta Pharmacologica Sinica* 1992 Sep; **13** (5) : 427–430

Scavenging effects of phenylpropanoid glycosides on superoxide and its antioxidation effect[1]

LI Ji, ZHENG Rong–Liang[2], LIU Zi–Min[3], JIA Zhong–Jian[3] (*Department of Biology*[3], *Institute of Organic Chemistry, Lanzhou University, Lanzhou 730000, China*)

ABSTRACT The antioxidative activities of six phenylpropaniod glycosides (PPG) extracted from *Pedicularis striata* and *Pedicularis lasiophrys* for inhibiting the lipid peroxidation induced by Fe^{2+} / ascorbic acid in mouse liver microsome may be related to the number and steric position of phenolic hydroxyl groups (PHG) they possess (32.5 μmol · L^{-1} to 65.0 μmol · L^{-1}). The scavenging effects of PPG for superoxide produced by NBT / PMS / NADH system may be related to both the number of PHG and their conjugated system (16.0 μmol · L^{-1} to 65.0 μmol · L^{-1}).

KEY WORDS phenylpropanoid glycosides; superoxide; free radical scavengers; antioxidants

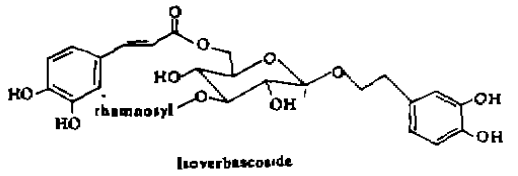
Pedicularis is used in folk medicine as cardi–tonics for treatment of collapse, exhaustion and senility[1], and is usually called "pseudo–ginseng" by local inhabitants of northwestern China. PPG, a class of constituents of *Pedicularis*, showed antiviral[2] and antiplatelet properties[3], inhibited leukotriene B_4 formation[4], but had little effect on blood pressure, heart rate, microbial growth, or prostaglandin biosynthesis[4]. Phenolic compounds possess both antiradical and antioxidative properties. We have reported the antioxidative and scavenging activities of 7 natural hydroxylated flavonoids[5] and 6 phenols[6]. Since PPG are polyphenols, we investigated the superoxide–scavenging and antioxidative effects of the 6 natural PPG.

MATERIALS AND METHODS

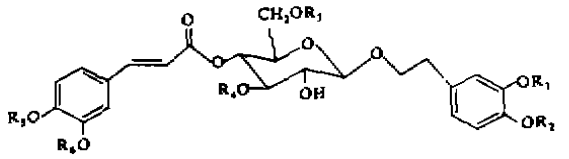
Agents Isoverbascoside, verbascoside, echinacoside and pedicularioside A (new compound) were extracted from *Pedicularis striata*, and cistanoside D

Received 1991–11–09 Accepted 1992–07–01
[1] Project supported by the National Natural Science Foundation of China, № 38970238.
[2] To whom correspondence should be addressed.

was extracted from *Pedicularis lasiophrys*, and permethyl verbascoside was modified from verbascoside[1,7]. 2-Thiobarbituric acid (TBA) and β-nicotinamide adenine dinucleotide (reduced form, NADH) were from the Sigma Chemical Co, USA. Ascorbic acid was from Xi-an Chemical Co. *N*-Methylphenazonium methosulphate (PMS) was produced by Shanghai Qianjing Chemical Co. All the other reagents were of AR.



