Cardiovascular Effects of Trilinolein, a Natural Triglyceride Isolated from the Herb Sanchi (Panax Notoginseng)

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Trilinolein is a natural triacylglycerol isolated from the traditional Chinese herb, *Panax notoginseng*, and has been found to have several pharmacological effects. Through various mechanisms, trilinolein has been demonstrated to have anti-ischemic, antiarrhythmic, and antioxidant properties. This article summarizes our research on the myocardial protective effects of trilinolein, which may explain some of the benefits of the natural herb in treating circulatory disorders which have been perceived by empirical practice.

Key Words: Cardioprotection • Sanchi • Trilinolein

INTRODUCTION

In Chinese populations, traditional Chinese medicine (TCM) is often used to maintain good health rather that to cure illness once it has developed, in a similar way to the use of vitamin or mineral supplementation or herbal preparations in Western countries. Trilinolein (Figure 1) was isolated from the traditional Chinese herb, *Sanchi [Sangi]*, which comes from the plant, *Panax notoginseng*.[1] *Panax notoginseng* has been shown to possess a wide spectrum of pharmacological activities, such as hemostatic activity, platelet aggregation inhibitory activity, anti-inflammatory activity, and therapeutic effects in cardiac infarction, cardiac ischemia, and angina pectoris.[2] Trilinolein has linoleic acid as the fatty acid residue at all 3 esterified positions of glycerol, and it has been shown to have various beneficial effects, including improvement of erythrocyte deformability and inhibition of platelet aggregation.[3,4] We have also demonstrated that it possesses an effective antioxidant action in various experimental models.[5-9] Furthermore, our *in vivo* studies have demonstrated that trilinolein has antiarrhythmic effects.[9] All of these findings suggest that trilinolein is a medication with potential applications for the treatment of cardiovascular diseases.

ANTI-ISCHEMIC EFFECT

We investigated the myocardial-protective effects of trilinolein in a rat coronary artery ligation model.[9] Pretreatment with trilinolein, at a dose of 0.1 μg/kg given 15 minutes prior to coronary ligation, resulted in a significant reduction in infarct size. It was concluded that trilinolein may protect the myocardium against ischemic injury during ischemia and reperfusion. Moreover, the mechanism of the myocardial protective effect of trilinolein was further investigated in isolated cardiomyocytes to determine if inhibition of calcium influx and alteration of the activity of superoxide dismutase were involved.[5] In isolated cardiomyocytes, Ca^{2+} influx stimulated by hypoxia/normoxia was effectively re-

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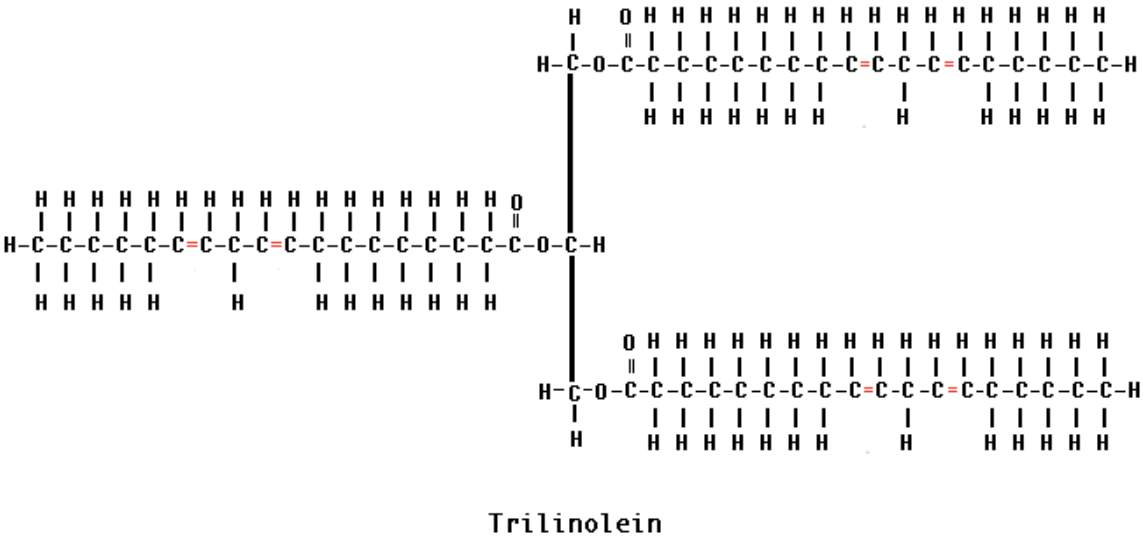

