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Protective effects of trilinolein extracted from *Panax notoginseng* against cardiovascular disease

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ABSTRACT

Trilinolein is a triacylglycerol purified from a commonly used traditional Chinese medicine *Panax notoginseng*. Trilinolein has been reported to provide a number of beneficial effects including reducing thrombogenicity and arrhythmias and increasing erythrocyte deformability. Additionally, trilinolein has been reported to be an antioxidant, which can counteract free radical damage associated with atherogenesis, and myocardial damage seen with ischaemia and reperfusion. These pharmacologic effects may explain the perceived benefits derived from treating circulatory disorders with the herb over the centuries.

INTRODUCTION

Trilinolein, isolated from the traditional Chinese herb *Sanchi* (*Panax notoginseng*)[1], has been used in treating circulatory disorders among Chinese for hundreds of years. In Chinese culture, traditional Chinese medicine is often used to maintain good health rather that to cure illness once it has developed, much in the same way that vitamin or mineral supplements or herbal preparations are used in Western countries. Trilinolein is a triacylglycerol, with the fatty acid linoleic acid, which carries two unsaturated bonds (C 18:2), at all three esterified positions of glycerol (Fig 1). Trilinolein has been shown to have various beneficial effects, including reducing thrombogenicity, erythrocyte deformability and arrhythmias and having antioxidant effects in various experimental models. Similarly, there are epidemiological data to suggest that linoleic acid may play a protective role in vascular disease, which is supported by *in vitro* studies and animal models. For instance, a higher intake of either linoleic or linolenic (C 18:3) acid was inversely related to the prevalence of coronary artery disease[2]. Treatment with dietary li-

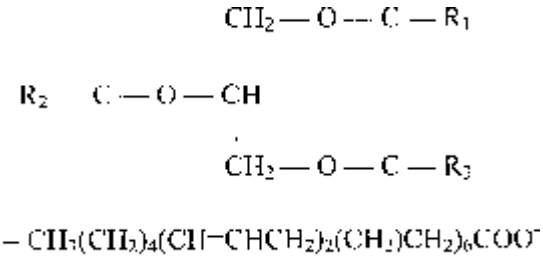

Fig 1. Chemical structure of trilinolein, a triacylglycerol with three linoleate ($C_{18}H_{32}O_2$) residues.

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noleic acid reduced the progression of atherogenesis and also caused a 30 % regression of established atherosclerosis in high fat fed rabbits[3]. However, interventional studies are still not available with either trilinolein or linoleic acid and as such the beneficial effects on vascular disease remain speculative[4,5]. Additionally, linoleic acid has been reported to be a strong anticarcinogen in a number of animal models at concentrations close to those achieved by normal dietary intake[4,6,7].

EFFECTS ON THROMBOGENICITY

Trilinolein inhibited platelet aggregation induced by epinephrine but not by collagen, thrombin, ADP or arachidonic acid at concentrations ranging from 0.001-1 μmol/L[8]. Although, the inhibition was accompanied by reduced thromboxane B_2 formation and ATP release, the mechanism of action was unclear. However, concentration-response curves for the interaction between trilinolein and adrenaline showed that trilinolein was unlikely to be a competitive antagonist of adrenaline.

Trilinolein (0.01-1 μmol/L) increased cyclic GMP formation and decreased cyclic AMP formation in washed human platelets[9], which was attenuated by both N^G-nitro-*L*-arginine methyl ester (*L*-NAME) and methylene blue, suggesting the effects of trilinolein are nitric oxide-mediated. Adrenaline decreased not only the production of cyclic AMP but also that of cyclic GMP. Trilinolein antagonized the inhibitory effect of adrenaline on cyclic GMP formation, but potentiated the inhibitory effect of adrenaline on cyclic AMP accumulation[9]. Both trilinolein and adrenaline increased intracellular calcium, but less than that produced by thrombin. The anti-platelet effect of trilinolein was reported to be mediated through an increase in cyclic GMP[9], following stimulation of platelet nitric oxide synthesis.

