The serotonin-O-sulfate as a potential plasma surrogate biomarker

Raimonds Lozda, Indulis Purviņš

Pharmacology course of Department of Internal diseases at Riga Stradin’s University

Correspondence: Raimonds Lozda
E-mail: raimonds@farma.lv
Received: August 01, 2014
Published online: October 15, 2014

According to the latest European regulatory policy a close attention is paid to research into the use of biomarkers and surrogate markers in the development of pharmaceuticals. Since early sixties of the last century when a sulphation of serotonin was described from which the biotransformation product serotonin-O-sulfate (5-HT-SO4) was formed, is assumed it accentuates the intensity of serotonin metabolism in the central nervous system. Not so many researches are done with this compound particularly in humans, but taking into account serotonin-o-sulfate is able to reflect serotonin pathways it has a potential to be employed as a surrogate biomarker to follow the effects of a specific serotonergic treatment. Hereby we summarize the literature evidence of 5-HT-SO4 appearance in vivo. So far this indolemine was found in animal neurons, endothelial cells, urine and the only site of detection in humans was cerebrospinal liquid. Probably due to its absence in the easy accessible body fluids the clinical significance of 5-HT-SO4 has thus far been lessened. In result of our latest research we developed a suitable liquid chromatography–mass spectrometry (LC-MS) method, found this neurotransmitter degradation product in the human plasma and performed the first in humans clinical trial detecting it in healthy volunteers. According to the earlier animal research it is hypothesised that 5-HT-SO4 release is site specific and emphasizes central nervous system specific serotonin metabolism, therefore a further research is necessary to define the origin of plasmatic 5-HT-SO4 in humans.

Keywords: serotonin-o-sulfate; biomarkers; liquid chromatography

To cite this article: Raimonds Lozda, et al. The serotonin-O-sulfate as a potential plasma surrogate biomarker. Neurotransmitter 2014; 1: e281. doi: 10.14800/nt.281.

Introduction

The monoamine-deficiency theory affirms that the pathophysiological mechanism of depression is a deficiency of the neurotransmitters serotonin (5-HT), norepinefrine or dopamine in the central nervous system [1]. The patients with depression may not respond to antidepressants therapy for a few weeks or longer and thus a biomarker that predicted treatment effectiveness after a short time could be clinically useful [2]. There have been many publications issued describing the application of biomarkers in pharmaceutical development but the nomenclature to characterize distinct aspects of this process varies. The most widely used definition of a biomarker is a factor that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention, whereas a surrogate endpoint is a biomarker that is intended to substitute for a clinical endpoint. In this case, a surrogate endpoint is expected to predict clinical benefit [3]. The well-known marker of serotonin metabolism in the brain is 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid (CSF). Low levels of 5-HIAA in the CSF and suicidal behavior have been reported and a correlation between them
has been established \[4\]. Additionally, the effectiveness of antidepressant treatments has been evaluated based on the CSF 5-HIAA approach. However, the use of lumbar puncture is restricted for medical and ethical reasons. On the other hand, increased plasma levels of 5-HIAA and 5-HT have been observed in depressed patients and that plasma 5-HIAA directly correlates with the severity of depression \[5\]. Thus, the laboratory value of 5-HIAA as a serotonin metabolism biomarker is defined by the ability to measure this compound both in CSF and plasma, though the clinical significance of 5-HIAA in CSF is greater than that in plasma.

From the practical convenience point of view, a biomarker emphasizing central nervous system (CNS) -specific 5-HT metabolism that does not require a spinal puncture would be the most valuable and for such a role, the serotonin catabolite, 5-HT-SO4, could be evaluated. Hereby we would like to summarize the literature knowledge and our personal experience regarding 5-HT-SO4 and its potential biomarker value.

**Formation of serotonin-O-sulfate**

The formation of 5-HT-SO4 belongs to the minor metabolic pathways of serotonin.

Serotonin sulfotransferase catalyzes the formation of serotonin-O-sulfate. This enzyme is present in liver, lung, and kidney as well as brain. The enzyme is localized in the supernatant fraction of cells, and it uses 3'-phosphoadenosine, 5'-phospho- sulfate as sulfate donor. Serotonin-O-sulfate is not biologically active \[6\].

