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Targeting tryptophan and tyrosine metabolism by liquid chromatography tandem mass spectrometry

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ABSTRACT

An imbalance in tryptophan (Trp) and tyrosine (Tyr) metabolites is associated with neurological and inflammatory disorders. The accurate and precise measurement of these compounds in biological specimens is a powerful tool to understand the biochemical state in several diseases. In this study, a rapid, accurate and sensitive method based on liquid chromatography–tandem mass spectrometry (LC–MS/MS) for the targeted analysis of the metabolism of Trp and Tyr has been developed and validated. The method allows for the adequate quantification of Trp, Tyr and, eight Trp metabolites, three Tyr metabolites, together with four competitive large neutral amino acids. Serotonin, 5-hydroxyindoleacetic acid, kynurenine, kynurenic acid, dopamine, and homovanilic acid were among the targeted compounds. Sample preparation, chromatographic separation and mass spectrometric detection were optimized in human urine, human plasma and mice prefrontal cortex extracts. The method was shown to be linear ($r > 0.98$) in the range of endogenous concentrations for all studied metabolites. In general, the limits of detection were suitable for the detection of the endogenous levels. Intra- and inter-assay precisions below 25% and accuracies ranging from 80 to 120% were found for most of the analytes. The use of labeled internal standards corrected the moderate matrix effect observed for some compounds. The applicability of the method was confirmed by analyzing urine samples collected from 13 healthy volunteers and comparing the results with previously established normal ranges. In addition, urine samples from two patients and a heterozygous carrier of a family with disturbed monoamine metabolism due to a loss of function mutation in the *MAOA* gene (X-linked) were analyzed and compared with samples from controls. All data together show the potential of the developed approach for targeted metabolomic studies.

1. Introduction

The aromatic and essential amino acids phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Trp) are converted to catecholamines and serotonin (5-HT) by enzymes in adrenal, intestinal and nervous tissue [1]. Imbalances on these pathways are associated with several neurological and inflammatory disorders.

Trp is an essential amino acid necessary for protein biosynthesis and also the precursor of a large number of biologically active metabolites [2]. Trp is metabolized via several pathways, the major ones being the 5-HT and kynurenine (Kyn) pathways (Fig. 1). Trp depletion or the imbalance in its metabolic products can have pathophysiological implications [3–5].

5-HT, is an important neurotransmitter that modulates numerous behavioral and physiological functions such as sleep, mood, appetite, learning, and memory [6]. Recent studies have demonstrated decreased levels of cortical 5-HT in neonatal animals with cerebral palsy induced by maternal-fetal inflammation [7]. An elevated urinary concentration of its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), is used as a biochemical test for the diagnosis of a carcinoid tumor [8], as well as for the diagnosis of phenylketonuria, and migraine [9].

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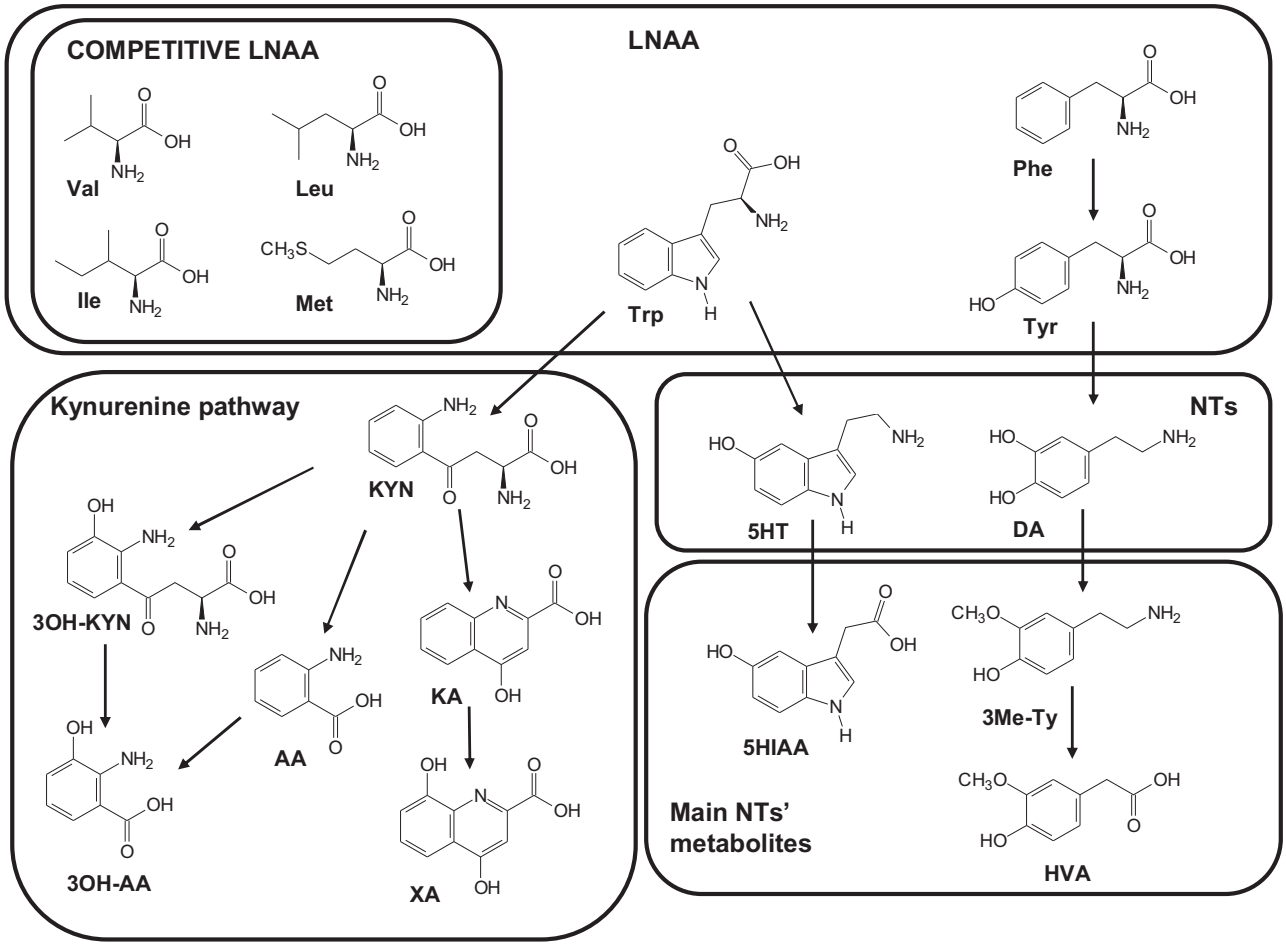

