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Chronotherapeutic effect of fisetin on expression of urea cycle enzymes and inflammatory markers in hyperammonaemic rats

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ABSTRACT

Background: Elevated blood ammonia leads to hyperammonaemia that affects vital central nervous system (CNS) functions. Fisetin, a naturally occurring flavonoid, exhibits therapeutic benefits, such as anti-cancer, anti-diabetic, anti-oxidant, anti-angiogenic, neuroprotective and neurotrophic effects.
Methods: In this study, the chronotherapeutic effect of fisetin on ammonium chloride (AC)-induced hyperammonaemic rats was investigated, to ascertain the time point at which the maximum drug effect is achieved. The anti-hyperammonaemic potential of fisetin (50 mg/kg b.w. oral) was analysed when administered to AC treated (100 mg/kg b.w. i.p.) rats at 06:00, 12:00, 18:00 and 00:00 h. Amelioration of pathophysiological conditions by fisetin at different time points was measured by analysing the levels of expression of liver urea cycle enzymes (carbamoyl phosphate synthetase-I (CPS-I), ornithine transcarbamoylase (OTC) and argininosuccinate synthetase (ASS)), nuclear transcription factor kappaB (NF-κB p65), brain glutamine synthetase (GS) and inducible nitric oxide synthase (iNOS) by Western blot analysis.
Results: Fisetin increased the expression of CPS-I, OTC, ASS and GS and decreased iNOS and NF-κB p65 in hyperammonaemic rats. Fisetin administration at 00:00 h showed more significant effects on the expression of liver and brain markers, compared with other time points.
Conclusions: Fisetin could exhibit anti-hyperammonaemic effect owing to its anti-oxidant and cytoprotective influences. The temporal variation in the effect of fisetin could be due to the (i) chronopharmacological, chronopharmacokinetic properties of fisetin and (ii) modulations in the endogenous circadian rhythms of urea cycle enzymes, brain markers, redox enzymes and renal clearance during hyperammonaemia by fisetin. However, future studies in these lines are necessitated.

Introduction

The common feature of urea cycle disorder (UCD) is a defect in ammonia elimination leading to hyperammonaemia and hepatic encephalopathy (HE). The increased level of ammonia in the blood leads to an increase in cerebral uptake across the blood–brain barrier (BBB) [1]. Excess ammonia is toxic to the central nervous system (CNS); elevated cerebral ammonia results in a severe accumulation of glutamine in astrocytes, causing oedema and encephalopathy [2], and leading to a spectrum of neuropsychiatric and neurological symptoms (impaired memory, shortened attention span, sleep–wake inversions, brain oedema, intracranial hypertension, seizures, ataxia and coma) with high mortality [3].

Abbreviations: AC, ammonium chloride; ASS, argininosuccinate synthetase; CPS-I, carbamoyl phosphate synthetase-I; GPx, glutathione peroxidase; GS, glutamine synthetase; GSH, reduced glutathione; GST, glutathione-S-transferase; HE, hepatic encephalopathy; IκB, inhibitor kappaB; NF-κB p65, nuclear factor kappaB subunit p65; NMDA, *N*-methyl-D-aspartate; NO, nitric oxide; NOS, nitric oxide synthase; iNOS, inducible nitric oxide synthase; OTC, ornithine transcarbamoylase; ROS, reactive oxygen species; SOD, superoxide dismutase; TNF-α, tumour necrosis factor-α; UCD, urea cycle disorder.

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Endogenous 24 h rhythmicities of urea cycle enzymes such as carbamoyl phosphate synthetase-I (CPS-I), ornithine transcarbamoylase (OTC) and argininosuccinate synthetase (ASS) in rat liver and serum have been reported; the enzyme levels are low in light period but exhibit a significant increase in darkness [4,5]. The increase in cerebral ammonia concentration may stimulate glutamine synthetase (GS) activity to metabolise excess ammonia and glutamate into glutamine and thus thwarting neurotoxicity [6]. GS has a robust circadian rhythm in liver and kidney; increased mRNA expression occurs in the dark period when the animal begins activity and feeding coinciding with amino acid metabolism [7]. In humans, the rhythmic pattern is relatively reversed [8].

Nitric oxide (NO) produced by inducible nitric oxide synthase (iNOS) possesses microbicidal, antiviral, antiparasitic and antitumoural effects. However, abnormal iNOS induction seems to be involved in the pathophysiology of a variety of human diseases. In addition to ammonia and glutamate levels, the NOS and NO system might also be involved in the brain responses to HE [9]. The acrophases of NO and NOS in plasma, brain, kidney, testis and lung occur at midnight, corresponding to the behavioural activity and increased reactive oxygen species (ROS) production [10,11].

NF-κB/Rel proteins, a family of ubiquitous transcription factors, participate in immunological responsiveness, inflammatory processes and cell growth regulation. ROS seem to play a dual role in the NF-κB activation cascade. NF-κB helps to regulate various inflammatory genes in different target cells [12]. The nuclear content of subunit of NF-κB p65 was found to exhibit circadian rhythm in animals [13]. The circadian oscillator components regulate the immune response, and the absence of the core clock component, cryptochrome, caused elevation of NF-κB p65, suggesting a link between circadian rhythm disruption and increased susceptibility to chronic inflammatory diseases [14].

