JOURNAL OF FUNCTIONAL FOODS 22 (2016) 578–587

Available online at www.sciencedirect.com

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Enhanced bioavailability and relative distribution of free (unconjugated) curcuminoids following the oral administration of a food-grade formulation with fenugreek dietary fibre: A randomised double-blind crossover study

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ARTICLE INFO

Article history:
Received 9 December 2015
Received in revised form 18 January 2016
Accepted 20 January 2016
Available online

Keywords:
Curcumin
Curcuminglucuronide
Conjugated curcumin metabolites
Bioavailability
Pharmacokinetics
Oral administration

ABSTRACT

Despite the various reports on enhanced bioavailable formulations of curcumin, systemic oral bioavailability of unconjugated curcuminoids remains a challenge. Considering the differences in plasma bioactivity and membrane permeability of free curcuminoids over conjugated metabolites, herein we report a randomised double-blinded crossover study (n = 50) to investigate the relative bioavailability and pharmacokinetics of free curcuminoids following the oral administration of high (1000 mg) and low (250 mg) doses of a food-grade formulation of curcumin with fenugreek dietary fibre as curcumagalactomannosides (CGM), which was reported to exhibit improved blood-brain – barrier permeability in rats. CGM administration provided over 45.5-fold enhancement in free curcuminoids bioavailability with improved pharmacokinetics when compared to unformulated standard curcumin. Further investigations with and without enzymatic hydrolysis of plasma collected over 5 h post-administration of CGM at 1000 mg dose revealed higher free curcuminoids in plasma (74 ± 8%) as compared to conjugated curcuminoids (26 ± 12%) indicating a significant distribution of free curcuminoids over conjugated curcumin metabolites.

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http://dx.doi.org/10.1016/j.jff.2016.01.039
1756-4646/

1. Introduction

In spite of the excellent safety profile and health benefits, as demonstrated by numerous preclinical evaluations, the poor oral bioavailability of curcuminoids [the bioactive principle of turmeric (*Curcuma longa* L.)] has been regarded as the most significant factor which limits its use as an efficient natural therapeutic/functional agent (Anand, Kunnumakkara, Newman, & Aggarwal, 2007; Gupta, Patchva, & Aggarwal, 2013; Prasad, Tyagi, & Aggarwal, 2014). Systematic analysis of the physicochemical characteristics of the three closely related curcuminoids, namely curcumin, demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC) has revealed the properties such as poor water solubility (11 ng/L), hydrophobicity (log P: 3.28), and instability under varying pH conditions of the gastrointestinal tract as the main reasons for its poor bioavailability (Anand et al., 2007; Gupta et al., 2013). The α, β-unsaturated polyphenolic structure of curcuminoids with a β-diketone moiety and an active methylene group has been identified as the mechanistic centre, which makes curcuminoids a highly labile substrate for various enzymes responsible for the rapid intestinal biotransformation and first pass metabolism (Lin, 2007; Prasad et al., 2014). Pan, Huang, and Lin (1999) employed the method of glucuronidase hydrolysis of the plasma samples to demonstrate the predominant existence of glucuronide/sulfate conjugates of curcuminoids, in addition to a number of reduced metabolites such as di, tetra and hexahydrocurcuminoids and their conjugates (Lao et al., 2006; Pan et al., 1999). Thus, it has been hypothesised that the *in vivo* therapeutic efficacy of curcumin is mainly emanating from the bioactivity of conjugated metabolites, congeners and degradation products (Kurita & Makino, 2013).

