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Curcumin and linseed oil co-delivered in phospholipid nanoemulsions enhances the levels of docosahexaenoic acid in serum and tissue lipids of rats

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ABSTRACT

Docosahexaenoic acid (DHA) is an important long chain omega-3 polyunsaturated fatty acid (PUFA) primarily found in marine fishes. The diets of vegetarian population do not contain preformed DHA, but they can derive it from shorter chain α-linolenic acid (ALA) found in plant oils. However, the conversion efficiency of ALA to DHA is minimal in human adults. This may cause insufficiency of DHA in the vegetarian population. Curcumin, diferuloyl methane found in the spice turmeric, has the potential to increase the formation of DHA from ALA by activating the enzymes FADS2 and elongase 2. The present study was designed to prepare curcumin nanoemulsion using phospholipid core material (Lipoid™) and exploring the possibility of enhancing its bioavailability and its impact on DHA levels in rats. Curcumin was dissolved in coconut oil (CNO, MCFA rich), Sunflower oil (SNO, n-6 PUFA rich) or Linseed oil (LSO, n-3 PUFA rich) and nanoemulsions were prepared after mixing with Lipoid™ using high pressure homogenizer. The nanoemulsions were fed to weaning rats for 60 days along with AIN-93 diets. Rats fed nanoemulsion containing curcumin in LSO showed high levels of curcumin in serum liver, heart and brain. Significant increase in DHA levels of serum and tissue lipids were observed in rats given LSO with curcumin in nanoemulsions. Therefore, supplementation of diets with ALA rich LSO and curcumin could increase DHA concentrations in serum, liver, heart and brain lipids which have implications for meeting the DHA requirements of vegetarian populations.

1. Introduction

The benefits of consuming a diet containing Docosahexaenoic acid (DHA) in improving cardiovascular health, prevention of inflammatory conditions and slowing the progression of certain types of cancer is well documented in the literature [1–4]. DHA is also an important constituent of lipids in brain and heart. The levels of DHA are significantly reduced in many neurodegenerative diseases such as Alzheimer, Parkinson, Dementia, Schizophrenia and Huntington disease [5]. Low levels of DHA in central nervous system also results in the impairment of cognitive and photoreceptor functions [6]. A reduced deposition of DHA in brain and retina during the last trimester of fetal development may affect learning abilities of the infant during later stages of life [7]. A recent study has shown that more than 70% of middle aged women in Germany are at increased risk for heart diseases because of low intake of omega-3 fatty acids [8]. Preformed DHA is found in breast milk, marine fishes and in algae. However, a vegetarian diet seldom contains long chain omega-3 fatty acids such as DHA. Vegetarian population primarily depend on plant oils such as flax seed oil, soybean oil, canola oil and green leafy vegetables for fulfilling the omega-3 fatty acid requirements. However, the plant oils contain only α-linolenic acid (ALA) and DHA is not detected in plant oils. ALA needs to be converted to eicosapentaenoic acid (EPA) and DHA to derive benefits attributed to long chain omega-3 fatty acids. Studies have shown that majority of the ingested ALA is diverted to β- oxidation pathway and the conversion efficiency of ALA to DHA is very minimal. Less than 5% of ingested ALA is converted to eicosapentaenoic acid and overall only 0.5% of ALA is converted to DHA [9]. This exposes the vegetarian populations to long chain omega-3 PUFA insufficiency. This underscores the need for finding ways to increase the conversion of ALA to DHA or to engineer plant oils to contain DHA to meet the DHA requirements for vegetarian populations.

A recent study by Walsh et al. [10] has shown that it is possible to engineer canola plants with a microalgal polyketide synthase - like

Abbreviations: CNO, Coconut oil; SNO, Sunflower oil; LSO, Linseed oil; LA, Linoleic acid (18:2, n-6); ARA, Arachidonic acid (20:4, n-6); ALA, α-linolenic acid (18:3, n-3); DHA, Docosahexaenoic acid (22:6, n-3)

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system to produce canola oil enriched in DHA. However such approaches need further refinement and await regulatory clearances before commercializing the product. It is also observed that the intake of curcumin, a diferuloyal methane found in spice turmeric could enhance the DHA levels in rats by activating the enzymes such as FADS2 and elongase 2 [11]. These enzymes are involved in the synthesis of DHA from ALA. Turmeric is a common spice used as food adjunct in Asian sub continent. Turmeric contains 4–5% curcumin.

We have recently demonstrated that the bioavailability of ALA rich oil such as Linseed oil (LSO) is significantly enhanced in rats when ingested in the form of phospholipid based (Lipoid ™) nanoemulsions [12,13]. It was also observed in this study that the DHA levels in serum and tissue lipids was significantly enhanced when ALA rich LSO was provided in nanoemulsion form. However, the combined effects of LSO with Curcumin on DHA levels have not received adequate attention. Curcumin has poor solubility in aqueous media and it has low bioavailability. This needs to be improved for exploiting the potentials of curcumin for health benefits. Nanoemulsion approach has been tried to improve the solubility and bioavailability of curcumin [14].

In this investigation, we report a protocol for the preparation and characterization of curcumin nanoemulsion in phospholipid core material. We further evaluated the bioavailability of curcumin by monitoring its distribution in plasma, liver, heart and brain when rats ingested curcumin in nanoemulsion form. The consequence of this on DHA levels in plasma and tissue lipids was also evaluated. This approach was taken to assess whether the vegetarian population could derive sufficient amounts of DHA from ALA rich oil to bridge the gap in the assimilation of DHA in the lipids of vital organs without changing their dietary habits.

