ANALYSIS OF ANALGESICS AND THEIR METABOLITES IN HUMAN URINE USING CAPILLARY ELECTROPHORESIS

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Kelvin Henry

May 2009
ABSTRACT

Acetylsalicylic acid (Aspirin) is rapidly metabolized to salicylic acid (salicylate) and other compounds, including gentisic acid and salicyluric acid. Monitoring of salicylate and its metabolites is of toxicological, pharmacological and biomedical interest. The separation of acetylsalicylic acid and its metabolites has been carried out by using non-aqueous capillary electrophoresis system with reversed electroosmotic flow. The flow is reversed by adding polycation hexadimethrine bromide and using a negative voltage. This method provides a fast and effective separation of the acetylsalicylic acid and its metabolites. The separation of all urine samples was performed on a fused-silica capillary (59.3 cm x 50, μm ID; 49.3 cm to detector). The result was favorable when using 214 nm detection, temperature 25°C and voltage -30 kV. By comparing blank urine and urine samples taken after administration of 500 mg aspirin tablet shows that there were changes in number of peaks appear in the electropherogram of urine sample taken after administration of 500 mg aspirin. This new peaks can be considered as the metabolites of acetylsalicylic acid. Comparison of electropherogram of urine samples 2 and 6 hour after administration of aspirin shows that there were changes in the height of the peaks between the samples.
ABSTRAK

ANALISIS ANALGESIA DAN METABOLISMANYA DI DALAM AIR KENCING MANUSIA DENGAN MENGGUNAKAN KAPILLARI ELEKTROPORESIS

Asid asetilsalisilik (Aspirin) termetabolism dengan pantas kepada asid salisilik (salisilat) dan sebatian lain. Pemantauan salisilat dan metabolismnya merupakan satu tarikan dalam bidang tosikologi, farmakologi dan bioperubatan. Pemisahan asid asetilsalisilik dan metabolismnya telah dijalankan menggunakan sistem kapillari elektroforesis bukan aqueous dengan aliran elektroosmotik berbalik. Aliran disongsang dengan menambah polikation hexadimethrine bromida dan menggunakan voltan negatif. Dengan menggunakan kaedah ini, pemisahan asid asetilsalisilik dan metabolismnya dapat dijalankan dengan pantas dan efektif. Pemisahan semua sampel air kencing telah dijalankan dengan menggunakan silika-flus kapillari (59.3 cm x 50, μm ID; 49.3 cm kepada pengesan). Keputusan adalah sesuai dengan menggunakan 214 nm pengesan, suhu 25°C dan voltan -30kV. Dengan membandingkan air kencing yang diambil sebelum dan selepas mengambil 500 mg pil aspirin menunjukkan terdapat perubahan bilangan puncak yang muncul dalam elektropherogram selepas pengambilan 500 mg pil aspirin. Puncak-puncak baru yang muncul dianggap sebagai metabolisma asid asetilsalisilik. Perbandingan elektropherogram sampel air kencing 2 dan 6 jam selepas mengambil pil aspirin menunjukkan perubahan dalam ketinggian puncak diantara sampel.
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<td>Acetylsalicylic acid</td>
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<td>CE</td>
<td>Capillary electrophoresis</td>
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<tr>
<td>CGE</td>
<td>Capillary gel electrophoresis</td>
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<tr>
<td>COX</td>
<td>Cyclo-oxygenase</td>
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<tr>
<td>cm</td>
<td>Centimeter</td>
</tr>
<tr>
<td>CIF</td>
<td>Capillary isoelectric focusing</td>
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<tr>
<td>CITP</td>
<td>Capillary isotachophoresis</td>
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<td>cmc</td>
<td>Critical micelle concentration</td>
</tr>
<tr>
<td>CZE</td>
<td>Capillary zone electrophoresis</td>
</tr>
<tr>
<td>E</td>
<td>Electric field strength</td>
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<tr>
<td>ECD</td>
<td>Electrochemical detector</td>
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<td>EOF</td>
<td>Electroosmotic flow</td>
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<tr>
<td>g</td>
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<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
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<tr>
<td>i.d</td>
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<td>kPa</td>
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<td>kV</td>
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<td>mol</td>
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<td>NACE</td>
<td>Non-aqueous capillary electrophoresis</td>
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<td>NAPQI</td>
<td>N-acetyl-p-benzoquinoneimine</td>
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<td>nm</td>
<td>Nanometer</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>Nonsteroidal anti-inflammatory drugs</td>
</tr>
<tr>
<td>pI</td>
<td>Isoelectric point</td>
</tr>
<tr>
<td>psi</td>
<td>Pound per square inch</td>
</tr>
<tr>
<td>s</td>
<td>Second</td>
</tr>
<tr>
<td>SA</td>
<td>Salicyclic acid</td>
</tr>
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</tr>
<tr>
<td>SPE</td>
<td>Solid Phase Extraction</td>
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<td>U/mL</td>
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<td>UV</td>
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<td>μg/mL</td>
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A. Preparation of solution concentration

