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Review Article

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Glyceryl Tribenzoate: A Food Additive with Unique Properties to Be a Substitute for Cinnamon

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Abstract

Cinnamon is a regularly used natural seasoning and flavouring material throughout the world for eras. Recent laboratory studies have demonstrated that oral cinnamon may be beneficial for different neuroinflammatory and neurodegenerative disorders such as multiple sclerosis (MS), Parkinson's disease (PD), Alzheimer's disease (AD), and Lewy body diseases (LBD). However, cinnamon's certain limitations (e.g. unavailability of true Ceylon cinnamon throughout the world, impurities in ground cinnamon, etc.) have initiated an interest among researchers to find an alternate of cinnamon that can potentially deliver the same efficacy in the diseases mentioned above. Glyceryl tribenzoate (GTB) is a U.S. Food and Drug Administration (FDA)-approved flavoring ingredient that is used in food and food packaging industries. It has been found that similar to cinnamon, oral GTB is capable of upregulating regulatory T cells and suppressing the autoimmune disease process of experimental autoimmune encephalomyelitis, an animal model of MS. Moreover, both GTB and cinnamon metabolite sodium benzoate (NaB) have the potency to attenuate neurodegenerative pathology in a mouse model of Huntington disease (HD). Here, we have also demonstrated anti-inflammatory property of GTB in astrocytes and macrophages, a property that is also seen with cinnamon and its metabolite sodium benzoate (NaB). Therefore, here, we have made a sincere attempt to discuss the similarities and dissimilarities between cinnamon and GTB with a focus whether GTB has the potential to be considered as a substitute of cinnamon for neuroinflammatory and neurodegenerative disorders.

Introduction

In preclinical studies as well as clinical trials, cinnamon, the brown bark of cinnamon tree and a widely-used spice, has shown competency in the treatment of different autoimmune (MS and rheumatoid arthritis) and neurodegenerative (PD, AD and LBD) disorders [1-11]. However, cinnamon's limited natural existence and varieties have created a dilemma among the users. Although there are different types of cinnamon, Chinese cinnamon (Cinnamonum cassia) and original Ceylon cinnamon (Cinnamonum verum or Cinnamonum zeylanicum) are two major types of cinnamon that are currently available in the world. Mass spectrometry analysis show the presence of cinnamaldehyde in both Cinnamonum cassia and Cinnamonum verum [1]. However, Cinnamonum cassia contains greater levels of unwanted components such as styrene, benzene, 1,1'-(2-butene-1,4-diyl)bis-, benzene, 1,1'-(1,2-cyclobutanediyl)bis-, 4-phenylbutyl chloride, and (2,3-diphenylcyclopropyl)methyl phenyl sulfoxide than Cinnamonum verum [1]. Moreover, Cinnamonum cassia, but not Cinnamonum verum, holds 1-benzopyran-2-one or coumarin, a hepatotoxic molecule [1]. As a result, prolonged consumption of unwanted compounds through Cinnamonum cassia may lead to compromised body systems. Although there are differences in composition, both types of cinnamon have negligible appearance variations. Therefore, people are perplex in identifying the genuine or pure variety of cinnamon and accordingly, delineation of an alternative compound that do not contain the unwanted molecules, but capable of rendering the same effect as cinnamon can be a milestone in the treatment of autoimmune and neurodegenerative diseases.

Recent studies demonstrate that glyceryl tribenzoate (GTB), a white crystalline chemical compound, has the potency to inhibit the pathogenesis of autoimmune and neurodegenerative disorders [12, 13]. According to reaffirmation by Federal Emergency Management Agency (FEMA), GTB is a flavoring agent based on self-limiting properties, absorption, rapid metabolic detoxication, and secretion in human and other animals. GTB is also used in the pharmaceutical industry as a safe and nontoxic additive in different dosage forms. The availability of pure GTB is abundant as it can be synthesized in the laboratory. This study has been undertaken to analyse whether GTB can be repurposed for cinnamon.

Metabolism of Cinnamon and GTB

In addition to cinnamaldehyde, cinnamon contains cinnamyl acetate, cinnamyl alcohol and cinnamic acid. After intake, cinnamaldehyde, cinnamyl acetate and cinnamyl alcohol are converted into cinnamic acid by oxidation and hydrolysis, respectively. Then cinnamic acid is β- oxidized to benzoate in the liver [1, 6, 11, 14]. This benzoate exists as sodium salt (sodium benzoate or NaB) or benzoyl-CoA. This NaB is a FDA-approved drug for urea cycle disorders and glycine encephalopathy in children [15, 16].

Since GTB is a benzoic acid ester, which might be metabolized to benzoate in the body, in a recent study [12], we have monitored the level of NaB in mouse brain after oral GTB treatment. HPLC analysis from cortical tissues shows the presence of NaB peak in the brain from GTB-fed, not vehicle-treated, mice [12]. Therefore, similar to cinnamon, GTB is also metabolized to NaB.

