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Stable isotope dilution ultra-high performance liquid chromatography–tandem mass spectrometry quantitative profiling of tryptophan-related neuroactive substances in human serum and cerebrospinal fluid

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ABSTRACT

Many compounds related to L-tryptophan (L-TRP) have interesting biological or pharmacological activity, and their abnormal neurotransmission seems to be linked to a wide range of neurodegenerative and psychiatric diseases. A high-throughput method based on ultra-high performance liquid chromatography connected to electrospray tandem mass spectrometry (UHPLC–ESI-MS/MS) was developed for the quantitative analysis of L-TRP and 16 of its metabolites in human serum and cerebrospinal fluid (CSF), representing both major and minor routes of L-TRP catabolism. The combination of a fast LC gradient with selective tandem mass spectrometry enabled accurate analysis of almost 100 samples in 24 h. The standard isotope dilution method was used for quantitative determination. The method's lower limits of quantification for serum and cerebrospinal fluid ranged from 0.05 to 15 nmol/L and 0.3 to 45 nmol/L, respectively. Analytical recoveries ranged from 10.4 to 218.1% for serum and 22.1 to 370.0% for CSF. The method's accuracy ranged from 82.4 to 128.5% for serum matrix and 90.7 to 127.7% for CSF matrix. All intra- and inter-day coefficients of variation were below 15%. These results demonstrate that the new method is capable of quantifying endogenous serum and CSF levels of a heterogeneous group of compounds spanning a wide range of concentrations. The method was used to determine the physiological levels of target analytes in serum and CSF samples from 18 individuals, demonstrating its reliability and potential usefulness in large-scale epidemiological studies.

1. Introduction

Interest in L-tryptophan (L-TRP) metabolites has increased over the last few decades due to their influence on human health. Many L-TRP metabolites exhibit biological and/or pharmacological activity, including kynurenines, indoleamines and kynuramines. A simplified scheme of tryptophan metabolism is shown in Fig. 1.

The major route of L-TRP catabolism is regulated by an enzymatic cascade known as the kynurenine pathway (KP) [1]. The principal branch of the KP generates quinolinic acid (QA) and nicotinamide (NAm), while the side branches generate kynurenic acid (KA) and xanthurenic acid (XA). In mammals, L-kynurenine (KYN) is the pivotal metabolite of the KP and represents the predominant degradation product of ingested L-TRP. KYN is further converted to the neuroprotective compound KA via irreversible transamination mediated by kynurenine aminotransferases (KATs). Alternatively, it

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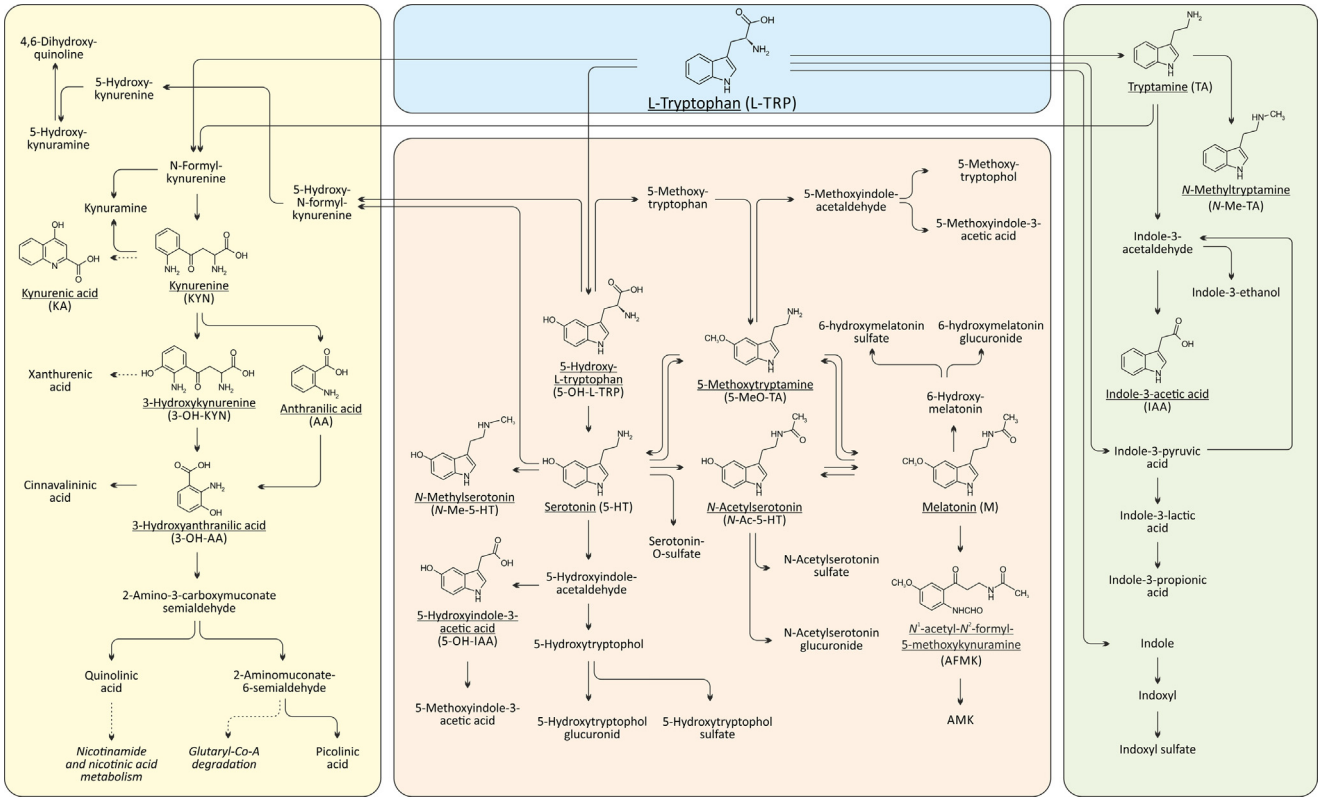