Isoverbascoside



Verbascoside	$R_1=R_2=R_3=R_5=R_6=H$	
	R_4=rhamnosyl	
Echinacoside	$R_1=R_2=R_5=R_6=H$	
	R_3=glucosyl	R_4=rhamnosyl
Pedicularioside A	$R_1=R_2=R_5=R_6=H$	
	R_3=rhamnosyl	R_4=apiosyl
Cistanoside D	$R_2=R_3=R_5=H$	
	$R_1=R_6=Me$	R_4=rhamnosyl
Permethyl verbascoside	$R_1=R_2=R_5=R_6=Me$	
	$R_3=H$	R_4=rhamnosyl

Phenylpropanoid glycosides

Superoxide generation Superoxide were generated in 1.5 ml of samples which contained NADH 73 μmol · L^{-1}, PMS 15 μmol · L^{-1}, nitrobluetetrazole (NBT) 50 μmol · L^{-1} and PPG of varying concentrations in Tris-HCl buffer 16 mmol · L^{-1}, pH 8.0 at 20℃. The blank sample with no PMS had no absorbance (*A*) at 560 nm. After *A* at 560 nm had reached a maximum and became stable in the control tube without PPG, the *A* was read[8]. Each test had 3 replicates. All PPG were dissolved in deionized water.

Lipid peroxidation in mouse liver microsome Twenty Kunming mice of either sex, weighing 20 ± *s* 2 g were killed by cervial dislocation. The liver was repidly homogenized in ice-cold sucrose 0.25 mol · L^{-1} and centrifuged at 9810 × *g* for 20 min. The supernatant was centrifuged at 95850 × *g* for 40 min, the microsomal pellet was rinsed with ice-cold KCl 0.15 mol · L^{-1} to remove any adsorbed supernatant (as the sucrose interferes with the determination of malondialdehyde). Protein content was measured colorimetrically[9]. The microsomal pellet was either stored at −20℃ or resuspended in ice-cold KCl 0.15 mol · L^{-1}. The samples consisted of pH 7.4 potassium phosphate buffer solution (PBS) 0.2 mol · L^{-1}, $FeSO_4$ 10 μmol · L^{-1}, 200-400 μg of microsomal protein in a final volume of 1.0 ml. The reaction was initiated by the addition of ascorbic acid 0.1 mmol · L^{-1}. After incubation at 37℃ for 1 h with constant shaking, the reaction was stopped by 20% (wt / vol) trichloroacetic acid (TCA) 1.0 ml and 0.67% (wt / vol) TBA 1.5 ml in succession. PBS 0.1 ml containing the compounds to be tested was added to inhibit the microsomal lipid peroxidation. PBS 0.1 ml alone was added serving as the control and TCA addition before incubaiton for blank sample.

Statistical comparisons were made by *t* test.

RESULTS

Inhibition of microsomal lipid peroxidaition Among the 6 tested compounds, isoverbascoside, verbascoside, echinacoside, and pedicularioside A, which possess 4 phenolic hydroxyl groups (PHG), inhibited microsomal lipid peroxidation concentration-dependently and efficiently. Their inhibitory effect was stronger than that of cistanoside D, GSH, and gallic acid. Gallic acid possesses 3 PHG. Isoverbascoside with a 50% inhibition concentration of 5.57 μmol · L^{-1} was the strongest antioxidant, while permethyl verbascoside possessing no PHG did not inhibit microsomal liqid peroxidation even at a concentration of 65.0 μmol · L^{-1} (Tab 1).

Tab 1. Effect of phenylpropanoid glycosides on microsomal lipid peroxidation. $n=3$, $\bar{x}\pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

	Concn / $\mu mol \cdot L^{-1}$	MDA formation A_{532}	Inhibition %
Control		0.456 ± 0.025	
GSH	32.5	0.427 ± 0.026*	6.4
	65.0	0.402 ± 0.015**	11.8
Gallic acid	32.5	0.386 ± 0.005**	15.4
	65.0	0.322 ± 0.022**	29.4
Isoverbascoside	32.5	0.000 ± 0.000***	100
	65.0	0.000 ± 0.000***	100
Verbascoside	32.5	0.370 ± 0.010**	18.9
	65.0	0.055 ± 0.015***	87.9
Echinacoside	32.5	0.34 ± 0.03**	24.8
	65.0	0.07 ± 0.03***	85.1
Pedicularioside A	32.5	0.385 ± 0.003**	15.6
	65.0	0.102 ± 0.016***	77.6
Cistanoside D	32.5	0.40 ± 0.03**	12.9
	65.0	0.39 ± 0.03**	15.4
Permethyl verbascoside	32.5	0.446 ± 0.024*	2.2
	65.0	0.448 ± 0.018*	1.8
Glucose	65.0	0.445 ± 0.016*	2.4
Rhamnose	65.0	0.450 ± 0.027*	1.3
Apiose	65.0	0.45 ± 0.03*	1.3