Similar Functions of Cinnamon and GTB

Since both cinnamon and GTB are metabolized to NaB, both the compounds have a number of similar functions, which are outlined below.

Decrease in Pro-Inflammatory Molecules by Cinnamon and GTB:

Chronic or acute inflammation has become a hallmark in the pathogenesis of many human disorders including autoimmune as well as neurodegenerative diseases [2, 17-23]. Therefore, controlling inflammation is an important area of research. It has been seen that NaB inhibits the activation of p21ras and suppresses the expression of proinflammatory molecules in glial cells [2]. Accordingly, oral cinnamon treatment is also capable of increasing the level of NaB in the brain [1, 24] and reducing inflammation in vivo in the CNS of mice with EAE [5]. Oral cinnamon also suppresses glial activation and inflammation in mouse models of PD, AD and LBD [7, 9, 24].

Since it is not known whether GTB can suppress the level of proinflammatory molecules in cultured cells, here, we examined the effect of GTB on the formation of proinflammatory molecules in cultured macrophages and astrocytes.

Reagents

Cell culture materials (DMEM/F-12, L-glutamine, Hanks' balanced salt solution, 0.05% trypsin, and antibiotic/antimycotic) were purchased from ThermoFisher. Fetal bovine serum (FBS) was obtained from Atlas Biologicals. GTB was purchased from Spectrum Chemical (New Brunswick, NJ). Griess reagent, bacterial lipopolysaccharides (LPS) and polyinosinic-polycytidilic acid (poly IC) were procured from Sigma-Aldrich (St. Louis, MO).

Isolation of Primary Mouse Astrocytes

Astrocytes were isolated from 2- to 3-day-old mouse pups as described by us in several studies [25-28]. Briefly, on day 9, the mixed glial cultures were subjected to shaking at 240 rpm for 2 h at 37 °C on a rotary shaker to remove microglia followed by another round of shaking on day 11 at 180 rpm for 18 h to remove oligodendroglia and residual microglia. The adherent cells were washed and seeded onto new plates for further studies.

RAW 264.7 Macrophages

RAW 264.7 cells (purchased from ATCC) were also maintained in DMEM/F-12.

ELISA

Levels of TNFα and IL-1β were measured in supernatants using ELISA kits from Thermo Fisher following manufacturer's protocol as described [27, 29].

Statistical Analysis

Statistical analyses were performed with one-way ANOVA using Prism 8 (GraphPad Software). Data represented as mean ± SD. A level of $p<0.05$ was considered statistically significant.

GTB inhibits the production of proinflammatory molecules from activated RAW 264.7 macrophages.

To understand the effect of GTB on the activation of macrophages, RAW 264.7 cells (ATCC) preincubated with different concentrations of GTB for 4 h were stimulated with bacterial LPS. As expected, we found marked increase in the levels of nitrite (Figure 1A), TNFα (Figure 1B) and IL-1β (Figure 1C) in LPS-stimulated RAW 264.7 cells. However, GTB, at different doses tested, significantly inhibited LPS-induced production of nitrite (Figure 1A), TNFα (Figure 1B) and IL-1β (Figure 1C). Next, we were prompted to investigate whether GTB was also capable of suppressing the level of proinflammatory molecules in RAW 264.7 macrophages induced with other stimuli. Therefore, cells were challenged with double-stranded RNA in the form of poly IC (one of the etiological reagents for viral encephalopathy). Similar to LPS, poly IC also induced the production of nitrite (Figure 2A) and TNFα (Figure 2B) in RAW 264.7 cells, which were inhibited GTB (Figure 2).

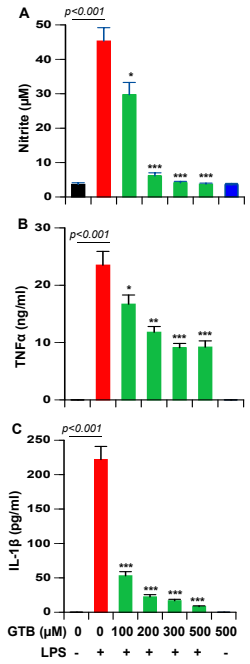

Figure 1: GTB inhibits the production of proinflammatory molecules from lipopolysaccharides (LPS)-stimulated mouse RAW 264.7 macrophages. Cells preincubated with different concentrations of GTB for 4 h were stimulated with 1 μg/ml LPS under serum-free condition. After 24 h, level of nitrite (A) was measured in supernatants by Griess reagent. Levels of TNFα (B) and IL-1β (C) were quantified in supernatants by ELISA. Results are mean ± SD of three independent experiments. *p < 0.05; **p < 0.01; ***p < 0.001 vs LPS.