Sulfonate conjugation was first described in 1876 and has since been shown to be a significant pathway in the biotransformation of many neurotransmitters \[7\]. This biotransformation is initiated by a super gene family of sulfotransferases (SULTs). Two broad classes of SULTs have been identified so far:

a) membrane-bound SULTs that are located in the Golgi apparatus of the cell and are responsible for the sulfonation of peptides, proteins, lipids, and glycosaminoglycans, affecting both their structural and functional characteristics;

b) cytosolic SULTs responsible for the metabolism of neurotransmitters and other compounds \[7\].

In humans a subclass of SULT1A3 gene expressed protein plays a distinct role in sulfoconjugation of catecholamines. The latest study results indicate the apparent involvement of sulfation in the biotransformation of 7-hydroxyserotonin and

![Figure 1. Formation of 5-HT-SO4 in vivo.](http://www.smartscitech.com/index.php) Formation of 5-HT-SO4 belongs to the minor pathways of 5-HT metabolism and occurs thanks to the sulfotransferase - SULT1A3.

6-hydroxydopamine. By serving as substrates for SULT1A3, previously mentioned monoamines may interfere with the homeostasis of endogenous serotonin \[8\].

During later years in animal experiments it was approved that 5-HT-SO4 is the final product of serotonin metabolism which is rapidly excreted from the organism \[9; 10\]. Later similar compound was found in human urine, cerebrospinal liquor and platelets \[11, 12; 13\].

Schematically formation of 5-HT-SO4 in vivo can be summarized by a following figure.

More detailed mechanism is following. Monoamine oxidase (MAO) A is primarily responsible for the oxidative deamination of serotonin to 5-hydroxyindole acetaldehyde although monoamine oxidase B can function in its absence. A serotonin found in the central blood circulation is predominantly originating from peripheral tissues and is mainly metabolized in the liver to 5-hydroxyindole acetate, which is excreted in the urine. The minor metabolite 5-hydroxytryptophol is more hydrophobic and is first sulfated, or glucurononated before urinary excretion. Low levels of 5-HT-SO4, produced by phenol (aryl) sulfotransferase have been identified in both the central and enteric nervous systems and isoforms SULT1A1, SULT1A3 and SULT1C#2 have been shown to sulfate serotonin. 5-hydroxyindole thiazolidine carboxylate is a condensation product of 5-hydroxyindole acetaldehyde and L-cysteine that has been shown to form in the central and enteric nervous systems, but does not accumulate presumably due to the action of mitochondrial aldehyde dehydrogenase \[14, 15, 16\].

During the last years a few researches were done with marine mollusks determining 5-HT-SO4 in their nervous system. Findings showed that the 5-HT-SO4 forms from
5-HT uptake and metabolism in central ganglia and other structures of nervous system, but not in hemolymph per se. The scheme of metabolism according to the Stuart et al. is showed below \cite{17,18}.

Serotonin can also be glucurononidated by action of the enzyme uridine diphosphate glucurononyltrans- ferase. The enzyme has high activity in many tissues, but liver has the highest activity likely due to its absence in the peripheral blood circulation and the better-established 5-HIAA method \cite{6}.

**Literature evidence of detection of 5-HT-SO4 in animals and humans**

The aim of our literature search was to define a serotonin metabolism biomarker, which is the most potential for little invasive laboratory method and emphasizes CNS specific metabolism.

Nine online literature searches (via NCBI Entrez databases) were used to identify all studies from 1955 till June 2008 which investigated metabolism of serotonin with further formation of metabolites such as homovanillic acid (HVA), 5-hydroxyindolacetic acid and serotonin-O-sulfate. Also involvement of tryptophan hydroxylase genes (TPH1 and TPH2) in 5-HT metabolism was reviewed. Search limits included English, German languages. The first search included the key words serotonin metabolism CNS, the second search included the key words CNS 5-hydroxyindolacetic acid (CNS 5-HIAA), the third- words CNS homovanillic acid (CNS HVA), the fourth- 5-HTP CNS 5-HIAA, the fifth - 5-HTP CNS homovanillic acid, the sixth - Tph2 CNS, the seventh - serotonin-o-sulfate, the eighth search included the key words phenol sulfotransferase serotonin and the ninth - words sulfoconjugation catecholamine. Articles selected for inclusion in the final review contained information related to specific serotonin metabolism resulting in formation of 5-HT-SO4.