MDA: malondialdehyde; *A*: absorbance

Inhibition of superoxide production

Isoverbascoside, verbascoside, echinacoside, and pedicularioside A, all possessing 4 PHG, were more effective than cistanoside D which possesses only 2 PHG. Surprisingly, permethyl verbascoside has no PHG, but its inhibitory effect was stronger than that of cistanoside D (Tab 2).

Glucose, rhamnose, and apiose alone did not scavenge the superoxide nor inhibit the microsomal lipid peroxidation (Tab 1 and 2).

Tab 2. Scavenging activities of phenylpropanoid glycosides for superoxide (O_2^-) produced by NBT / PMS / NADH system. $n=3$, $\bar{x}\pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

	Concn / $\mu mol \cdot L^{-1}$	Superoxide generation A_{560}	Inhibition %
Control		0.294 ± 0.020	
GSH	16.0	0.233 ± 0.012**	20.7
	32.5	0.218 ± 0.007**	25.9
	65.0	0.211 ± 0.010**	28.2
Gallic acid	16.0	0.215 ± 0.020**	26.9
	32.5	0.189 ± 0.022**	35.7
	65.0	0.169 ± 0.004**	42.5
Isoverbascoside	16.0	0.154 ± 0.017**	47.7
	32.5	0.125 ± 0.012***	57.6
	65.0	0.111 ± 0.021***	61.2
Verbascoside	16.0	0.175 ± 0.013**	40.6
	32.5	0.137 ± 0.012***	53.6
	65.0	0.103 ± 0.025***	63.9
Echinacoside	16.0	0.165 ± 0.012**	43.8
	32.5	0.145 ± 0.023***	53.6
	65.0	0.096 ± 0.027***	66.6
Pedicularioside A	16.0	0.153 ± 0.013**	47.8
	32.5	0.126 ± 0.021***	56.8
	65.0	0.088 ± 0.025***	69.4
Cistanoside D	16.0	0.253 ± 0.008**	13.9
	32.5	0.212 ± 0.013**	27.8
	65.0	0.162 ± 0.028**	44.9
Permethyl verbascoside	16.0	0.196 ± 0.019**	33.5
	32.5	0.164 ± 0.009**	44.2
	65.0	0.154 ± 0.015**	46.4
Glucose	65.0	0.290 ± 0.021*	1.4
Rhamnose	65.0	0.29 ± 0.03*	2.0
Apiose	65.0	0.292 ± 0.009*	0.7

A: absorbance

DISCUSSION

As phenolic compounds PPG may react with superoxide via a one-electron transfer mechanism or hydrogen abstraction mechanism to give the semiquinones[10], hence, the scavenging activities of PPG for superoxide may due to their reductive activities which may be related to the number of PHG and the conjugated system.

The activities of PPG for inhibiting lipid peroxidation may depend mainly on the number and steric position of PHG[11]. On the basis of their stereochemistry, the PHG of caffeoyl moiety on verbascoside, echinacoside, and pedicularioside A would be easier to form "H-bond" with the hydroxyl groups of rhamnosyl or apiosyl than that on isoverbascoside, thus the latter reserved more PHG than the former 3 compounds did. That

is why the isoverbascoside got the strongest inhibitory activity.