The reappearance of symptoms and serious adverse effects after the discontinuation of treatment are serious drawbacks of many anti-hyperammonaemic agents/therapies. These drugs or therapies are inadequately effective. Therefore, the screening and development of drugs for anti-hyperammonaemic activity are still in progress. This can be achieved by focusing research on the active principles of flavonoids by increasing their efficacy by time dependent administration of drugs at which the effect is maximal. The structural features of flavonoids are the presence of a B-ring catechol group and the presence of a C2–C3 double bond in conjugation with an oxo-group at C4; the first serves to donate a hydrogen/electron to stabilise a radical species, and the second serves to bind transition metal ions such as iron and copper [15]. The anti-oxidant activity of flavonoids and their glycosides has been associated with their capacity to (i) scavenge reactive oxygen and nitrogen species [16], (ii) chelate transition metals that may induce oxidative damage through the Fenton reaction [17], (iii) inhibit pro-oxidant enzymes [18] and (iv) induce anti-oxidant enzymes [19]. Fisetin (3,3′,4′,7-tetrahydroxyflavone) is a dietary flavonoid widely distributed in strawberries, apples, persimmons, grapes, onions and cucumbers and displays anti-oxidant [20], anti-allergic [21], anti-inflammatory [22], anti-cancer [23,24], neuroprotective [25], neurotrophic [26] and anti-angiogenic [27] activities.

Our earlier studies showed preservation of hepatocellular architecture, normalisation of oxidative stress markers, anti-oxidant enzymes, liver marker enzymes (alanine transaminase, aspartate transaminase and alkaline phosphatase) and astrocytic marker enzyme (soluble guanylyl cyclase) and modulation of their temporal variation towards normalcy in fisetin treated AC-induced rats, although no temporal variations are noticed in AC-alone treated groups [28,29].

Chronotherapy is one of the best therapeutic approaches used to deliver medications in response to endogenous biological rhythms according to the pathophysiology of disease states to optimise treatment outcomes or limit adverse effects. Earlier reports described administration-time differences in the pharmacokinetics of many drugs [8,30]. The maximum clearance of gentamicin, amikacin, and isepamicin was higher when injected during the activity period [31,32]. A higher rate of urinary excretion of ciprofloxacin was found at the beginning of the day [33]. Most laboratory and clinical observations showed that the clearance of drugs was lower during the rest span and higher during the activity span.

Circadian variations have been reported in different populations of T total, T helper, and T killer lymphocytes in inflammatory reactions [33]. Further, circadian oscillations in the immune system and the inflammatory response play a major role in the temporal changes in the body's response to infection and other pathological conditions. Circadian rhythms of activity with gene expression changes have been reported in redox pathway enzymes, including NOS, haem oxygenase (HO), superoxide dismutase (SOD), catalase, reduced glutathione (GSH), glutathione-S-transferase (GST) and glutathione peroxidase (GPx) [34–37]. Modified circadian rhythms of activity have been reported in hyperammonaemic rats [38].

Endogenous 24 h oscillations in gastrointestinal, liver, kidney and other bodily processes are of great importance for therapeutics and for selecting the phase for drugs administration, due to rhythm influences on the pharmacokinetics, effect-duration, efficacy, adverse effects and beneficial outcomes of medications [39]. Investigations on the chronotherapeutic efficacy of anti-hyperammonaemic drugs are completely lacking. However, the time dependent administration of drugs would be helpful in increasing the efficacy and minimising the side effects of drugs. Hence, the present study was designed to assess the chronotherapeutic modulations of liver urea cycle enzymes, NF-κB p65, brain GS and iNOS by Western blotting analysis in chronic experimental hyperammonaemic rats.

Materials and methods

Experimental animals

Adult male Wistar rats (180–200 g) obtained from the Central Animal House, Faculty of Medicine, Annamalai University were maintained in air-conditioned room (25 ± 3 °C) with a 12 h light/12 h dark cycle. Feed and water were provided *ad libitum* to the animals. The study protocols were approved by the Institutional Animal Ethics Committee (Reg. No. 160/1999/CPCSEA, Approval No. 737: 2.9.2010), Annamalai University as per the guidelines of Indian Council of Medical Research, New Delhi.

Chemicals

Fisetin was purchased from Shanxi Jintai Biological (China). Ammonium chloride and other chemicals used in this study were of analytical grade and obtained from Merck and HiMedia, India. The primary polyclonal antibodies (anti-rat CPS-I, anti-rat OTC, anti-rat ASS, anti-rat NF-κB p65, anti-rat GS, anti-rat iNOS and anti-rat β-actin) were purchased from Santa Cruz Biotech, CA, USA. The secondary antibodies were purchased from Bangalore Genei, Bangalore, India.

Experimental induction of hyperammonaemia

Hyperammonaemia was induced in Wistar rats by intraperitoneal injections of AC (100 mg/kg b.w.) thrice a week for 8 weeks [40,41].