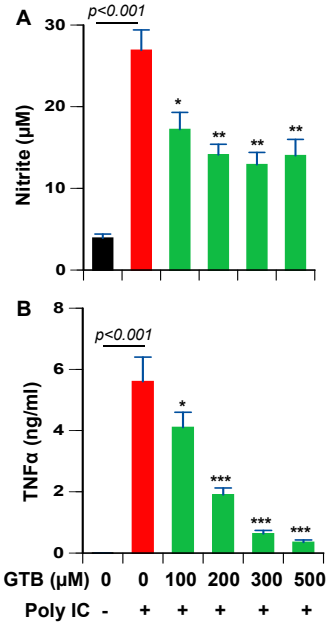

Figure 2: GTB suppresses the production of proinflammatory molecules from poly ICstimulated mouse RAW 264.7 macrophages. Cells preincubated with different concentrations of GTB for 4 h were stimulated with 50 μg/ml poly IC under serum-free condition. After 24 h, level of nitrite (A) was measured in supernatants by Griess reagent. Level of TNFα (B) was quantified in supernatants by ELISA. Results are mean ± SD of three independent experiments. *p < 0.05; **p < 0.01; ***p < 0.001 vs poly IC

GTB Inhibits the Production of Proinflammatory Molecules from Activated Mouse Primary Astrocytes

Next, to understand whether GTB attenuates the induction of proinflammatory molecules in primary cells, we used mouse primary astrocytes. Astrocytes are the major glial cells in the central nervous system (CNS), and astrocytic activation also plays an important role in various neuroinflammatory and neurodegenerative disorders [17, 23, 30, 31]. As expected, both LPS (Figure 3A) and poly IC (Figure 3B) induced the production of TNFα in astrocytes. However, similar to that found in RAW 264.7 macrophages, GTB significantly inhibited the production of TNFα in astrocytes stimulated by both LPS (Figure 3A) and poly IC (Figure 3B). Together, these results suggest that GTB is capable of suppressing the production of proinflammatory molecules in different cells.

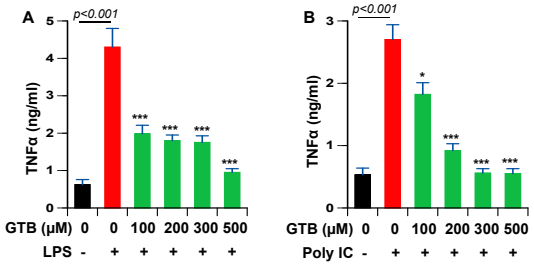

Figure 3: GTB inhibits the production of TNFα from LPS-stimulated primary mouse astrocytes. Cells preincubated with different concentrations of GTB for 4 h were stimulated with either 1 μg/ml LPS (A) or 50 μg/ml poly IC (B) under serum-free condition. After 24 h, level of TNFα was measured in supernatants by ELISA. Results are mean ± SD of three independent experiments. *p < 0.05; ***p < 0.001 vs LPS or poly IC.

Protection and/or Upregulation of Tregs by Cinnamon and GTB: Regulatory T cells (Tregs) are considered as the master regulator of immune responses due to the unique ability of modifying the immune system, preserving the tolerance to self-antigens, and preventing autoimmune diseases [32, 33]. It has been reported that Tregs are downregulated in patients with relapsing-remitting MS as compared to age-matched healthy subjects [34]. Consistently, Tregs are also lowered in patients with RA than control subjects [35, 36]. Therefore, maintenance and/or protection of healthy Tregs is beneficial for autoimmune disorders. Interestingly, oral cinnamon treatment upregulated and/or restored Tregs in mice with EAE, an animal model of MS [5]. It is found that nitric oxide plays an important role in the maintenance of Tregs [37, 38] and that cinnamon treatment restores Tregs via suppression of NO production [5]. Similarly, according to Mondal et al, GTB treatment also upregulates and/or normalizes Tregs in EAE mice. Transforming growth factor β (TGF-β) is known to induce Tregs from non-Tregs [39]. Interestingly, GTB increases the level of TGF-β to protect and/or upregulate Tregs [13].

Attenuation of Th17 Response by Cinnamon and GTB: Th17 cells capable of secreting IL-17A, IL- 17F, IL-21, and IL-22 cytokines play a more prominent role than Th1 cells in the pathogenesis of autoimmune diseases [19, 40-42]. Concentration of IL-17 is higher in patients with RRMS and PRMS compared to healthy individuals [43]. Accordingly, higher plasma level of IL-17 & IL-22 is observed in serum of RA patients as compared to age-matched healthy controls [44]. However, cinnamon treatment is capable of downregulating CD4+IL-17+ Th17 cells in EAE mice [5]. Since oral GTB reduces clinical symptoms and the incidence of EAE, Mondal et al have examined the effect of GTB on Th17 cells in EAE mice and found significant inhibition of Th17 cells in EAE mice. Therefore, both cinnamon and GTB are capable of reducing and/or normalizing Th17 cells.

