乐秀芳、韩家娴、沈祖铭、杨蔚怡、陆丽娟
(中国科学院上海药物研究所、上海 200031、中国)
李炳钧、王 春、许秀坤
(中国科学院昆明植物研究所、昆明 650204、中国)

提要 特利回生(trewiasine、TWS)是美登素类化合物. 体外研究表明对4种人体肿瘤细胞株有明显抗癌活性，其中对白血病U937最为敏感，1 $\mu g \cdot ml^{-1}$ 抑制率大于90% ($P<0.01$). 特利回生对多种实验动物肿瘤如腹水型的S180，肝癌，U14及实体型的Lewis肺癌等均有明显疗效. 剂量为10 $\mu g \cdot kg^{-1}$ 及50 $\mu g \cdot kg^{-1}$ 时，对小鼠白细胞无明显影响. 特利回生也不诱导SCE形成.

关键词 特利回生；美登素；培养的肿瘤细胞；实验性肿瘤；植物性抗肿瘤药

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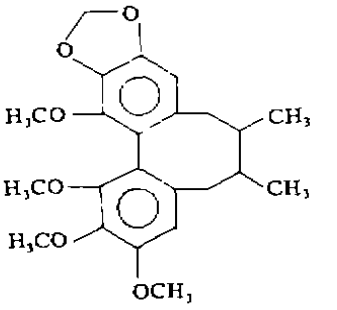
Effect of schisandrin B on lipoperoxidative damage to plasma membrane of rat liver *in vitro*

ZHANG Tie-Mei[1], WANG Bao-En[1], LIU Geng-Tao[2] (*Department of Pharmacology, Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing 100050, China*)

ABSTRACT The effect of schisandrin B (Sin B) on oxygen free radicals-induced lipoperoxidative damage to plasma membrane of rat hepatocytes was investigated. When the plasma membrane of rat hepatocytes was incubated with iron / cysteine or Vit C / NADPH, the production of malondialdehyde (MDA) and consumption of NADPH increased, while the membrane fluidity reduced. Addition of Sin B (3-25 $\mu g \cdot ml^{-1}$) to the incubation mixture inhibited all these alterations of the plasma membrane induced by iron / cysteine and Vit C / NADPH. The results indicated that Sin B could maintain membrane stability of rat hepatocytes under oxidative stress.

KEY WORDS schisandrin B; membrane fluidity; lipid peroxidation; liver; cell membrane; malondialdehyde; NADP

Lipid peroxidation induced by oxygen free radicals is a deteriorative reaction, which results in cell injury[1]. Plasma membrane of cells is rich in polyunsaturated fatty acid and sensitive to lipid peroxidation. Our previous studies demonstrated that schisandrin B (SinB) with the following structure, one of the active components isolated from *Fructus Schisandrae Chinensis* (Turcz) Baill, has significant protective effect against lipoperoxidative damage to cultured rat hepatocytes[2]. The release of transaminase and lactic dehydrogenase (LDH) and malondialdehyde (MDA) production as well as damages of cell surface of hepatocytes were all counteracted[2]. In this paper, the effects of Sin B on MDA production, NADPH consumption and decrease of membrane fluidity of plasma membrane of rat hepatocytes



Schisandrin B

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[1] *Beijing Friendship Hospital, Beijing 100050, China.*
[2] To whom correspondence should be addressed.

induced by iron / cysteine and Vit C / NADPH were further studied.

MATERIALS AND METHODS

Wistar rats weighing 182 ± *s* 25 g were used. 1,6–Diphenyl–1,3,5–hexatriene (DPH) and NADPH were purchased from Sigma Chemical Co. Cysteine was obtained from E Merck, Darmstadt. Sin B, isolated from *Fructus Schiandrae Chinensis* (Turca) Baill, was kindly provided by professor LI Lian–Niang of our institute and its purity was over 98%. The other reagents were purchased from Beijing Chemical Reagent Plant.

Preparation of plasma membrane of rat liver The plasma membrane was prepared from liver homogenate of rats through sucrose gradient centrifugation[3]. Plasma protein content was determined by the method of Lowry[4].

Determination of malondialdehyde (MDA) Phosphate buffer 1 ml containing plasma membrane protein 4 mg was incubated with $FeSO_4$ 50 μmol · L^{-1} / cysteine 200 μmol · L^{-1} or Vit C 250 μmol · L^{-1} / NADPH 130 μmol · L^{-1} at 37℃ water bath for 30 min. The amount of MDA product in the incubation mixture was determined by fluorometrically[5].