Fig. 1. Structures of studied analytes involved in the tryptophan and tyrosine pathways. LNAA: large neutral aminoacids, NTs: neurotransmitters, Val: valine, Leu: leucine, Ile: isoleucine, Met: methionine, Trp: tryptophan, Phe: phenylalanine, Tyr: tyrosine, KYN: kynurenine, 3OH-KYN: 3-hydroxykynurenine, KA: kynureninc acid, AA: anthranilic acid, 3OH-AA: 3-hydroxyanthranilic acid, XA: xanthuneric acid, 5HT: serotonin, DA: dopamine, 5HIAA: 5-hydroxyindoleacetic acid, 3Me-Ty: 3-methoxytyramine, HVA: homovanillic acid.

The kynerunine pathway is composed of two branches, leading to either the formation of kynurenic acid (KA) and xanthurenic acid (XA) or to the generation of 3-hydroxyanthranilic acid (3OH-AA) and quinolinic acid [10]. Whereas KA, an *N*-methyl-D-aspartate receptor antagonist, is considered to be neuroprotective [11], the metabolic products of the other branch, including 3OH-AA and quinolinic acid, are considered to be neurotoxic [12]. Imbalances in the kynurenine pathway have been related with several pathological conditions like schizophrenia [13], major depression [14], autism and epilepsy [15], or Alzheimer disease [16].

On the other hand, Tyr is synthesized from the essential amino acid Phe by the action of the enzyme phenylalanine hydroxylase. Plasma Tyr has been proposed as a useful assessment of thyroid function [17]. The main metabolic pathway for Tyr involves its transformation in the neurotransmitter dopamine (DA). DA is subsequently metabolized into several metabolites like 3-methoxytyramine (3Me-Ty) and homovalinic acid (HVA) (Fig. 1). Quantification of the major urinary product HVA and others catecholamine metabolites is routinely performed in patients suspected of having neuroblastoma or pheochromocytoma [18].

The rate of brain 5-HT and DA synthesis depends on the Trp and Tyr concentration respectively [19,20], which in turn depends on the ratio between plasma concentration of Trp/Tyr and other large neutral amino acids (LNAAs) that compete for blood–brain barrier (BBB) transport [21]. These LNAAs are Phe, leucine (Leu), isoleucine (Ile), valine (Val) and methionine (Met) [19,22]. It has been proved that, depending on the dietary intake of these amino acids, there can be substantial differences in the plasmatic ratios Trp/LNAAs and Tyr/LNAAs, with subsequent consequences on the catecholamine synthesis in the brain [23].

In summary, the accurate and precise measurement of these compounds in biological specimens is a powerful tool to understand the biochemical state in several diseases. To date, a number of methods for the analysis of Trp and its metabolites have been developed, mostly based on liquid or gas chromatography with various detection modalities, such as UV absorbance, fluorescent, and electrochemical [24–28]. However, if multiple species need to be analyzed with high sensitivity, which is very useful when a small amount of sample is available, then the use of mass spectrometers is preferred [29]. Thus, several LC–MS/MS methods have been recently developed for the detection of Trp and some of its metabolites [30–32], as well as for Tyr and its metabolites [33].

The use of LC–MS/MS instruments allows for the simultaneous quantification of targeted components of different metabolic routes, thus providing a more comprehensive picture of the biological changes in a single analysis. Moreover, these targeted strategies can easily incorporate some other analytes of interest that are not directly involved in the Trp and Tyr metabolic routes, such as the rest of LNAAs. To our knowledge, there is not any published method for the simultaneous determination of the complete panel of Trp and Tyr metabolites and LNAA competitors.