Figure 1. *The chemical structure of trilinolein.*

duced by 34% after pretreatment with trilinolein at a low concentration of 10^{-9} M. Furthermore, in isolated perfused rat hearts subjected to 60 minutes of global hypoxemia without reperfusion, pretreatment with 10^{-7} M trilinolein for 15 minutes reduced infarct size by 37%.[9]

Another effect which may contribute to the anti-ischemic activity of trilinolein is the improvement of the deformability of red blood cells. It was suggested that the improved red cell deformability might occur through the modification of membrane fluidity rather than competitive antagonism with calcium ions. The *in vitro* effect of trilinolein on erythrocyte deformability measured with a filtration method was studied in blood samples collected from 12 patients before and after cardiopulmonary bypass.[10] The reduction in the index of erythrocyte deformability after cardiopulmonary bypass was reversed when the blood samples were mixed with trilinolein at a concentration of 10^{-7} M.

The vasodilatory effects of trilinoloein were also evaluated in large vessels. In studies using isolated rat aorta, trilinolein, at concentrations ranging from 10^{-10} to 10^{-6} M, concentration-dependently relaxed phenylephrine-induced constriction. The concentration-response curves for the interaction between trilinolein and phenylephrine showed that trilinolein was unlikely to be a competitive antagonist of phenylephrine. The vasorelaxant effect of trilinolein was dependent on the presence of intact endothelium.[11] Both NG-nitro-L-arginine methyl ester and methylene blue antagonized this vasorelaxant effect.[11] L-arginine partially reversed the effect of L-NAME on the action of trilinolein. Linoleic acid had no vasorelaxant effect in the isolated rat aorta. This study suggests that trilinolein is an endothelium-dependent vasorelaxant, and the underlying mechanisms may be through stimulation of the nitric oxide and cyclic GMP pathways. In addition, the inhibitory effect of trilinolein on neutrophil adhesion may play a role in its myocardial-protective activity.

In other studies, trilinolein at concentrations ranging from 10^{-9} to 10^{-6} M inhibited human platelet aggregation induced by epinephrine but not by collagen, thrombin, ADP, or arachidonic acid.[3] This inhibition was accompanied by reduced ATP release and thromboxane B2 formation, but, as with the phenylephrine-induced vasoconstriction, concentration-response curves for the interaction between trilinolein and epinephrine-induced platelet aggregation showed that trilinolein was not likely to be a competitive antagonist of epinephrine. The mechanism for this anti-aggregatory effect still remains obscure. Epinephrine decreased not only the production of cyclic AMP but also cyclic AMP accumulation. Both trilinolein and epinephrine activated intracellular calcium mobilization, but the increment was less than that induced by thrombin. It was considered that the antiplatelet effect of trilinolein was mediated through an increase in cyclic GMP, and that the change in cyclic GMP results from stimulation of nitric oxide synthesis in platelets.[11]

We identified another effect that may contribute to

the anti-ischemic activity of trilinolein in isolated perfused rat hearts, which had been subjected to 60 minutes of global ischemia, where pretreatment with trilinolein at a concentration of 10^{-7} M for 15 minutes preserved the integrity of the rat heart mitochondria as demonstrated by examination under an electron microscope.[12]

ANTIARRHYTHMIC EFFECTS

The antiarrhythmic effects of trilinolein were investigated in models of ventricular arrhythmia due to cardiac glycoside toxicity or coronary artery ligation.[9,13] Ventricular arrhythmia is a frequent complication in the use of digitalis. The mechanism underlying the generation of delayed after-depolarization by cardiac glycosides is complex. It involves the overloading of intracellular Ca^{2+} stores, caused by the inhibitory effect of digitalis on the Na^+/K^+ pump, which subsequently activates the reverse mode of the Na^+/Ca^{2+} exchanger.[14] After pretreatment with 0.1 mg/kg trilinolein, strophanthidine-induced ventricular extrasystoles decreased significantly.[13] It was shown that trilinolein effectively reduced Ca^{2+} influx at low concentrations in isolated rat cardiomyocytes, therefore the strophanthidine-induced ventricular extrasystoles may be suppressed through the inhibition of calcium influx.[13]

