The Potential for Tocotrienols in Inflammation

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INTRODUCTION

Inflammation has close relationships with almost all diseases from fever to allergy and cancer. While inflammation can bring on white blood cells to ward off minor infections and promote healing, the same process may lead to devastating damage if not controlled. Inflammatory diseases such as rheumatoid arthritis cause lifelong suffering to patients and, as a result, economic loss through decline in productivity. From these perspectives, there is an urgent need to address the problem of inflammation. Dietary modification is one viable approach as it is a simple yet effective way to tackle the problem. Increasing dietary intake of antioxidants in particular may be helpful in relieving inflammatory conditions. For instance, the anti-inflammatory potential of tocotrienols looks promising enough to merit further study.

As a process, inflammation is a complex immune response intended to protect the body from various harmful agents such as microbes and toxins. It is characterized by five cardinal features, namely, heat (*calor*), redness (*rubor*), swelling (tumour), pain (*dolor*) and loss of function (function laesa) (Mitchell and Cotran, 2003). The sensation of heat is caused by increased blood flow through dilated blood vessels, resulting in redness of the area. Subsequently, fluid moves from the leaky blood vessels into surrounding tissues, causing the area to swell. Swelling or oedema leads to stretching of sensory nerves, causing pain to the affected area. In addition to that, other inflammatory mediators released during the process may contribute to the pain sensation. All these result in loss of function, which refers to either a simple loss of joint mobility due to oedema or pain, or to the replacement of functional cells with scar tissue (Punchard *et al.,* 2004).

The outcome of an immune response may be beneficial or deleterious. It is good for the host if foreign agents are phagocytosed or neutralized as this will lead to recovery from the infection and healing. On the other hand, it may be harmful if it leads to chronic inflammation without resolution of the underlying injury (Furst and Munster, 2001). The inflammatory process is regulated by both mediators that initiate and maintain inflammation, and mediators that will stop it (Choy and Panayi, 2001). During chronic inflammation, cellular damage arises due to imbalance between these mediators as those that cause inflammation become unregulated. Such damage can be observed in various chronic diseases such as rheumatoid arthritis, atherosclerosis and cancer.

INFLAMMATION AND CHRONIC DISEASES

Inflammation has been linked to many chronic diseases, contributing to the increase in worldwide morbidity and mortality rates. A hypothesis has been made from cohorts that the decline in old-age mortality is promoted by the reduced burden of infections and inflammation (Crimmins and Finch, 2006). It suggests that reduced levels of inflammation delays the ageing process by reducing the occurrence of chronic diseases. Due to the long-term and debilitating effects of chronic diseases, the need for treatment is especially important.

Cancer

The relationship between inflammation and cancer was postulated in 1863 by Rudolf Virchow (Balkwill and Mantovani, 2001). Since then, many studies have supported the hypothesis. Today, the idea that inflammation is related to cancer is well established. For instance, inflammatory bowel disease has been identified as a high risk factor for the development of colorectal adenomas and cancer (Smith *et al.*, 2001a). Meanwhile, environmental pollutants and tobacco smoke lead to inflammation of the lungs, greatly increasing the risk of lung cancer (Mitchell and Cotran, 2003a). Cyclooxygenase (COX)-2, the inducible COX enzyme that synthesizes prostaglandins (some inflammatory mediators) via the inflammatory COX pathway, is expressed in various diseases including cancer (DuBois *et al.,* 1998). A review stated that COX-2 expression is increased in lung as well as other types of cancer. It also suggested that COX-2 may have a major impact on lung tumour progression and tumour-

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associated inflammation (Brown and DuBois, 2004).

Atherosclerosis

Cardiovascular disease is the principal cause of death in the United States and, perhaps, worldwide (Braunwald, 1997). Undeniably, it is also linked to inflammation. Atherosclerosis, once being understood as the accumulation of lipids within the artery wall, has now been accepted as an inflammatory disease (Mehta *et al*., 1998; Ross, 1999; Libby *et al*., 2002). This view is supported by many studies, which proved that the presence of inflammatory markers and mediators is associated with cardiovascular events. One example is the Rotterdam study, a population-based cohort study of the elderly aiming to investigate the incidence and risk factors for chronic disabling diseases (van der Meer *et al.*, 2002). In the study, it was found that C-reactive protein (CRP), a sensitive marker of inflammation, is associated with the severity of atherosclerosis at various sites of the arterial tree.