Each article was selected for the review and independently evaluated by 2 specialists in the field. We constructed a following matrix to compile the data for each reviewed article: research date, research name, results, analytical method applied.

Below mentioned table summarizes the key evidence related to 5-HT-SO4 appearance in the animals and humans.

The most recent finding was that metabolism of serotonin in mollusks with further formation of 5-HT-SO4 depends upon release location. Thus it was concluded that 5-HT-SO4 detected in hemolymph is most probably originating from the nervous system \cite{17,18}. This suggests us that given compound could emphasizes intensity of serotonin metabolism in the nervous system. Moreover the evidence of neural origin of the compound was found in monkeys too \cite{13}.

As far similar sulfotransfares exists in mammals an equal process should be theorized in humans \cite{8}. Based on the literature review we hypothesized that 5-HT-SO4 could be detected by modern HPLC method in human plasma too. As far there were no literature data available for detection of 5-HT-SO4 in the human plasma by HPLC method we decided to develop such a method. As the most suitable method described in literature for detection of indoleamines we found LC-MS.

**Detection of 5-HT-SO4 in human plasma by LC MS**

Due to lack of literature data regarding detection of 5-HT-SO4 in the human plasma by LC-MS method we decided to develop such a method. The method was developed and validated through evaluation of specificity, linearity, sensitivity (concentration range), accuracy and precision in accordance with current bioanalytical method validation guidelines \cite{19}.

During development of the method a naturally occurring 5-HT-SO4 was found in pooled human plasma samples and further studies involving healthy volunteers were performed.

Overall thirteen healthy volunteers were involved. The studies consisted of two stages – the first a Pilot study where we detected 5-HT-SO4 in the plasma samples of healthy
Table 1. Summary of the evidence found related to 5-HT-SO4 appearance in animal and humans

<table>
<thead>
<tr>
<th>No</th>
<th>Date</th>
<th>Research name</th>
<th>Results</th>
<th>Analytical method applied</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2008</td>
<td>5-HT and 5-HT-SO4, but not tryptophan or 5-HIAA levels in single feeding neurons track animal hunger state</td>
<td>Changes in levels of 5-HT-SO4 in the metacerebral giant neurons of Pleurobranchaea californica related to feeding were observed</td>
<td>Capillary electrophoresis with laser-induced wavelength-resolved fluorescence detection (CE-LIF)</td>
</tr>
<tr>
<td>2</td>
<td>2006</td>
<td>Serotonin catabolism in the central and enteric nervous systems of rats upon induction of serotonin syndrome</td>
<td>Serotonin sulfate showed surprisingly large increases in rat intestinal tissues after induction of serotonin syndrome</td>
<td>Capillary electrophoresis with laser-induced native fluorescence detection (CE-LINF)</td>
</tr>
<tr>
<td>4</td>
<td>2003</td>
<td>Serotonin catabolism depends upon location of release: characterization of sulfated and gamma-glutamylated serotonin metabolites in Aplysia californica</td>
<td>The pathway of serotonin inactivation with further formation of 5-HT-SO4 depends upon the type of neuronal tissue subjected to neurotransmitter incubation.</td>
<td>CE-LIF</td>
</tr>
<tr>
<td>6</td>
<td>1986</td>
<td>Amine sulfate formation in the central nervous system</td>
<td>Origin of the central nervous system of amine sulfates (also 5-HT-SO4) in monkeys and humans observed. 5-HT-SO4 was detected in CSF of monkeys and humans but not in the plasma</td>
<td>High performance liquid chromatography (HPLC) with electrochemical detection</td>
</tr>
<tr>
<td>7</td>
<td>1985</td>
<td>Free and Conjugated Amines in Human Lumbar Cerebrospinal Fluid</td>
<td>5-HT-SO4 was detected in the CSF of healthy humans</td>
<td>HPLC with electrochemical detection</td>
</tr>
<tr>
<td>8</td>
<td>1983</td>
<td>Exploration of the role of phenolsulfotransferase in the disposition of serotonin in human platelets: implications for a novel therapeutic strategy against depression</td>
<td>Existence of phenolsulfotransferase in the platelets and formation of 5-HT-SO4 verified.</td>
<td>The assay technique- purified alveolysin toxin</td>
</tr>
<tr>
<td>9</td>
<td>1966</td>
<td>Isolation of serotonin-O-sulfate from human urine</td>
<td>5-HT-SO4 isolated from human urine</td>
<td>Ion exchange resins</td>
</tr>
</tbody>
</table>

volunteers for the first time and the Main study to confirm results obtained earlier.