Decrease in Inflammatory Infiltration by Cinnamon and GTB: Membranous structures blood-brain barrier (BBB) and blood-spinal cord barrier (BSB) protect the brain and the spinal cord, while facilitating essential molecules to pass and preventing chemicals in the blood from entering the CNS [14, 19, 41]. However, in active MS and EAE, breakdown of both BBB and BSB is seen probably due to increase in inflammation, thus allowing the passage of chemicals as well as autoreactive T cells and mononuclear cells into the brain and spinal cord [14, 19, 41]. Accordingly, H&E staining of EAE mice indicates an extensive inflammatory infiltration in the spinal cord and brain [5, 45-48]. However, cinnamon treatment restores the integrity of BBB and BSB and strongly halts the infiltration of inflammatory molecules into the CNS of EAE mice [5]. Similarly, it has been seen that oral administration of GTB is also capable of improving the integrity of BBB and BSB and inhibiting infiltration of mononuclear cells in adoptively transferred EAE mice [13].

Cinnamon and GTB in EAE and MS

MS is a devastating autoimmune disease of the CNS caused by demyelination of neurons and EAE serves as an animal model for MS [19, 41, 49]. Interestingly, Brahmachari et al [3] demonstrates that cinnamon metabolite NaB provided through drinking water modifies functions of T cells at multiple steps and inhibits the adoptive transfer of EAE in female SJL/J mice. Accordingly, oral administration of Cinnamonum verum powder also decreases the incidence of EAE and ameliorates the disease process of relapsing-remitting EAE in female proteolipid protein (PLP)-T cell receptor (TCR) transgenic mice [5], suggesting that oral cinnamon may have importance in the treatment of MS. Similar to cinnamon, GTB treatment also reduces the disease process of relapsing-remitting EAE in two different models in female mice and chronic EAE in male mice [13], indicating that this indirect food additive may be explored for beneficial interference in MS.

Cinnamon and GTB in A Mouse Model of Huntington Disease

Huntington disease (HD) is a rare genetic disorder associated with deposition of mutant huntingtin protein and loss of the neurons in the striatum and cortex of the brain, ultimately leading to physical, mental and behavioural decline. Although HD can develop at any time, people first experience HD symptoms when they are in their 30s or 40s. There is no effective treatment available for HD. Clinically approved treatments only offer symptomatic relief together with exhibiting a number of side effects. Interestingly, we have seen that oral administration of GTB significantly reduces mutant huntingtin level in striatum, motor cortex as well as hippocampus, inhibits glial activation and increased the integrity of viable neurons in transgenic N171-82Q mouse model of HD [12]. Accordingly, cinnamon metabolite NaB also decreases HD-related pathology and improves motor performance in transgenic N171-82Q mice [12]. Therefore, these treatments may be repurposed for HD.

Differences between Cinnamon and GTB

While there are many similarities between cinnamon and GTB as described above, some differences are also there between the two. First, cinnamon is naturally found as brown bark of cinnamon tree and GTB is manufactured in the laboratory. Second, cinnamon, but not GTB, has sweet and spicy taste. Third, cinnamon is usually medium shade of brown in color, whereas GTB is colorless. Fourth, in addition to containing cinnamaldehyde as the active ingredient, cinnamon contains a number of other unwanted molecules such as styrene, benzene, 1,1'-(2-butene-1,4- diyl)bis-, etc. On the other hand, being chemical synthesized, GTB is pure. Fifth, upon metabolism, GTB generates glycerol having laxative effect to help digested food move through our gut more smoothly. This glycerol is not present in cinnamon. Sixth, GTB, but not cinnamon, is reported to stimulate TGFβ in splenocytes [13].

Conclusion

Studies show that both cinnamon and GTB prevent inflammation and inhibit neurodegenerative pathways via same mechanisms. Oral treatment of cinnamon and GTB also upregulates and/or protects the Tregs, preserves the BBB and BSB, and inhibits inflammatory infiltration in the CNS of EAE mice. Since cinnamon is naturally available, people can always initiate self-treatment with this as a supplement without the guidance of health professionals. However, due to the presence of various constituents like coumarin as a hepatotoxin, cinnamon may cause undesirable influence on the body systems upon prolonged usage. Under that scenario, in the absence of pure cinnamon, GTB, synthesized in the laboratory in uncontaminated form, may come into the picture as a substitute of cinnamon to render beneficial effects against neurodegenerative and autoimmune disorders. GTB treatment may also have the additional advantage as laxative that can gravitate overall relief in patient's wellbeing.

Data Availability: This is a mainly review and the readers can access the entire published article supporting the conclusions of this study through PubMed.

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