Measurement of NADPH consumption Phosphate buffer (0.1 mol · L^{-1}, pH 7.4) 1 ml containing plasma protein 1 mg (0.2 ml) and NADPH 380 μmol · L^{-1} was incubated with $FeSO_4$ 50 μmol · L^{-1} / cysteine 200 μmol · L^{-1} or Vit C 250 μmol · L^{-1} / NADPH 380 μmol · L^{-1} in the presence or absence of Sin B 25 $\mu g \cdot ml^{-1}$ in a cuvette at 37℃. The value of absorption of NADPH at 340 μmol · L^{-1} was monitored for 20 min immediately after mixing the suspension. The consumption of NADPH is expressed as reduction of absorbance within 20 min.

Measurement of membrane fluidity The plasma membrane suspension 1.5 ml (10 μg protein · ml^{-1}) was mixed with 1.5 ml of the lipid probe DPH (10 μmol · L^{-1}). $FeSO_4$ 100 μmol · L^{-1} and cysteine 750 μmol · L^{-1} were added to this incubation mixture in the presence or absence of Sin B (3–30 $\mu g \cdot ml^{-1}$). The mixture was incubated at 25℃ for 30 min. The fluorescence intensity was measured at excitation and emission wave–lengths of 360 and 432 nm, respectively. The fluorescence polarization and microviscosity were calculated as described in reference (6).

RESULTS

Effect of CCl_4 and $FeSO_4$ on MDA formation by the plasma membrane of rat liver There was an absorption peak at 532 nm when the plasma membrane was incubated with $FeSO_4$ / cysteine. Whereas the plasma membrane incubated with CCl_4 and NADPH, no abosrption peak at 532 nm was seen (Fig 1). This result indicated that $FeSO_4$ / cysteine induced MDA production by the plasma membrane, while CCl_4 did not induce formation of MDA in the same system. Therefore, $FeSO_4$ / cysteine was used as a system to induce lipid peroxidation of plasma membrane in the following experiments.

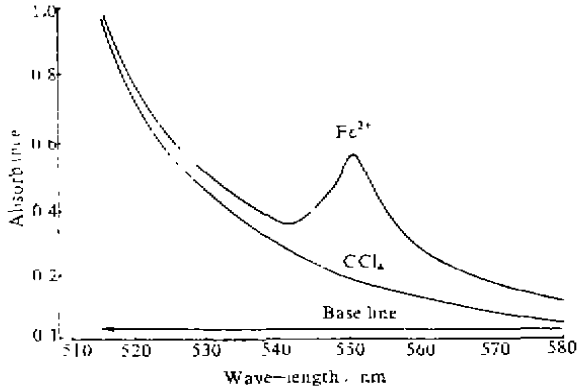

Fig 1. Absorption spectrum of malondialdehyde formation by plasma membrane of rat hepatocytes induced by $FeSO_4$ / cysteine and CCl_4 *in vitro*.

Effect of Sin B on MDA production by plasma membrane of rat liver induced by

$FeSO_4^{2+}$ / cysteine and Vit C / NADPH MDA formation markedly increased after the plasma incubated with Fe^{2+} / cysteine or Vit C / NADPH for 30 min. Addition of Sin B to the incubation mixture significantly inhibited MDA production (Tab 1). The inhibitory rates of MDA formation by Sin B were 69 and 78%, respectively.

Tab 1. Effect of Sin B on lipid peroxidation of plasma membrane of rat hepatocytes induced by $FeSO_4$ / cysteine and Vit C / NADPH *in vitro*. $n=4$, $\bar{x} \pm s$, $^{***}P<0.01$.

Sin B / $\mu g \cdot ml^{-1}$	MDA, nmol / mg protein	
	$FeSO_4$ / cysteine	Vit C / NADPH
0	3.25 ± 0.27	3.23 ± 0.28
25	$1.24 \pm 0.26^{***}$	$0.82 \pm 0.15^{***}$

Effect of Sin B on NADPH consumption When the plasma membrane were incubated with Fe^{2+} or Vit C / NADPH together at 37℃, increase of NADPH consumption was associated with lipid peroxidation. Addition of Sin B reduced NADPH consumption (Fig 2).

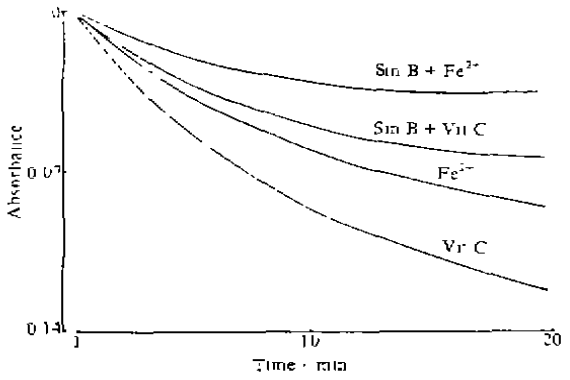

Fig 2. Inhibitory effect of Sin B on NADPH consumption of plasma membrane of rat hepatocytes induced by $FeSO_4$ / cysteine and Vit C.

