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Original article

Effects of turmeric curcuminoids and metformin against central sensitivity to pain in mice

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ABSTRACT

The reported experimental study was conducted to compare the effects of repeated daily oral doses of curcuminoids (CLE) with metformin as potential antidepressants and analgesics. Effects of a single and ten daily oral doses of CLE (5, 20, 80 mg/kg/day) and of 50 mg/kg/day metformin (MET) were compared in mice hot plate test (HPT) for analgesics. On the 11th treatment day, all animals were subjected to foot shock stress triggered hyperthermia test, and on the 12th treatment day to tail suspension test (TST) for antidepressants. Immediately thereafter, their blood levels of glucose, insulin and cortisol were quantified. Dose dependent analgesic activity of CLE was observed in HPT, whereas the metformin dose tested suppressed only pain hypersensitivity in the test. But statistically significant effects of both of them were observed in TST, and both of them also afforded protections against body weight loss and slight elevation in core temperatures induced by daily handling and repeated testing. CLE or metformin had no significant effects in foot shock stress triggered transient hyperthermic responses or on blood glucose, insulin and cortisol levels. Reported results reveal that curcuminoids as well as metformin are stress response modifiers with antidepressants like activities, but only low dose curcuminoids possess centrally acting analgesics like activities. They suggest that the bio-assay system used in this study is well suited for identifying curcuminoids like plant metabolites with analgesic and anti-stress activities, and that low dose curcuminoids are more effective as analgesics than low dose metformin.

1. Introduction

Central hypersensitivity to pain is often encountered in patients suffering from chronic inflammatory diseases as well as diverse spectrums of somatic symptom disorders.[1,2] In traditionally known Indian and Chinese systems of medicine and health care, turmeric (*Curcuma longa* rhizomes) is often used for prevention and cure of such medical conditions. Curcumin and structurally analogous diarylheptanoids encountered in turmeric, often collectively referred to as turmeric curcuminoids, are now attracting considerable attention of modern drug discoverers as therapeutic leads potentially useful for prevention and cure of chronic diseases commonly associated with mental health problems and central sensitivity to pain,[3,4] and number of reports reaffirming antinociceptive effects of curcumin and other turmeric derived products in animal models and clinical trials have continued to increase since late decades of the 20th century.[5,6] It has often been suggested also that curcuminoids formulations or their derivative and analogs with improved bioavailability and adverse effect potentials could be universally more acceptable and reliable therapeutic alternatives for prevention and cure of numerous chronic diseases, including cancer and Alzheimer's disease.[3,7,8] Such suggestions often neglect that like numerous non-systemic[9] or covalent

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drugs,[10,11] blood levels of curcuminoids are not very reliable predictors of their therapeutic potentials or effectiveness.

Oral bioavailability studies conducted with curcuminoids have consistently revealed that even after very high oral doses (up to 1 g/kg) their observed blood levels are often undetectable.[12,13] On the other hand, it has often been reported that low oral doses of curcumin possess antidepressant and stress response suppressing effects in laboratory rodents.[14–16] A report on dose dependant antinociceptive effects of curcumin after its single fairly low oral doses has appeared also.[17] Other dose finding studies have suggested though, that oral 500 mg/kg curcumin could be its optimal pharmacologically interesting dose in laboratory animals.[18] Therefore, in the realm of our ongoing psychopharmacological studies with traditionally known herbal remedies and their bioactive constituents[19] we used curcumin and diverse types of curcuminoids enriched turmeric extracts to define their stress response suppressing dose ranges and dosing regimen.[20,21] They have reconfirmed that repeated daily oral 5 mg/kg curcumin or turmeric curcuminoids are high enough for increasing stress resistance in mice and have revealed that several low dose effects of turmeric curcuminoids are qualitatively analogous those of metformin and diverse other food chemicals commonly consumed with every day meals.[22–26] Metformin is currently the antidiabetic drug of first choice for prevention and cure of diverse spectrums of comorbidities associated with diabesity[27] and it is also often used for treatments of neuropathic pain.[28] Numerous, but not all, therapeutically interesting bioactivities of metformin known to date[29] are quite analogous to those of curcuminoids, and it has been reported also that repeated daily oral curcumin administrations also suppresses neuropathic pain in diabetic animals.[30,31]

However, like in numerous pharmacological studies with curcumin and turmeric curcuminoids, most preclinical studied revealing their no-conceptive potentials were conducted with their relatively high doses and arbitrarily chosen dosing regimen.[5] Therefore, the question whether daily oral intake of lower stress resistance promoting or adaptogenic doses of turmeric curcuminoids could also suppress central hypersensitivity to pain still remain open, or can be speculatively answered only. Results of an experiment conducted to more rationally answering this question has been described and discussed in this communication. Implication of the preclinical observation made to date with low dose curcuminoids for better understanding of Ayurvedic concepts of nutritional therapies or for more rational medicinal uses of turmeric curcuminoids are also pointed out in this article.