2. Materials and methods

2.1. Materials

Curcumin (> 99% pure) was purchased from Flavours and Essences Limited (Mysore, India). Linseed oil (LSO) (~54% 18:3 n-3 PUFA) was obtained from Kamani flax omega industries, Mumbai, India. Sunflower oil (SNO) (~58% 18:2, n-6 PUFA) and coconut oil (CNO) (~92% saturated fatty acids) were purchased from local super market. Lipoid S75-3 (69% phosphatidylcholine and 10% phosphatidylethanolamine) was a gift from Lipoid™ (Ludwigshafen, Germany). Sodium chloride and sodium azide were procured from Himedia, Mumbai, India. β−17 estradiol acetate, polyethylene glycol, lauric acid, myristic acid, α-linolenic acid, linoleic acid, eicosapentaenoic acid and docosahexaenoic acid were procured from Sigma Chemical Company, St. Louis, MO, USA. All the solvents used were of analytical grade and distilled before use.

2.2. Preparation of phospholipid based nanoemulsions of curcumin taken in different oils

Curcumin taken in one of three vegetable oils (CNO, SNO or LSO) was encapsulated in phospholipid based Lipoid™ S75-3 according to the method of Sugasini and Lokesh [12]. The components added to prepare emulsions were Lipoid (0.075%), polyethylene glycol (3%) and NaCl (0.9%) dissolved in distilled water. Curcumin (0.2 wt%) in 1 mL of CNO, SNO or LSO was added to lipoid containing solution and thoroughly mixed by using a vortex mixer. The emulsions were homogenized for 5 min at 6000 rpm using a laboratory mixer (Silverson L4RT, Silverson Machines Ltd, Chesham, Bucks, England) and sonicated for 60 s. These emulsions were further homogenized at 900 bar for eleven cycles at 4 °C using a high pressure homogenizer (Panda, GEA NiroSoavi, Italy). These emulsions were collected and stored at −20 °C. Curcumin in above mentioned oils but not subjected to any emulsification step were labelled as native curcumin.

2.3. Encapsulation efficiency, particle size and topography of curcumin in nanoemulsions

Efficiency of binding material to encapsulate curcumin was determined as described by Maiti et al. [15]. The curcumin adsorbed on nanoemulsions were calculated as described by Yen et al. [16]. The percentage of curcumin entrapped was determined after centrifuging the nanoemulsion at 15,000×g for 15 min. The pellet was washed twice with water and dissolved in acetonitrile. The curcumin in the pellet was estimated by HPLC method of Suresh and Srinivasan [17]. The amount of the curcumin entrapped in the nanoemulsions was calculated as (%) encapsulation=[(total curcumin−adsorbed curcumin)×100]/total curcumin.

The particle size distribution of the emulsified curcumin droplets was measured by using a Mastersizer fitted with small volume sample presentation unit and integration software (Nano-ZS, Malvern Instruments Ltd., Worcestershire, UK). The particle size distributions were calculated based on a relative particle refractive index of 1.350 as described by Jafari et al. [18]. Phosphate buffer (5 mM, pH. 7.0) was used as the dispersant for the determination of the globule size distribution of curcumin in emulsion. The mean droplet diameter was expressed as the Volume mean diameter. Each sample was analyzed in triplicate.

The topography of curcumin nanoemulsions was evaluated using Atomic force microscopy (AFM). Prior to taking AFM imaging, emulsion samples were diluted 30-fold with de-ionized water. Experiments were performed in tapping mode using a Silicon cantilevers with 100–300 kHz resonance frequency. All images were recorded at room temperature at a scan speed of 1 Hz and this was done three times for each treatment. 10 fields were used for one single plate. Images were obtained by displaying the amplitude signal of the cantilever in the trace direction, and the height signal in the retrace direction, both signals being simultaneously recorded.

2.4. Animal experiments

2.4.1. Dietary studies

Weaning male rats [OUB-Wistar, IND-cft (2c)] weighing 45 ± 4 g were grouped (six rats in each group) by random distribution and housed in individual cages, under a 12 h light/dark cycle, in an approved animal house facility at the CSIR-Central Food Technological Research Institute in Mysore, India. The rats were fed AIN-93 diet [Reeves et al. [19]] for 60 days. The rats had free access to food and water throughout the study. In addition, the rats were administered 1 mL curcumin (65 ± 3 mg) in native form or as nanoemulsion by gavage once a day at 10AM for 60days. After 60days, rats were fasted overnight and sacrificed under ether anesthesia. Blood was drawn by cardiac puncture and serum was separated by centrifugation at 1100*g* for 20 min. Liver, heart and brain were harvested, rinsed thoroughly with ice-cold saline to remove traces of blood, blotted, weighed and stored at −20 °C until analyzed. Fecal matter was collected during the last 3 days before sacrificing the animals. The protocols for animal experiments were approved by Animal ethics committee recognized by Government of India.

2.5. Estimation of curcumin

The curcumin was extracted from serum, tissues and fecal matter using ethyl acetate and methanol. The curcumin from the extract was analyzed as described by Suresh and Srinivasan [17] using HPLC (Model Shimadzu LC-20 A, UV visible detector (SPD-20 A), Pump (LC-20 AT), Shimadzu, Japan) fitted with injection valve of 20 μL sample loop (Model 7125, Rheodyne, CA, USA). An aliquot of serum (200 μL) was mixed with water (80 μL), 20 μL of β−17-estradiol acetate (internal standard 6 μg/mL) and vortexed for 30 s. The curcumin was then extracted with 1 mL of ethyl acetate: methanol (95:5, v/v) and the

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