In another study, we showed that trilinolein inhibits ischemia-induced ventricular arrhythmias.[9] Male Sprague-Dawley rats were anesthetized with urethane and were subjected to coronary ligation. Trilinolein, at dosages ranging from 10^{-4} to 10^{-7} μg/kg, was administered intravenously 15 minutes before ligation of the coronary artery. Also, the effect of trilinolein on arrhythmias was studied by ligating the coronary artery for 30 minutes, then reperfusing the myocardium for 10 minutes. During the 30-minute period of ischemia, trilinolein reduced the incidence, rate, and duration of ventricular tachycardia and the number of ectopic beats. At a dose of 10^{-7} μg/kg, trilinolein completely suppressed all ventricular arrhythmias. During the 10-minute in reperfusion, ventricular arrhythmias were also reduced by trilinolein at similar dosages.

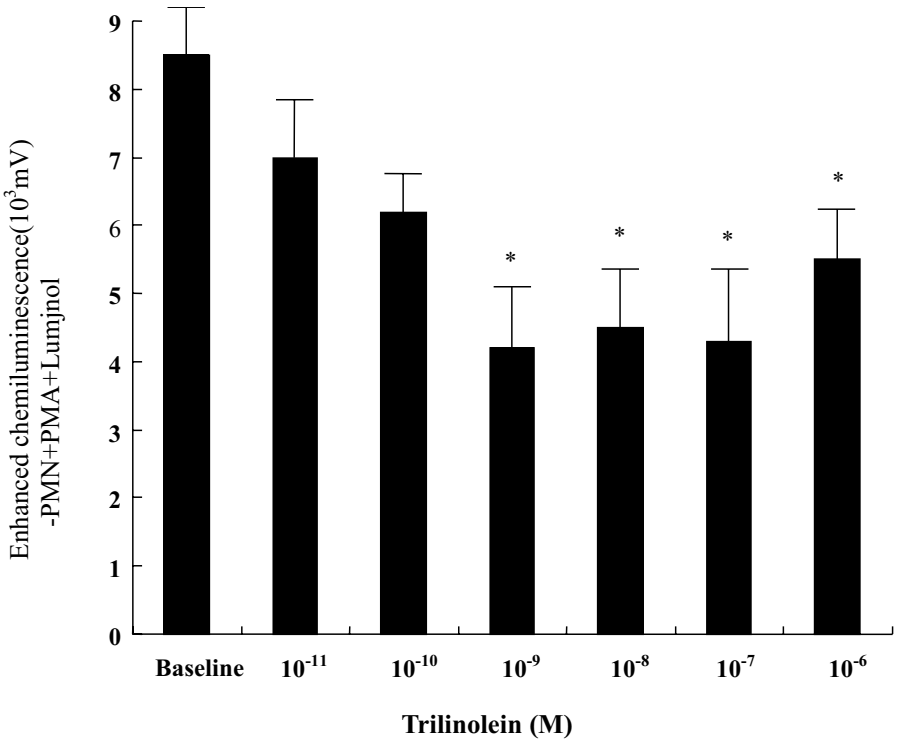

Figure 2. *Antioxidant activity of trilinolein as measured by enhanced chemiluminescence-polymorphonuclear neutrophil (PMN) + phorbol myristic acetate (PMA) + luminol. Trilinolein caused a mean maximal percentage decrease in free radicals of 48%. * $p < 0.05$, compared with the baseline.*

ANTIOXIDANT EFFECT

The development of atherosclerosis involves the infiltration of low-density lipoprotein (LDL) particles and monocytes into the vascular intima.[15] Oxidatively modified LDL, but not native LDL, has been shown to be a potent chemoattractant for circulating monocytes.[15,16] Oxygen-derived free radicals (OFRs) are also thought to mediate the injury to the myocardium brought about by ischemia and reperfusion. Various OFR scavengers have been shown to reduce this damage during myocardial ischemia/reperfusion, and some may have a clinical role in certain situations.[17]