Fig. 1. Scheme of L-TRP biosynthesis and metabolism in human Metabolites included in presented study are underlined and accompanied by the structure. Particular enzymes are described in the section "Introduction".

can serve as a precursor of the neurotoxic 3-hydroxy-L-kynurenine (3-OH-KYN) and QA. The intermediates of the KP are proposed to be involved in the pathogenesis of serious neurological diseases, such as Parkinson's [2] and Alzheimer's diseases [3] and in diseases of viral origin [4]. They also play roles in immunoregulation and communication between the immune and nervous systems [5,6], and are suggested to mediate the immune escape of tumors [7].

The second important process of L-TRP catabolism is the methoxyindole pathway (MIP), which produces the neuroactive compounds serotonin (5-HT; 5-hydroxytryptamine) and melatonin (M; *N*-acetyl-5-methoxytryptamine). About 1–2% of dietary tryptophan is catabolized via this pathway [8]. These endogenous indoleamines are synthesized enzymatically from L-TRP via a number of key intermediates and metabolized by several metabolic pathways (Fig. 1) [9–12]. Since the discovery of 5-HT and M, an enormous amount of experimental evidence has shown that they play pivotal roles in a bewildering diversity range of behavioral and physiological processes. It is now well known that 5-HT is involved in smooth muscle contraction, blood pressure regulation and both peripheral and central nervous system neurotransmission [13]. Abnormalities in serotonin-related processes underlie the pathophysiology of some neuropsychiatric disorders, such as anorexia and anxiety [13], and dysfunctions of serotonergic systems are also involved in hypertension, migraine, the genesis of cardiac arrhythmias, Raynaud's disease, fibrotic syndromes and some symptoms of the carcinoid syndrome [13]. M, which is classified as a chemical mediator of the darkness signal [14,15], also has several important physiological functions including circadian rhythm modulation, reproductive regulation in seasonal breeders, and sleep promotion [16,17]. Melatonin deficiencies are associated with diseases including cancer, severe pain, metabolic syndrome, type 2 diabetes, rheumatoid arthritis, and some mood disorders. Pathological changes in the distribution of M are also frequently related to sleeping problems and disturbances of the circadian rhythm [18–20]. Moreover, M is a potent endogenous free-radical scavenger and antioxidant that counteracts the deleterious effects of reactive oxygen and nitrogen species in different systems [21].

The kynuramines, such as N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK), represent a unique class of biogenic amines among the metabolites of L-TRP [22]. AFMK and its secondary product, N^1-acetyl-5-methoxykynuramine (AMK), are bioactive and neuroprotective metabolites that have been shown to scavenge reactive oxygen and nitrogen species and to protect tissues from damage induced by reactive intermediates in various models. They may also play a role in immunomodulation and have been shown to possess cell-protective properties including beneficial effects on mitochondria [11].

In addition to the major metabolic routes, L-TRP can be degraded via an intestinal bacterial pathway. Approximately 4–6% of ingested tryptophan undergoes bacterial degradation that produces indole, indoxyl (3-hydroxyindole), indoxyl sulfate (3-IS), tryptamine (TA) and indole derivatives such as indole-3-acetic acid (IAA), indole-3-pyruvic acid (IPyA), indole-3-propionic acid (IPA), and indole-3-lactic acid (ILA) [23].

The use of sensitive and highly selective tandem mass spectrometry (MS/MS) based detection can markedly reduce interference from endogenous compounds, yielding accurate measurements [24–27]. Moreover, the application of ultra-high performance liquid chromatography (UHPLC) instead of more conventional high-performance liquid chromatography (HPLC) confers further improvements in separation efficiency and shorter analysis times. The efficient separation achieved with UHPLC is particularly advantageous because it means that it is often possible to monitor multiple metabolically related compounds from the same sample in a single experiment, even when only small-volume samples (10–100 μL) are available [28].