Effect of Sin B on fluidity of plasma membrane of rat hepatocytes under lipid peroxidation The fluorescence polarization and microviscosity of plasma membrane of rat livers increased under $FeSO_4$ / cysteine–induced lipid peroxidation, indicating that the fluidity of the plasma membrane reduced. Pre–incubation with Sin B (3–30 $\mu g \cdot ml^{-1}$) counteracted this reduction of membrane fluidity (Tab 2). However, no influence of Sin B on normal fluidity of the plasma membrane was noted.

Tab 2. Effect of Sin B ($\mu g \cdot ml^{-1}$) on $FeSO_4$ / cysteine induced lipid fluidity of plasma membrane of rat hepatocytes *in vitro*. $n=4$, $\bar{x} \pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$.

Group	Fluorescence polarization	Microviscosity $\bar{\eta}$ / P
Normal control	0.179 ± 0.009	1.26 ± 0.08
Sin B	$0.170 \pm 0.027^{*}$	$1.21 \pm 0.23^{*}$
$FeSO_4$	$0.209 \pm 0.009^{**}$	$1.66 \pm 0.22^{**}$
$FeSO_4$ + Sin B		
3	$0.124 \pm 0.009^{**}$	$0.74 \pm 0.07^{**}$
15	$0.131 \pm 0.008^{***}$	$0.86 \pm 0.10^{***}$
30	$0.172 \pm 0.027^{***}$	$1.21 \pm 0.43^{***}$

DISCUSSION

Both $FeSO_4$ / cysteine and Vit C / NADPH systems generate hydroxyl radical and superoxide anion. CCl_4 generates methyltrichloride radical (CCl_3) by the activation of liver cytochrome P–450. The active oxygen radicals and non–oxygen radicals are all capable of initiating lipid peroxidation of biomembranes. The present study demonstrated that active oxygen free radicals induced MDA formation by plasma membrane of rat hepatocytes. CCl_4 did not induce MDA formation in the same membrane system, of which lack of cytochrome P–450 in the plasma membrane may be the cause. Peroxidation of polyunsaturated fatty acid in biomembranes has been proposed as one of the mechanisms by which free radicals induce structural and functional damages of membranes[7]. The inhibition by Sin B of MDA formation and

NADPH consumption as well as reduction of membrane lipid fluidity indicate that Sin B may stabilize the plasma membrane of rat hepatocytes. Our previous and present studies would be valuable for the explanation of the therapeutic effects of *Fructus Schisandrae* in the treatment of viral hepatitis.

REFERENCES

1 Halliwell B, Gutteridge JMC. *Free radicals in Biology and Medicine*. Oxford: Clarendon Press, 1989: 188–266.

2 Zhang TM, Wang BE, Liu GT. Action of schisandrin B, an antioxidant, on lipid peroxidation in primary cultured hepatocytes. *Acta Pharmacol Sin* 1989, **10**: 353–6.

3 Emmelot P, Bos CJ, van Hoeven RP, van Butterswijk WJ. Isolation of plasma membranes from rat and mouse livers and hepatomas. *Methods Enzymol*. NY: Academic Press, 1974; **31**: 75–9.

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

5 Yagi K. A simple fluorometric assay for lipoperoxide in blood plasma. *Biochem Med* 1976; **15**: 212–16.

6 Shinitzky M, Barenholz Y. Fluidity parameters of lipid regions determined fluroescence polarization. *Biochim Biophys Acta* 1978; **515**: 367–94.

7 Slater TF. Free-radical mechanisms in tissue injury. *Biochem J* 1984; **222**: 1–15.

(14) 255–258

五味子乙素对大鼠肝脏质膜过氧化损伤的影响

张铁梅[1], 王宝恩[1], 刘耕陶[2]
(中国医学科学院药物研究所, 北京 100050, 中国)

提要 本文研究了五味子乙素(Sin B)对氧自由基引起大鼠肝脏质膜过氧化损伤的影响. 大鼠肝脏质膜与铁离子和半胱氨酸或维生素 C 和 NADPH 体外温育后, 丙二醛的生成和 NADPH 的消耗量均增加, 而膜流动性降低. 温育中加入 Sin B 3–25 $\mu g \cdot ml^{-1}$, 质膜的上述变化均明显地被抑制. 结果表明 Sin B 能维持大鼠肝细胞膜在氧化性损伤状态下的稳定性.

关键词 五味子乙素; 膜流动性; 脂质过氧化; 肝脏; 细胞膜; 丙二醛; NADP