The *in vitro* effects of trilinolein on erythrocyte deformability of samples from 12 patients before and after cardiopulmonary bypass were measured using a filtration method effect[10]. Trilinolein (0.1 μmol/L) reversed the reduction in the index of erythrocyte deformability after cardiopulmonary bypass. Trilinolein was reported to improve the deformability of calcium-loaded red blood cells by modification of membrane fluidity rather than a competitive antagonism with calcium ions[1].

Trilinolein has also been reported to reduce thrombogenicity by reducing the adhesion of neutrophils to endothelial cells[11]. Pre-treatment of a cultured bovine endothelial monolayer with trilinolein (0.0001-1 μmol/L) significantly inhibited human neutrophil adhesion to endothelial cells. Trilinolein was less potent than sodium nitroprusside in inhibiting neutrophil adhesion[11]. Furthermore, the inhibitory effect of trilinolein was antagonized by methylene blue and *L*-NAME. The inhibitory effect of trilinolein was not mediated through linoleic acid because linoleic acid did not inhibit neutrophil adhesion. Pre-treatment of neutrophils with trilinolein did not reduce neutrophil adhesion. However, in neutrophils activated with *N*-formyl-methionyl-leucyl-phenylalanine, trilinolein inhibited the neutrophil adhesion to endothelial cells[11]. It appeared that trilinolein inhibited neutrophil adhesion to the endothelial monolayer by stimulating the nitric oxide and cyclic GMP pathways in endothelial cells. Another mechanism may be by scavenging free radicals. A further study supported the increases in cyclic GMP, but found trilinolein induced aggregation of human polymorphonuclear neutrophils, and pre-treatment with low concentrations of trilinolein (0.1 nmol/L) enhanced phorbol-12-myristate 13-acetate (PMA) induced aggregation[12]. In other experiments trilinolein presented a U-shaped antioxidant profile with higher concentrations having less effect than intermediate concentrations, but 0.1 nmol/L trilinolein did not significantly reduce free radical-mediated chemiluminescence[13].

ANTI ARRHYTHMIC EFFECTS OF TRILINOLEIN

In an *in vivo* rat model study, trilinolein was reported to reduce ischaemia-induced arrhythmias[14]. Male Sprague-Dawley rats were anaesthetised with urethane and were subjected to coronary ligation. Trilinolein (0.01-100 ng/kg) was administered intravenously 15 min before ligation of the coronary artery. At a dose of 100 ng/kg, trilinolein completely suppressed all ventricular arrhythmias. Trilinolein also reduced the incidence, rate and duration of ventricular tachycardia and the number of ectopic beats during the first 30 min of coronary artery ligation[14]. Additionally, similar doses of trilinolein also reduced ventricular arrhythmias during 10 min reperfusion of the myocardium, following the 30 min of coronary artery ligation[14].

Ventricular arrhythmia is a prevalent complication resulting from the use of digitalis. The generation of delayed after-depolarizations by cardiac glycosides in-

volves the overloading of intracellular calcium stores following inhibition of the Na^+/K^+ pump, which subsequently activates the reverse mode of the Na^+/Ca^{2+} exchanger[15]. Treatment of guinea pigs with trilinolein (0.1-100 μg/kg) prior to ligation of the coronary artery did not reduce strophanthidine-induced ventricular tachycardia, but did significantly reduce ventricular extrasystoles[16]. As low concentrations of trilinolein reduced Ca^{2+} influx in isolated rat cardiomyocytes, similar mechanisms may be involved in reducing strophanthidine-induced ventricular extrasystoles[17]. Trilinolein also dose-dependently narrowed the width of the QRS complex during ventricular tachycardia[16], possibly by improving conduction velocity between muscle fibres through the action of trilinolein on calcium metabolism.

PROTECTIVE EFFECTS OF TRILINOLEIN ON ISCHEMIC MYOCARDIAL DAMAGE

The effect of trilinolein on infarct size was evaluated by staining and weighing the infarct zone after occluding the coronary artery for 4 h. Pre-treatment with trilinolein (0.1 μg/kg) 15 min prior to the coronary ligation significantly reduced the size of the infarction[14]. It was concluded that trilinolein may protect the myocardium against ischaemic injury. The mechanism of the myocardial protection 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[17]. In isolated cardiomyocytes, $^{45}Ca^{2+}$ influx stimulated by hypoxia/normoxia was effectively reduced by 34 % after pre-treatment with trilinolein at a low concentration of 0.001 μmol/L. In isolated perfused rat hearts subjected to 60-min global hypoxaemia without reperfusion, pre-treatment with trilinolein 0.1 μmol/L for 15 min reduced infarct size by 37 %[17].