Rheumatoid Arthritis

Another common chronic inflammatory disease is the painful and disabling rheumatoid arthritis, in which inappropriate inflammatory response causes the destruction of bone and cartilage (Furst and Munster, 2001). It usually progresses to severe disability and results in shortening of life. Some features of rheumatoid arthritis are: increases in inflammatory mediators such as prostaglandins and cytokines, leukocytes accumulation, as well as tissue injury (Mitchell and Kumar, 2003). Previous studies using animal models for arthritis showed that anti-inflammatory effects were attained through inhibition of the inflammatory mediators (Portanova *et al.,* 1996; Jiang and Ames, 2003). Such reduction in the inflammatory mediators can be achieved using non-steroidal anti-inflammatory drugs (NSAIDs), which alleviate pain and slow down the damaging process (Furst and Munster, 2001).

FREE RADICALS AND DISEASES

Free radicals are highly reactive molecules that may react with the cell membrane, DNA and sulphohydryl bonds in protein to cause damage in the body (Machlin and Bendich, 1987). The highly reactive nature of free radicals is owed to the single unpaired electron at the outer orbital of the molecule. Due to the extremely unstable chemical state, free radicals will attack adjacent cellular components by acquiring electrons to fill the missing ones. In the process, considerable amount of damage to the cells and tissues ensues. These radicals play their part in the ageing process and illnesses such as cancer, cardiovascular disease and other inflammatory diseases.

Free radicals may come from both endogenous and exogenous sources. Within cells, free radicals are generated during normal physiological processes such as respiration and microbial defense. On the other hand, radiation exposure and metabolism of exogenous components such as pollutants, drugs and chemicals also contribute to the production of free radicals in the body (Machlin and Bendich, 1987; Mitchell and Cotran, 2003b).

Reactive oxygen and nitrogen species are examples of free radicals produced. Some examples of oxygen radicals are superoxide ($\cdot O_2^-$), hydroxyl (·OH), peroxyl ($RO_2\cdot$) and alkoxyl (RO·), whereas nitrogen radicals include nitric oxide (NO·) and nitric dioxide ($NO_2\cdot$) (Wiseman and Halliwell, 1996). Some of these free radicals react quickly with only few molecules while other may react with almost anything. Hydroxyl (·OH) is an example of a highly reactive radical. It is short-lived, reacting at the site of its formation (Conner and Grisham, 1996). Meanwhile, nitric oxide is a free radical gas produced by some white blood cells during anti-microbial activity that regulates many physiological processes.

ANTIOXIDANTS

Since free radicals have devastating effects on the body, there is a need to counteract these reactive molecules. This role is played by antioxidants, whose importance in health has been extensively researched. Antioxidants are categorized into exogenous and endogenous, with the exogenous sources being provided in the diet. Naturally occurring or endogenous antioxidants, on the other hand, are found in the body to overcome oxidative stresses that occur during normal metabolic processes (Hu *et al.,* 2000; Conner and Grisham, 1996). An example of an important endogenous antioxidant is superoxide dismustase (SOD) that attenuates tissue inflammation and injury by scavenging reactive oxygen species.

Food is an important source of antioxidants. Fruits, vegetables and some beverages are known to possess high antioxidant properties due to the presence of polyphenols and other phytochemicals. Polyphenols, being the most abundant source of dietary antioxidants, can be separated into several main classes, namely, phenolic acids, flavonoids, stilbenes and lignans (Tapiero *et al*., 2002). Among them, phenolic acids and flavonoids are the more important, being the major polyphenols in the diet. Caffeic acid is generally the most abundant phenolic acid and is found in large amounts in coffee and certain fruits such as kiwi

and apple. Meanwhile, flavonols, flavones, flavonones, isoflavones, anthocyanidins and flavanols are six sub-classes of flavonoids. Foods rich in these are onions, chocolate, green tea, red wine and tomatoes (Manach *et al.,* 2004).

Antioxidants have the ability to defend against oxidative damage that occurs in cancer, cardiovascular disease, brain dysfunction and cataracts (Ames *et al.,* 1993). Exogenous antioxidants are hypothesized to protect against endothelial dysfunction via a reactive oxygen species scavenging activity (Pratico, 2005). Besides, an *in vitro* study showed that the dietary flavonoid quercetin may play a role in redressing inflammatory responses associated with age (Hu *et al*., 2000). In addition, it was reviewed that nutritional antioxidants are beneficial in brain ageing by preventing and reversing age-related cognitive and motor deficits (Lau *et al*., 2005).