Our main interest performing studies on healthy volunteers was to apply the method developed in the first-in-humans study and further to ascertain quantitative differences of basal 5-HT-SO4 levels and intra-individual sensitivity of the quantitation. To define intra-individual sensitivity of the quantitation, after measurement of the basal 5-HT-SO4 levels, all subjects were exposed to serotonergic stimulation with per oral L-5-hydroxytryptophan (5-HTP) and blood samples analyzed. As already reported in six study subjects, a decrease in 5-HT-SO4 levels was observed 1 h after 5-HTP ingestion. Three subjects, however, showed an increase of 5-HT-SO4 1 h after 5-HTP ingestion. Paired Two Sample for Means analysis showed statistically significant differences between individual measurements. Results of the Main study are summarized in the table as below.

Taking into account above mentioned, we developed a suitable LC-MS method for the detection of 5-HT-SO4 in human plasma samples based on a minimally invasive laboratory method.

The Study phase confirmed the suitability of the method developed for clinical application by detecting basal 5-HT-SO4 levels in plasma samples and its ability to emphasize quantitative changes. As far these were exploratory studies with no therapeutic or diagnostic intent a control group was not used.

Discussion
The discovery of new biomarkers using minimally invasive techniques is highly appreciated to promote pharmaceutical development in the time when mechanism-based therapeutics plays a leading role.\textsuperscript{[2]}

The literature data and our work ascertain a possibility for 5-HT-SO\textsubscript{4} to be employed as a potential serotonin metabolism biomarker based on little invasive laboratory method. The outcome of our work is that we developed LC-MS method, which is specific to measurement of 5-HT-SO\textsubscript{4} in the samples of human plasma. Also it was the first time when 5-HT-SO\textsubscript{4} was detected in the samples of human plasma obtained from healthy volunteers. The clinical suitability of the method was justified. The sensibility of method to detect intraindividual changes of the compound in the healthy volunteers undergoing serotonergic stimulation was also confirmed. Method developed is specific to measurement of 5-HT-SO\textsubscript{4} in the samples of human plasma.

Regarding the issue whether 5-HT-SO\textsubscript{4} we found in plasma has CNS origin following concerns should be evaluated. As far l-aminoo acid decarboxylase acts both in the periphery and in the CNS, it means that ingested 5-HP can be converted into serotonin in the periphery of the body too.\textsuperscript{[20]} Nevertheless circulating serotonin mostly derived from peripheral tissues is primarily metabolized in the liver to 5-hydroxyindole acetate, which is excreted in the urine.\textsuperscript{[15; 16]} Also regarding serotonin appearing in the gastrointestinal tract is known that once serotonin reuptake transporter (SERT) has delivered 5-HT into the epithelial cells it is metabolized to 5-HIAA by MAO, localized to all epithelial cells of the intestine. Alternatively, 5-HT liberated into the lamina propria enters the portal circulation and can be detected either as free 5-HT or within platelets assisted by SERT. As the portal circulation goes thorough the metabolism by liver enzymes the free 5-HT is rapidly degraded. MAO degrades about one third to 5-HIAA, which is than detected in urine. The remaining two thirds of 5-HT is transformed to the metabolite 5-HT-O-glucuronide. Importantly, 5-HT absorbed by thrombocytes is secured from biotransformation by the liver enzymes and enters the general blood circulation.\textsuperscript{[21]} In a same time the only sites of serotonin sulfation identified so far in humans are the central and enteric nervous systems.\textsuperscript{[22, 13]} An enteric nervous system 5-HT-SO\textsubscript{4} was detected only after induction of serotonin syndrome.\textsuperscript{[22]}

It is known that isoforms of sulfotransferases SULT1A1, SULT1A3 and SULT1C2 have been shown to sulfate serotonin.\textsuperscript{[23]} The findings in sea mollusks indicates that metabolism of serotonin with further formation of 5-HT-SO\textsubscript{4}