In isolated, perfused rat hearts, which had been subjected to 60 min of global ischaemia, pretreatment with trilinolein at a concentration of 0.1 μmol/L for 15 min preserved the integrity of the rat heart mitochondria as demonstrated by examination under the electron microscope[18]. No swelling of the mitochondria occurred and there was good alignment of cristae and absence of amorphous density. Another mechanism of myocardial protection, in addition to the antioxidant effect, may therefore be by maintaining the membrane fluidity of cardiomyocytes.

In studies in isolated rat aorta, trilinolein at concentrations ranging from 0.1 nmol/L-1 μmol/L, concentration-dependently relaxed phenylephrine-induced constriction[19]. 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. Both *L*-NAME and methylene blue antagonised this vasorelaxant effect. *L*-arginine partially reversed the effect of *L*-NAME on trilinolein, suggesting that trilinolein is an endothelium dependent vasorelaxant and the underlying mechanism could be a stimulation of the nitric oxide and cyclic GMP pathway[19]. Linoleic acid had no vasorelaxant effect in that study. However linoleic acid induced relaxation and hyperpolarization of porcine coronary smooth muscle cells via a mechanism that involves activation of the Na^+/K^+-ATPase pump[20].

ANTIOXIDANT ACTIVITY OF TRILINOLEIN

The protective effect of certain dietary constituents is believed to be via antioxidant-mediated resistance to oxidative damage to key biomolecules, including DNA and polyunsaturated fatty acids, reducing the development of diseases, such as cancer or atherosclerosis[21]. Plant-based foods contain a variety of components with antioxidant activity, including vitamins C and E, carotenoids and flavonoids[21]. The development of atherosclerosis involves the infiltration of low-density lipoprotein (LDL) particles and monocytes into arterial intima. Macrophage uptake of oxidized LDL results in the formation of foam cells, fatty streaks and eventually fibrous plaques[22]. Oxidatively modified LDL, but not native LDL, has been shown to be a potent chemoattractant for circulating monocytes, which transform to produce further macrophages[23]. Lowering the serum cholesterol, especially the LDL-cholesterol levels, reduces the incidence of atherosclerotic cardiovascular disease and antioxidant therapy has also been shown to have positive effects in the prevention of atherosclerosis in some, but not all studies[24-28]. Oxygen-derived free radicals are also thought to mediate the injury to the myocardium brought about by ischaemia and reperfusion[29]. Various free radical scavengers have been shown to reduce this damage during myocardial ischaemia/reperfusion[30].

Pre-treatment with the antioxidants trilinolein and

various other saturated and unsaturated lipid compounds (0.01 nmol/L-1 μmol/L) quenched free radical-generated luminol chemiluminescence following the addition of phorbol myristic acetate (PMA) in medium containing leukocytes[13]. Trilinolein showed concentration-dependent antioxidant activity at concentrations between 0.1 nmol/L-1 μmol/L, with a maximal free radical reduction of 48.0 %, whereas trolox, a water-soluble analogue of vitamin E, showed a maximal mean reduction of 39.2 %[13]. Linoleic acid also showed a concentration-dependent scavenging of free radicals with a maximal mean reduction of 31.9 % when compared to baseline, whereas palmitic acid (C 16:0) showed only relatively weak antioxidant activity at concentrations of 0.1-1 μmol/L, with a maximum reduction of OFR of 15.2 %. The flavonol antioxidant catechin, extracted from tea, showed potent antioxidant activity (-40 %) as did the polyunsaturated triglyceride triolein (oleic acid, C 18:1, -31.9 %) whilst the saturated triglyceride tristearin (stearic acid, C 18:0) had only relatively weak antioxidant activity (-15.2 %)[13]. These compounds generally showed a concentration-dependent antioxidant effect, except for catechin, linoleic acid, triolein and to a lesser extent trilinolein (Fig 2), which showed U-shaped concentration-effect relationships with less antioxidant effect at higher concentrations. As expected, the antioxidant activity of the unsaturated compounds was generally greater than the saturated compounds[13]. Similarly pre-treatment with trilinolein quenched free radical-generated luminol chemiluminescence, with the most effective concentration being 0.1 μmol/L, which decreased the signal by 50 %, $P<0.01$[18]. The antioxidant effect had a concentration-response curve similar to alpha-tocopherol[18]. When the compounds were added to the polymorphonuclear leukocytes after the PMA the antioxidant effects were generally less than when they were added before the PMA and trolox actually behaved as an oxidant increasing chemiluminescence 45 %[13]. Trilinolein remained the most effective antioxidant reducing chemiluminescence by 33 % at 0.001 μmol/L[13].

