Tocotrienols: Stroke and Myocardial Infarction

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Although the current treatments for stroke and myocardial infarction contribute to an improvement in mortality rates, the consequences of reperfusion therapy have remained a challenge. Tocotrienols have been shown to exert beneficial effects on the brain and heart. We retrieved articles from Scopus, MEDLINE and PubMed from inception to June 2021, and included any studies using tocotrienols as a treatment for cerebral or myocardial I/R injury therapy. Observational studies and review articles were excluded, and the risk of bias was conducted using a specific tool for animal study (SYRCLE). The data were analyzed qualitatively. Twelve articles met the eligibility criteria. Tocotrienols significantly improved the structural, functional, and biochemical parameters in both cerebral and myocardial I/R injury models. In contrast, oxidative stress, inflammation, and apoptosis were markedly attenuated by tocotrienol treatment. Limitations to the analysis included marked differences in animal models, disease inductions, forms of tocotrienols, and an unclear risk of bias in certain types of bias. However, tocotrienols have the potential to serve as a supplement for reducing the impact of reperfusion injury.

myocardial infarction stroke tocotrienols

1. Burden of the Disease: IHD & Stroke

Ischemic heart disease (IHD) and stroke are the world's leading causes of death. The Global Burden of Disease study reported that around 126 million people have been affected with IHD, with nine million deaths in 2017 [1]. Moreover, around 80 million people were affected by stroke in 2016, mainly the ischaemic type (84.4%), and accounted for the deaths of about 5.5 million people worldwide in that year [2]. Although advancements in treatment significantly reduced the mortality rate, IHD became the most common cause of disability-adjusted life years (DALYs) in 2017, with more than two thousand people per hundred thousand being affected [1]. Stroke was the second-greatest contributor to DALYs in 2016, affecting more than a thousand people per hundred thousand [2]. Therefore, exploring potential approaches that promote additional protection against these diseases is essential to reduce debilitating effects after the condition's onset.

2. Tocotrienols as Nutraceuticals

Nutraceuticals in the forms of food or its components that are utilized for the prevention or treatment of diseases have been shown to exert various benefits on cardiovascular and cerebrovascular health [3][4]. Vitamin E is one of these nutraceuticals that can be found in most edible oils [5]. Two forms of vitamin E exist, namely, tocopherols and tocotrienols. The latter is primarily found in annatto, palm, and rice bran oils [5][6]. Tocotrienol differs from tocopherol in that it contains an isoprenoid side chain, making it polyunsaturated in nature [6]. Both isomers can be further

classified, based on the amount and position of the methyl group in the chemical structures, into alpha, beta, gamma, and delta groups [7].

A recent meta-analysis reported that vitamin E significantly reduced the risk of ischemic stroke but not hemorrhagic stroke [8]. However, this meta-analysis was limited to α-tocopherol treatment, as the authors retrieved no studies on tocotrienols. Another meta-analysis reported the remarkable effects of vitamin E in reducing myocardial infarction when used alone at a high dose concentration [9]. Again, no clinical data is available concerning tocotrienol supplementation for risk reduction.

Results in pre-clinical studies have reported various beneficial effects of tocotrienols as a cardioprotective agent [10][11][12][13][14][15][16] and neuroprotective agent [17][18][19][20][21]. The effects vary across different types of tocotrienols, and the gamma-type has been observed to produce remarkable effects compared to other types as a cardioprotective agent [10][11]. In contrast, a study using the cerebral ischemia-reperfusion (I/R) injury model reported that α- and γ-tocopherols and α-tocotrienol are neuroprotective. The latter two were found to be more potent than α-tocopherol [18]. The mechanisms of the protective effects of tocotrienols in cerebral and myocardial I/R injuries are attributable to their antioxidative, anti-inflammatory, and anti-apoptotic activities, making the brain and heart more resistant toward I/R injury [10][11][13][14][15][17][19][20][21].

Given their significant positive impact on the brain and heart, tocotrienols have a huge potential to be used as a supplement or incorporated into a diet as part of cerebral and coronary ischemic disease prevention and management. To our knowledge, no systematic review has been conducted that focuses on the effects of tocotrienol on both cerebral and myocardial I/R injury. Therefore, a systematic approach to analyzing the current state of evidence, drawing conclusions on the significance of tocotrienol use and addressing the current limitations for future research, is indispensable.