In our *in vitro* studies, trilinolein exhibited concentration-dependent antioxidant activity shown by enhanced chemiluminescence (Figure 2).[7] The addition of phorbol myristic acetate (PMA) to medium containing leukocytes produces OFRs which can be measured by changes in chemiluminescence. Addition of trilinolein at concentrations between 10^{-11} and 10^{-6} M to medium containing leukocytes preceding the addition of PMA suppressed the production of OFRs. The maximal mean reduction of OFR of 48.0% ($p < 0.001$) was seen with the concentration of trilinolein at 10^{-7} mol/L. The antioxidant effect had a concentration-response curve similar to that of the water-soluble form of alpha-tocopherol, trolox, which showed a maximal mean reduction of OFR of 39.2%.

In addition, we studied the effects of trilinolein on SOD activity and left ventricular pressure in isolated rat hearts subjected to hypoxia followed by normoxic perfusion.[18] In isolated rat hearts subjected to hypoxia for 10, 30, 60, and 90 minutes without subsequent normoxic perfusion, a significant decrease in Mn-SOD activity was seen throughout the period of hypoxia, whereas the CuZn-SOD activity was increased at 10 and 30 minutes, but did not differ from the baseline after 60 and 90 minutes of hypoxia. This phenomenon was confirmed in another study, in which incubation of rat aortic smooth muscle cells with low-concentration (0.1 μM) trilinolein increased both the activity and mRNA levels of SOD.[8]

ANTI-HYPERTROPHIC EFFECT

The protective effects of trilinolein on the myocardium during ischemia and reperfusion may be related to its antioxidant activity. However, the intracellular mechanism underlying the protective effect of trilinolein in the heart remains unclear. In a recent study, we investigated the effect of trilinolein on norepinephrine (NE)-induced protein synthesis in cardiomyocytes.[19] Cultured neonatal rat cardiomyocytes were stimulated with NE, then the protein content, [^{3}H]-leucine incorporation, and β-myosin heavy chain (β-MyHC) promoter activity were examined. The effect of trilinolein on NE-induced intracellular reactive oxygen species (ROS) generation was measured with a redox-sensitive fluorescent dye (2',7'-dichlorofluorescein diacetate) and extracellular signal-regulated kinase (ERK) phosphorylation by Western blotting. Trilinolein at concentrations of l and 10 μM effectively inhibited NE-increased ROS generation, protein synthesis, β-MyHC promoter activity, and ERK phosphorylation. These data indicate that trilinolein inhibits NE-induced protein synthesis in cardiomyocytes, which may involve the attenuation of ROS generation.

The effects of trilinolein on angiotensin II-induced intracellular ROS generation were also examined. Trilinolein significantly inhibited angiotensin II-increased protein synthesis, β-MyHC promoter activity, and intracellular heavy chain promoter activity.[20] Furthermore, trilinolein and N-acetylcysteine decreased angiotensin II-or hydrogen peroxide (H_2O_2)-avtivated phosphorylation of mitogen-activated protein kinases (MAPKs), and activator protein-1(AP-1)-[or nuclear factor-κB(NF-κB)]-reporter activities.[20] These data indicate that trilinolein inhibits angiotensin II-induced cardiomyocyte hypertrophy and β-MyHC promoter activity via attenuation of ROS generation.

CONCLUSIONS

Traditional Chinese medicines have been used for centuries, and their potential benefits have been identified by empirical usage.[2] Trilinolein, isolated from *Panax notoginseng*, has been shown to have cardioprotective effects in various experiments. In the studies summarized here, we have identified other pharmacological actions which might explain some of the positive effects of this herb in treating circulatory disease, for which it has been used for hundreds of years.[2]