### Table 2. Concentrations of plasma 5-HT-SO\textsubscript{4} obtained from the Main study subjects and data of statistical analysis.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration of serotonin-O-sulfate at baseline</th>
<th>Concentration of serotonin-O-sulfate 1 hour after 5-HTP ingestion</th>
<th>Relative change of concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average, ng/mL</td>
<td>Standard dev., ng/mL</td>
<td>RSD %</td>
</tr>
<tr>
<td>SF-2</td>
<td>20.6</td>
<td>1.2</td>
<td>5.0</td>
</tr>
<tr>
<td>SF-3</td>
<td>22.7</td>
<td>0.6</td>
<td>2.7</td>
</tr>
<tr>
<td>SF-4</td>
<td>23.6</td>
<td>2.4</td>
<td>10.1</td>
</tr>
<tr>
<td>SF-5</td>
<td>17.0</td>
<td>0.1</td>
<td>0.6</td>
</tr>
<tr>
<td>SF-6</td>
<td>28.1</td>
<td>1.1</td>
<td>3.8</td>
</tr>
<tr>
<td>SF-7</td>
<td>26.1</td>
<td>1.2</td>
<td>4.7</td>
</tr>
<tr>
<td>SF-8</td>
<td>11.6</td>
<td>0.3</td>
<td>2.9</td>
</tr>
<tr>
<td>SF-9</td>
<td>15.0</td>
<td>2.3</td>
<td>15.3</td>
</tr>
<tr>
<td>SF-10</td>
<td>8.1</td>
<td>0.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Average in the group</td>
<td>19.2</td>
<td>6.8</td>
<td>35.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>t-Test: Paired Two Sample for Means</th>
<th>P(T≤t) one-tail</th>
<th>t Critical one-tail</th>
<th>1.9</th>
<th>P(T≤t) two-tail</th>
<th>0.03</th>
<th>t Critical two-tail</th>
<th>2.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>t</td>
<td>0.02</td>
<td>t Critical two-tail</td>
<td>6.2</td>
<td>0.01</td>
<td>17.6</td>
<td>0.03</td>
<td>2.3</td>
</tr>
</tbody>
</table>

The calculation of average ng/mL and relative standard deviation (RSD) is based on 3 analyses – no 5-HT-SO\textsubscript{4} added, 28 ng/mL and 54 ng/mL of 5-HT-SO\textsubscript{4} added.
depends upon release location and the hemolymph 5-HT-SO₄ is most probably originating from the nervous system ⁴. Also earlier studies concluded that as far as 5-HT-O-SO₄ could not be detected in the plasma of monkeys or humans under normal conditions the 5-HT-O-SO₄ in ventriculocisternal perfusates undoubtedly originates in the central nervous system ⁹. Nevertheless significant finding was that 5-HT-O-SO₄ freely crosses blood-CSF barrier. Thus there are no physiological circumstances preventing appearance of CNS originating 5-HT-O-SO₄ in the venous blood circulation. Based on above-mentioned facts we are more likely to concern that 5-HT-SO₄ detected in the study mimics serotonin metabolism in CNS, but this should be justified by further studies.

The disputable outcome of our study is elevation or drop of 5-HT-SO₄. Majority of volunteers from the study phase (6 out of 9) had drop of plasma sulfate concentration. As far there is no data available regarding diurnal rhythmicity of 5-HT-SO₄ in the human plasma we are not able to confirm whether above mentioned changes are due to direct influence of serotonergic stimulation. Nevertheless in the light of literature data we tend to explain it by substrate inhibition of SULT1A3 because it was concluded that under normal circumstances quantity of serotonin synthesized and metabolized is kept under certain limits. Also another research showed that more serotonin did not lead to more potent swim motor action, implying that serotonin synthesis must be maintained within certain limits for the circuit to function properly. Alteration of neurotransmitter synthesis indicate serious consequences for the output of neural networks. The latter would correlate with the experiment made in Pleurobranchaea californica when hungry animals had significantly higher levels of 5-HT and 5-HT-SO₄ than their satiated partners. It remains for future investigations to determine whether 5-HT-SO₄ found in plasma has CNS origin and the reason of elevated or lowered sulfate levels after ingestion of 5-HTP.

References