2.1. Tocotrienol's Effects on Cerebral Ischaemia Reperfusion Injury

Reduction in cytotoxic edema was another structural improvement mediated by tocotrienol treatment [20]. Other than that, the improvement of collateral circulation was also evident in the same study that utilized a canine model [20]. Furthermore, various parameters were also reported to be significantly changed, including the attenuation of oxidative stress markers (oxidized glutathione (GSSG) to glutathione (GSH) ratio [21], 4-hydroxy-2-nonenal (4-HNE) [19][21], nitrotyrosine [21], 8-hydroxy-2′-deoxyguanosine (8-OHdG) [21], the receptor for advanced glycation end products (RAGE) [21], N (omega)-(carboxymethyl) arginine (CMA) [21], N (omega)-(carboxymethyl) lysine (CML) [21]), the regulation of antioxidant-related markers (increased NF-E2 p45-related factor 2 (Nrf2) [21]), reductions in inflammation parameters (tumor necrosis factor α (TNF-α) [17], monocyte chemotactic protein 1 (MCP-1), Iba-1, IgG [17], light chain 3 (LC3) II [21]) and a reduction of apoptotic marker (cleaved caspase-3 [21]). In addition, the extracellular matrix was significantly affected, as evidenced by the increase in collagen IV [17] and tissue inhibitor of metalloproteinase 1 (TIMP-1), and the reduction in matrix metalloproteinase (MMP)-9 (not MMP-2) levels [20]. Two transporters that were regulated by tocotrienol treatment were multidrug-resistant protein 1 (MRP1) [19][21] and

chloride intracellular channel protein 1 (CLIC1) [20]. Neurobehavioral assessment of the mice demonstrated a significant improvement in Rotarod time but a negligible change in the Bederson score and Corner test [17][21].

2.2. Tocotrienol's Effects in Myocardial Ischemia-Reperfusion Injury

Differing from the studies of cerebral I/R injury, the early reports regarding myocardial I/R injury focused on the use of palm oils containing both tocotrienols and tocopherols [12][13][16]. The more recent studies used individual tocotrienols, with the primary focus on γ-tocotrienol. Although both α- and γ-tocotrienol isomers have been reported to affect many parameters significantly, the latter isomer has the most prominent cardioprotective effects [10][11], particularly in females [11].

In addition, tocotrienol treatment remarkably reduced the oxidative stress markers (reactive oxygen species [15], lipid peroxidation product [16]), angiogenic factor (vascular endothelial growth factor [15]), collagen homeostasis regulators (MMP-2, MMP-9, transforming growth factor β (TGF-β) [11]) and the pro-apoptotic marker (cleaved caspase-3 [10]). In contrast, tocotrienols significantly increased the expression of the antioxidative enzyme of superoxide dismutase [11], the anti-apoptotic marker of b-cell lymphoma 2 (bcl-2) [14], mitochondrial-related proteins (ATP synthase, cytochrome-c oxidase 1, 2, 3) [11], and calcium pump-related proteins (calpain, calsequestrin, phospholamban) [11].

Red palm oil was reported to increase cyclic GMP levels after 10 min during the ischemic period [12][13]. However, a negligible effect was found on nitric oxide levels [13]. Furthermore, γ-tocotrienol was reported to increase the phosphorylation of Akt and reduce the phosphorylated mechanistic target of rapamycin (mTOR) [14]. The addition of wortmannin, an inhibitor of phosphatidylinositol-3-kinase (PI3K), reversed the effects of tocotrienols on Akt and mTOR [14]. Lekli et al. [14] also showed an elevated Beclin-1 level and the ratio of LCII to LCI. Other than that, tocotrienol treatment significantly increased the bindings of p38-mitogen-activated protein kinase (MAPK) α and SRC kinase, while reducing the endothelial nitric oxide synthase (eNOS) and heme oxygenase 1 (HO-1) with caveolin-1 [10]. In contrast, the interaction of p38MAPKβ and caveolin-3 was significantly attenuated in rats treated with tocotrienols [10].

3. Current Insight on Tocotrienols

Our review identified numerous beneficial effects of tocotrienols on many parameters regarding both the brain and heart. Tocotrienols, either used as individual isomers or when combined with other tocotrienols and tocopherol isomers, have various positive impacts as neuroprotective and cardioprotective agents.

Mishima et al. [18] reported that both α-tocotrienol and γ-tocopherol were more potent than α-tocopherol in cerebral protection, as evidenced by significant reductions of infarct volume. Similarly, Park et al. [19] found a remarkable reduction in infarct volume in the α-tocotrienol-treated group. The mechanism of neuroprotection might be attributable to the ability of tocotrienols to reverse oxidative stress conditions.