2. Materials and methods

2.1. Animals

Swiss albino male mice (20 ± 5 g) obtained from the Central Animal House of the Institute of Medical Sciences, Banaras Hindu University, Varanasi, India (Registration Number: 542/AB/CPCSEA) were housed in groups of six in polypropylene cages at an ambient temperature of 25 ± 1 °C and 45–55% relative humidity, with a 12:12 h light/dark cycle. They were always provided with commercial food pellets and water *ad libitum*, and were acclimatized for at least 1 week before using them for the experiments. Animals used in this study were pre-selected for their pain sensitivity in the hot plate test described later. For such purposes, reaction time of animals on a hot plate maintained at 55 ± 1 °C were recorded and only those mice reacting within 15 s on the hot plate and which did not show large variation when tested on four separate occasions (each 15 min apart), were randomly allotted to different experimental groups. Principles of laboratory animal care (NIH publication number 85-23, revised in 1985) guidelines were always followed, and prior approval from the Central Animal Ethical Committee of the University (CAECU) was taken for this study protocol (Dean/2014/CAEC/602, dated 30-05-2014).

2.2. Plant extract and other material used

The *C. longa* rhizome extract (CLE) highly enriched in turmeric curcuminoids (95.49%, w/w) used in this study was generously supplied by R&D Center of Natural Remedies Pvt. Ltd., Bengaluru, India. HPLC chromatogram of CLE is shown in Fig. 1. As quantified by the USP 37-NF32 method, CLE contained curcumin (>77.94%, w/w), demethoxycurcumin (>15.03%, w/w) and bisdemethoxycurcumin (>2.52%, w/w), Metformin (Ranbaxy Laboratories, Gurgaon, India), carboxymethyl cellulose (CMC) (Central Drug House, New Delhi, India) and other chemicals and reagents used in this study were of highest purity commercially available in India.

2.3. Animal grouping and drug treatments

Six groups of six animals each were randomly allotted to different experimental groups. Two of them used as controls viz. CON + HPT (subjected to hot plate test; i.e. HPT) and CON − HPT (not subjected to HPT) were treated orally with the vehicle (0.3% CMC; 10 ml/kg/day) for 11 consecutive days. The four others were similarly treated either with 50 mg/kg/day metformin or with 5 or 20 or 80 mg/kg/day CLE. Except for one of the CMC treated control groups (CON − HPT), all others were subjected to hot plate test on days 1, 5, 7, and 10 of the experiment. For oral administrations, metformin and CLE were suspended in 0.3% CMC. All tests were conducted one hour after the day's oral treatments and all oral treatments on all observational days were given after recording their body weights and rectal temperatures. Further details of the experimental procedure used are graphically summarized in Fig. 2.

2.4. Hot plate test (HTP)

One hour after days treatment, individual mice of a group was gently placed on a hot plate maintained at 55 ± 1 °C and its reaction time (in seconds) for forepaw licking or jumping (whichever occurred first) were recorded.[32] Immediately thereafter, the animal was placed back in its home cage, and 10 min thereafter its rectal temperature was recorded again. All rectal temperatures were recorded using a calibrated rectal probe and electronic thermometer. The numerical difference between the basal rectal temperature of an animal and its rectal temperature recorded 10 min after the hot plate test was calculated and used as an index for hot plate test induced hyperthermic response of the animal.

2.5. Foot shock stress induced hyperthermia test

All experimental groups were subjected to this test on the 11th day of the experiment. The experimental procedure used has been described in details elsewhere.[21] In short, individual mouse of a group was placed in a black box (24 × 29 × 40 cm) with a grid floor for 1 min. After 10 s of its stay in the box, five consecutive electric foot shocks of 2 mA at 10 s intervals were given through the grid floor (2 mA, 50 Hz of 2 ms duration). Immediately thereafter, the animal was placed back in its home cage and 10 min thereafter its rectal temperature was recorded again. Difference between the rectal temperature of the animal recorded 10 min after the foot shock stress and that of its basal one of that day was considered to be its foot shock stress induced hyperthermic response.

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