The PHG are able to inhibit lipid peroxidation through scavenging the microsomal peroxyl radicals induced by Fe^{2+} / Vit.C[11,12].

$$LOO^{\cdot} + PhOH \rightarrow LOOH + PhO$$

On the other hand, like other phenolic compounds such as rutin and quercetin[11], the iron chelation by hydroxyl-substituted but not methoxy-substituted PPG may play an important role in inhibiting the lipid peroxidation. This work is now in progress.

REFERENCES

1 Liu ZM, Jia ZJ. Phenylpropanoid and indoid glycosides from *Pedicularis striata*. *Phytochemistry* 1991; **30** : 1341-4.

2 Kong B, Dustmann JH. The caffeoylics as a new family of natural antiviral compounds. *Naturwissenschaften* 1985; **72** : 659-61.

3 Cano E, Veiga M, Jimenez C, Riguera R. Pharmacological effects of three phenylpropanoid glycosides from *Mussatia*. *Planta Med* 1990; **56** : 24-6.

4 Jimenez C, Villaverde MC, Riguera R, Castedo L, Stermitz F. Triterpene glycosides from *Mussatia* species. *Phytochemistry* 1989; **28** : 2773-6.

5 Chen YT, Zheng RL, Jia ZJ, Ju Y. Flavonoids as superoxide anion scavengers and antioxidants *Free Radic Biol Med* 1990; **9** : 19-21.

6 Zhou YC, Zheng RL. Phenolic compounds and an analog as superoxide anion scavengers and antioxidants. *Biochem Pharmacol* 1991; **42** : 1177-9.

7 Jia ZJ, Liu ZM, Wang CZ. Phenylpropanoid and indoid glycosides from *Pedicularis lasiophrys*. *Phytochemistry* 1991; **30** : 3745-7.

8 Ponti V, Dianzani MV, Cheeseman KJ, Slater TF. Studies on the reduction of nitroblue tetrazolium chloride mediated through the action of NADH and phenazine methosulphate. *Chem Biol Interact* 1978; **23** : 281-91.

9 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent *J Biol Chem* 1951; **193** : 265-75.

10 Afanas'ev IB, Polozova NI. One electron oxidation of *p*- and *o*-dihydroxybenzenes by oxygen radical anion in aprotic medium. *Zh Organ Khim* 1978; **14** : 1013-6.

11 Afanas'ev IB, Dorozhko AI, Brodskii AV, Kostyuk VA, Potopovich AI. Chelating and free radical scavenging mechanism of inhibitory action of rutin and quercetin in lipid peroxidation. *Biochem Pharmacol* 1989; **38** : 1763-9.

12 Chesseman KH. Effects of scavengers and inhibitions on lipid peroxidation in rat liver microsomes. In: McBrien DCH, Slater TF, editors. *Free Radicals, Lipid Peroxidation and Cancer*. New York: Academic Press, 1982 : 197-214.

427-430

苯丙素甙的抗氧化作用及对超氧化物的清除作用

R965.1

李 忌, 郑荣梁, 刘自民[1], 贾忠建[1] (兰州大学生物系, [1]兰州大学有机化学研究所, 兰州 730000, 中国)

提要 6种从马先蒿(*Pedicularis striata* 和 *Pedicularis lasiophrys*)中提取的苯丙素甙化合物(32.5-65.0 $\mu mol \cdot L^{-1}$)对小鼠肝微粒体脂质过氧化的抗氧化能力与其酚羟基数目和立体结构有关. 苯丙素甙(16.0-65.0 $\mu mol \cdot L^{-1}$)对超氧化物的清除能力则取决于酚羟基数目和共轭体系的强弱.

关键词 苯丙素甙; 超氧化物; 游离基清除剂; 抗氧化剂

抗氧化作用

Instructions to authors

Please read *Acta Pharmacol Sin* 1992 Jan; **13** (1) : 3-8.
Br Med J 1991 Fab 9; **302** (6772) : 388-41.
N Engl J Med 1991 Feb 7; **324** (6) : 424-8.