Cerebral I/R injury causes the excessive generation of reactive oxygen species (ROS), primarily from the electron transport chain [22]. ROS interacts with various cellular components, including DNA, proteins, and lipids, as evidenced by the significant increment of oxidative markers of 8–hydroxy-2′-deoxyguanosine (8-OHdG), nitrotyrosine, and 4-hydroxy-2-nonenal (4-HNE), respectively [19][21]. Membrane disruption following the insult causes the leakage of intracellular components, such as lactate dehydrogenase (LDH) [19][21].

The endogenous antioxidant system comprises catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S transferase (GST), and superoxide dismutase (SOD), which are distributed unevenly in the different brain regions [23]. SOD plays an essential role in converting superoxide into hydrogen peroxide, while CAT, GPx, and GST act in concert to neutralize hydroperoxides [23]. On the other hand, GR serves as a GSH regenerator, reducing the GSSG by transferring an electron from nicotinamide adenine dinucleotide phosphate (NADPH) to GSSG [23].

Cerebral I/R injury has been shown to affect antioxidant enzymes differently, depending on the region and duration of the insult. For instance, GR activity is reduced during ischemia in the cerebral cortex, cerebellum, and hippocampus but becomes elevated at one hour following reperfusion [23]. In contrast, Candelario-Jalil et al. [24] reported negligible hippocampus changes during ischemia, with a reperfusion period of up to 24 h. The inconsistent results might be attributable to the different durations of ischemia and probably to the different animal models [23][24]. Moreover, during the first six hours of reperfusion, the levels of GSH significantly decreased, along with a notable increase in GSSG levels. GSSG is a toxic metabolite and can be transported away via the multidrug resistance-associated protein 1 (MRP1) [21]. Shang et al. [21] reported that the I/R injury contributed to the increase in GSSG levels and MRP1 expression. In contrast, MRP1-deficient mice had significantly higher GSSG accumulation and infarct volume than non-deficient mice. α-tocotrienol upregulates MRP1, attenuating the elevation of the intracellular GSSG level and, thus, reducing the infarct volume [19][21].

Three included studies utilized a fixed-dose preparation of tocotrienols or in combination with tocopherol [17][20][21]. Rink et al. [20] used a canine model to elucidate the potential mechanism of combined tocotrienols in stroke via collateral circulation and reported significant improvements in cytotoxic edema and infarct volume in the first hour following the initiation of reperfusion. Furthermore, the infarct volume remained significantly lower in the combined tocotrienols group at 24 h [20]. The effects might be attributed to arteriogenesis, a process that takes place in an early phase due to enhanced blood flow in the existing collateral blood vessels [25]. This improvement in collateral circulation might be attributed to the regulation of the extracellular matrix, as evidenced by the increase in TIMP-1 and reductions in both MMP-2 and MMP-9 [17][20]. MMPs, together with plasmin, are essential for the degradation of the external elastic lamina and elastin, forming a space for expanding blood vessels [25]. Excessive MMP activity during the I/R event causes damage to the basal lamina, promoting blood-brain barrier disruption and inflammation [26][27]. TIMP-1, the inhibitor of MMP-9, was significantly elevated in the tocotrienol-treated group [20]. The downregulation of MMP-9 and the elevation of TIMP-1 are essential mechanisms that promote neuroprotection [27].

On the contrary, the level of vascular endothelial growth factor (VEGF) is not significantly changed by tocotrienol-enriched treatment [20]. VEGF is an essential factor for angiogenesis, the process of sprouting of new blood

vessels [28]. However, several studies had reported that the induction of angiogenesis in the early phase contributes to the aggravation of stroke, due to disruption of the blood-brain barrier and increased vascular permeability, resulting in worsening vasogenic edema and infarct size [28]. Thus, the downregulation of angiogenic factors, including VEGF by tocotrienols, promotes beneficial outcomes in ischemic stroke.

Another interesting finding is the increase in CLIC1 but not CLIC4 expression in the tocotrienol-enriched group [20]. A previous study by Chalothorn et al. [29] reported that CLIC4 was an essential factor for collateral circulation and demonstrated that mice that were deficient in CLIC4 genes (homozygous *Clic4*$^{-/-}$) had a fourfold-larger infarct size compared to the homozygous *Clic4*$^{+/+}$. However, they did not extend their study regarding CLIC1, as negligible changes were observed in the collateral density and diameter between the postnatal and adult period in *Clic1*$^{-/-}$ mice [29]. The exact reason for the contrasting expression of CLIC1 and CLIC4 remains to be elucidated, particularly with tocotrienol treatment.

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