REFERENCES

1. Hong CY, Lai LJ, Yeh SF. Linoleate-rich triacylglycerol in *Panax notoginseng* improves erythrocyte deformability in vitro. *Planta Med* 1992;59:323-5.
2. Chen FH. Panax notoginseng. In: Tang W, Disenbrand G, Eds. *Chinese Drugs of Plant Origin. Chemistry, Pharmacology and Use in Traditional and Modern Medicine*. Berlin, Heidelberg: Springer Verlag, 1992:745-51.
3. Lai LJ, Shen YC, Hong CY. Trilinolein inhibits epinephrine-induced human platelet aggregation. *Thromb Res* 1994;76: 281-7.
4. Shen YC, Hong CY. Effect of trilinolein on cyclic nucleotide formation in human platelets: relationship with its antiplatelet effect and nitric oxide synthesis. *Br J Pharmacol* 1995;116: 1644-8.
5. Chan P, Hong CY, Tomlinson B, et al. Myocardial- protective effect of trilinolein: an antioxidant isolated from the medicinal plant *Panax notoginseng*. *Life Sci* 1997;1999-2006.
6. Chiu WT, Chan P, Liao SS, et al. Effect of trilinolein on the activity and gene expression of superoxide dismutase in cultured rat brain astrocytes. *Neurosci Lett* 1999;269:17-20.
7. Chan P, Cheng JT, Tsao CW, et al. The *in vitro* antioxidant activity of trilinolein and other lipid-related natural substances as measured by enhanced chemiluminescence. *Life Sci* 1996;59:2067-73.
8. Chan P, Chang JJ, Chen FC, et al. Effects of trilinolein on superoxide dismutase activity and mRNA levels in aortic smooth muscle cells. *Clin Exp Pharmacol Physiol* 2000;27:1002-6.
9. Chan P, Tsai SK, Chiang BN, Hong CY. Trilinolein reduces infarct size and suppresses ventricular arrhythmias in rats subjected to coronary ligation. *Pharmacology* 1995;51:118-26.
10. Tsai SK, Chan P, Lee TY, et al. Trilinolein improves erythrocyte deformability during cardiopulmonary bypass. *Br J Clin Pharmacol* 1994;37:457-9.
11. Hong CY, Lain LJ, Huang YT. Endothelium-dependent vasorelaxant effect of trilinolein mediated by nitric oxide and cyclic GMP. *Life Sci* 1995;57:1309-15.
12. Chan P, Niu CS, Cheng JT, et al. Trilinolein preserves the ultrastructure of mitochondria in isolated rat heart subjected to global ischemia through antioxidant activity as measurement by chemiluminescence. *Pharmacology* 1996;52:216-25.
13. Chen YJ, Chen SA, Tai CT, et al. Effect of trilinolein on strophanthidine-induced ventricular tachycardia in guinea pigs. *Pharmacology* 1999;58:15-23.
14. Goldman RH, Coltart DJ, Friedman JP, et al. The inotropic effects of digoxin on hyperkalemia: relation to (Na+, K+)-ATPase inhibition in the intact animal. *Circulation* 1973;48:830-8.
15. Steinberg D, Parthasarathy S, Carew TE, et al. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med* 1989;320:915-24.
16. Jialal I, Devaraj S. The role of oxidized low density lipoprotein in atherogenesis. *J Nutr* 1996;126:1053S-1057S.
17. Stephens NG, Parsons A, Schofield PM, et al. Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). *Lancet* 1996; 347:781-6.
18. Chan P, Niu CS, Tomlinson B, et al. Effect of trilinolein on superoxide dismutase activity and left ventricular pressure in isolated rat hearts subjected to hypoxia and normoxic perfusion. *Pharmacology* 1997;55:252-8.
19. Liu JC, Chan P, Chen JJ, et al. The inhibitory effect of trilinolein on norepinephrine-induced β-myosin heavy chain promoter activity, reactive oxygen species generation, and extracellular signal-regulated kinase phosphorylation in neonatal rat cardiomyocytes. *J Biomed Sci* 2004;11:11-8.
20. Liu JC, Cheng TH, Lee HM, et al. Inhibitory effect of trilinolein on angiotensin II-induced cardiomyocyte hypertrophy. *Eur J Pharmacol* 2004;484:1-8.

三亞麻油酸：一種分離自中草藥三七的天然三酸甘油脂具有心血管保護效果

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三亞麻油酸 (Trilinolein) 是分離自傳統中草藥三七根部的一種天然三酸甘油脂，過去的研究顯示其有多種心血管藥理作用，包括心肌保護，抗心律不整及抗氧化作用。上述作用主要是經由抗氧化而產生。近期研究發現此物質更可抑制心肌細胞肥厚。顯示傳統中草藥用作於治療心血管疾病是有其科學基礎。

關鍵詞：心肌保護、三七、三亞麻油酸。