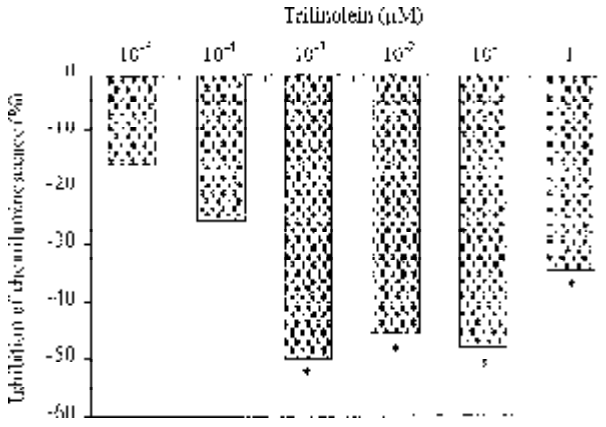

Fig 2. Antioxidant activity of trilinolein (0.01 nmol/L- 1 μmol/L) assessed using polymorphonuclear neutrophil free radical-generated luminol chemiluminescence following the addition of phorbol myristic acetate. The data describe percentage inhibition of chemiluminescence compared to baseline, $P<0.05$. (Adapted from Chan P *et al*, Life Sci 1996).

It has been proposed that decreased endogenous superoxide dismutase (SOD) activity may be one of the factors that contribute to free radical-mediated reperfusion injury of the ischaemic myocardium[31]. In isolated rat hearts subjected to hypoxia for 10, 30, 60 and 90 min without subsequent normoxic perfusion, a significant decrease in Mn-SOD activity was seen throughout the period of hypoxia, whereas the Cu.Zn-SOD activity was increased at 10 and 30 min, but was not different from the baseline after 60 and 90 min of hypoxia[32]. In rat hearts pre-treated with 0.1 μmol/L trilinolein and subjected to 60 min of hypoxia, Cu.Zn-SOD activity was increased compared with baseline and compared with hearts subjected to 60 min of hypoxia without trilinolein. After trilinolein treatment, the activity of Mn-SOD was still reduced compared with baseline, although less so than after 60 min of hypoxia without trilinolein[32]. Pre-treatment with trilinolein was associated with better preservation of left ventricular function during hypoxia and a more rapid return to recovery during the subsequent normoxic perfusion. The myocardial protective effect of trilinolein may thus be related to an antioxidant effect through potentiation of SOD, particularly Cu.Zn-SOD during hypoxia[32].

After 2-d incubation with 0.1 μmol/L trilinolein, the activity and mRNA levels of SOD were increased in rat aortic smooth muscle cells, but not with 1 μmol/L trilinolein. In contrast, after 7-d incubation with trilinolein, both the activity and mRNA levels of SOD were lowered in a dose-dependent manner, emphasising the importance of choosing an optimal dosage for supplementation with antioxidants for scavenging oxygen free radicals[33]. The antioxidant capacity of trilinolein has also been demonstrated in brain astrocytes, liver and spleen[33,34]. The studies in isolated cardiomyocytes and isolated perfused rat heart also suggested that the benefits might be related to antioxidant activity and inhibition of $^{45}Ca^{2+}$ influx[17].

CONCLUSION

Traditional Chinese medicines have been used for centuries and their potential benefits have been identified by empirical usage. Trilinolein, isolated from *Panax pseudoginseng*, has been shown to have an antioxidant effect and other pharmacologic actions, which supports its long history of use in treating circulatory disorders.

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