However, the investigations on the relative bioavailability of free unconjugated curcuminoids and their metabolites in plasma have received recent interest owing to the various reports that the conjugated curcumin metabolites possess low bioactivity and permeability as compared to their unconjugated forms (Ji & Shen, 2014; Krishnakumar et al., 2015; Sandur et al., 2007; Sharma, Gescher, & Steward, 2005). In an attempt to explain the *in vivo* bioactivity of curcuminoids, Vareed et al. (2008) postulated the possibility of de-conjugation of curcumin conjugates at the selected target sites to make the free curcuminoids available for activity. A recent cell-based study reported no anti-inflammatory and anti-proliferative effects to curcuminglucuronides (Pal et al., 2014). Moreover, the ability of free curcumin to reduce IL-1β levels, amyloid plaques and tau deposits have also been reported (Begum et al., 2008; Ma et al., 2013; Yang et al., 2005). However, oral administration of standard curcumin (natural curcuminoids isolated from dried turmeric rhizomes with 95% purity), even at doses as high as 10 to 12 g per day could not provide significant plasma levels of free curcuminoids when administered orally (Garcea et al., 2004; Lao et al., 2006). Thus, drug delivery systems capable of delivering significant levels of free unconjugated curcuminoids in plasma and tissues assumes great significance, since the absorption and tissue distribution of the bioactive form of a molecule at significantly higher concentration has been identified as a key factor to mediate efficacy of the molecules, which undergo rapid biotransformation.

Although a large number of drug delivery techniques and formulations comprising the nanoparticles suitable for transdermal, nasal, intravenous, or intraperitoneal, or intramuscular administration have been developed over the years (Kurita & Makino, 2013; Prasad et al., 2014), food-grade formulations capable of providing therapeutically significant levels of free curcuminoids upon oral administration were limited; except a solid-lipid nanoformulation, which has shown to detect 22.43 ng/mL of curcumin in plasma when supplemented at 650 mg dose (Gota et al., 2010). Most of these oral bioavailable formulations were developed on the basis of either the solubility enhancement or synergistic effects of multiple ingredients. A recent study on a water dispersible formulation of curcuminoids with fenugreek dietary fibre rich in galactomannans as 'curcumagalactomannosides' (hereinafter referred to as 'CGM') has reported to exhibit improved BBB-permeability and tissue distribution of all the three free curcuminoids (curcumin, DMC and BDMC) following its oral administration to rats (Krishnakumar et al., 2015). In the present contribution, a double-blinded randomised crossover study (n = 50) is described to compare the bioavailability and pharmacokinetics of free curcuminoids following a high (1000 mg) and low (250 mg) dose administration of CGM in comparison with the similar doses of standard curcumin, employing Ultra performance liquid chromatography coupled with electrospray ionisation tandem mass spectrometry (UPLC-ESI-MS/MS). Further, an investigation (n = 10), was also carried out to measure the ratio of the free to conjugated curcuminoids in plasma over 5 h of post-administration time period and hence to better understand the degree of bioavailability of free curcuminoids over the curcumin conjugates upon CGM administration.

2. Subjects and methods

2.1. Materials

Commercial grade natural unformulated curcuminoids (standard curcumin) with 95.08% purity and CGM (patented and registered formulation under the trademark, CurQfen®) containing 39.1% curcuminoids were obtained from M/s Akay Flavours & Aromatics Pvt Ltd, Cochin, India, in powder forms. Purity and composition of curcuminoids in both standard curcumin and CGM were determined by a validated HPLC procedure employing Shimadzu M20 model HPLC fitted with photodiode array (PDA) detector (Shimadzu Analytical India Pvt Ltd, Mumbai, India) and reverse-phase C18 column (250 × 4.6 mm, 3 μm) (Phenomenex, Hyderabad, India). Analytical reference standards of curcumin (CAS# 458-37-7; purity >98%), DMC (CAS# 22608-11-3; purity >98%) and BDMC (CAS# 33171-05-0; purity >95%) and *Helix pomatia* β-glucuronidase enzyme were obtained from Sigma-Aldrich, Bangalore, India. All solvents used for mass spectrometry and HPLC analyses were obtained from Merck, Mumbai, India.

2.2. Subjects

Healthy adult human volunteers (45 males and 9 females; aged between 24 and 46 years), who were not under any medica-