Vitamin E

Apart from polyphenols, other dietary antioxidants that are equally important for health include vitamins and micronutrients. Tocopherols and tocotrienols (vitamin E), ascorbic acid (vitamin C) and carotenoids function as antioxidants by scavenging free radicals (Sies and Stahl, 1995). Tocopherols and tocotrienols, the main components of fat-soluble vitamin E, are found abundantly in oil palm fruit (*Elaeis guineensis*) (Sundram *et al.*, 2003). Both tocopherols and tocotrienols, can be further separated into different compounds, namely, α-, β-, γ- and δ-tocopherol and α-, β-, γ- and δ-tocotrienol. In recent years, numerous studies have been done on vitamin E and their antioxidant properties. In one of the studies, γ -tocopherol was postulated to trap membrane-soluble electrophilic nitrogen oxides and other electrophilic mutagens *in vivo* (Christen *et al.,* 1997). Furthermore, several *in vitro* studies showed that tocotrienol rich fractions (TRF) are more powerful than α-tocopherol in terms of antioxidant capabilites (Mutalib *et al*., 2002). Besides functioning as antioxidants, Vitamin E also possesses other properties that are beneficial to health. Tocotrienols were shown to inhibit the growth of human breast cancer cell lines *in vitro,* suggesting an anti-cancer role for vitamin E (Nesaretnam *et al.*, 1995; 1998; 2000). Other than that, tocotrienols, particularly α-tocotrienol, were found to be the most effective vitamin E in reducing endothelial expression of adhesion molecules and adhesion to monocytes, two events involved in the pathogenesis of atherosclerosis (Theriault *et al*., 2002).

Vitamin E and Inflammation

It is a known fact that inflammation is closely associated with free radicals. Free radicals can mediate certain types of inflammatory tissue injury and vice versa, the process of inflammation itself generates free radicals that cause tissue damage (Conner and Grisham, 1996; Mitchell and Cotran, 2003c). Therefore, it is not surprising that antioxidants are widely used in the alleviation of inflammatory conditions. As mentioned earlier, vitamin E exhibits free radical scavenging activities and, thus, can be useful in the therapy of inflammatory conditions. It was shown in a study that in contrast to α-tocopherol, dietary γ-tocopherol and its metabolite, 2,7,8-trimethyl-2-(β-carboxyethyl)-6-hydroxychroman (γ-CEHC), inhibits COX-2 activity in macrophages and epithelial cells (Jiang *et al.*, 2000). The evidence showed that γ-tocopherol and its metabolite have anti-inflammatory properties *in vitro*. Subsequently, an *in vivo* study was performed and the results showed that γ-tocopherol inhibits prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4) production, two major products of the inflammatory arachidonic acid pathway (Jiang and Ames, 2003). Besides, γ-tocopherol also decreases the tumour necrosis factor, (TNF)-α, and inflammation-mediated damage in experimental rats. These findings demonstrate that besides functioning as free radical scavengers, γ-tocopherol also has significant anti-inflammatory actions.

All this while, less attention has been paid to tocotrienols compared to tocopherols, proven by the relatively low number of research papers published on the topic. However, the situation is changing as tocotrienols begin to garner more interest and are being compared to tocopherols in many studies. This is due to the unique functions of tocotrienols that are distinct from those of tocopherols (Sen *et al.*, 2006). In this review, all previous studies on tocotrienols were compiled and it shows that tocotrienols have different properties that are not seen in tocopherols, such as neuroprotection, anti-angiogenesis and anti-breast cancer activity. One of the anti-inflammatory studies done demonstrated that α-tocotrienol was more potent in inhibiting expression of the adhesion molecule compared to α-tocopherol (Theriault *et al.*, 2002). The result was confirmed in another study where α-tocotrienol was shown to reduce vascular cell adhesion molecule-1 (VCAM-1) expression and adhesion of THP-1 monocytes to endothelial cells (Noguchi *et al*., 2003).

In addition, a study in our laboratory demonstrated that treatment with different concentrations of TRF attenuates the expression of COX-2 in interleukin-1 beta (IL-1β)

1 Control (without IL-1β stimulation)
2 Control (with IL-1β stimulation)
3 Control (EtOH)
4 5 μg ml^{-1}
5 8 μg ml^{-1}
6 10 μg ml^{-1}
7 15 μg ml^{-1}
8 5 μg ml^{-1}
9 Beta actin (control)
10 Beta actin (experimental)

Figure 1. Expression of COX-2 in IL-1β stimulated A549 lung epithelial cancer cells treated with different concentrations of TRF.

stimulated A549 lung epithelial cancer cells (unpublished data). As can be seen from *Figure 1*, stimulation with IL-1β increases COX-2 expression. The Band intensity decreases with increasing concentration of TRF, indicating a reduction in COX-2 expression. The result suggests that TRF possesses anti-inflammatory effects that target the cyclooxygenase pathway.

CONCLUSION

While increasing the dietary intake of antioxidants has been shown to be helpful in relieving inflammatory conditions, more comprehensive studies should be undertaken to determine their actual mode of action. The anti-inflammatory potential of tocotrienols looks promising and further studies should be spear-headed. Better understanding of the properties of tocotrienols and other antioxidants would lead to greater benefits and options when planning for the health of the public.

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