1. Preparation of buffer stock solution
   a. 1 M sodium acetate
   b. 1 M ammonium acetate

2. Preparation of working buffer solution
   a. 0.15 M sodium acetate
   b. 0.05 M ammonium acetate

3. Preparation 0.02% (w/v) HDB stock solution

4. Preparation of working buffer solution with methanol and acetonitrile (1:1,v/v) and 0.002% (w/v) HDB

B. Electropherogram of urine samples using 50 μm i.d

1. Blank urine sample
2. Urine samples taken 2 hour after administration 500 mg aspirin tablet
3. Urine samples taken 6 hour after administration 500 mg aspirin tablet

C. Electropherogram of urine samples using 70 μm i.d

1. Blank urine sample
1.1 Capillary Electrophoresis (CE)

CE has emerged as a powerful and versatile analytical separation technique during the past 25 years since its introduction by Jorgenson and Lukacs in 1981 (Scriba, 2007). CE is a very rapidly growing microseparation technique and this is mainly due to the following advantages; high separation efficiency, short analysis time, very small buffer and sample volumes are required, environmentally friendly technique due to minor organic solvent consumption, CE techniques are easily on-line combinable, diverse application range. It is well known that separations in CE are predominantly driven by efficiency while in High Performance Liquid Chromatography (HPLC) by selectivity (Mikuš et al., 2006).

However, the irreproducibility of migration behavior due to surface adsorption and the lack of control of electroosmotic flow (EOF) limit its use in real sample analysis. In order to improve the separation performance or expand the types of application in CE, a great number of studies have focused on the modification of
capillary surface for better controlling EOF and or reducing surface adsorption (Hsieh et al., 2002).

In the last two decades, various CE modes have been developed, such as capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), micellar electrokinetic capillary chromatography (MECC), capillary electrochromatography (CEC), capillary isoelectric focusing (CIEF), and capillary isotachophoresis (CITP). These CE modes have also been successfully adapted to microchip format in the last decade. In addition, various samples ranging from simple inorganic ions to biomolecular ions, such as proteins and peptides, can easily be analyzed with CE (Wu, 2003).

Clinical laboratories are under tremendous pressure to develop assays that are accurate, precise, fast, capable of being automated, and yet, inexpensive. For electrophoresis, this has been difficult. However, by using CE many, if not all, of these goals can potentially be attained. This is because CE, even though based on the movement of molecules in an electric field, is not restricted to areas classically assigned to electrophoresis, namely separation of large molecules based on size or charge. It can also separate molecules that have low molecular weight, in addition to neutral compounds, such as steroids (Petersen et al., 2003). Particularly advantageous for biomedical applications is the excellent peak resolution which enables performance of analyses directly in body fluids and the small sample volumes needed for the assays (Heitmeier et al., 1999).
1.2 The importance's of analgesic metabolites analysis in urine

Nonopioid analgesics such as paracetamol (acetaminophen), acetylsalicylic acid, antipyrine (phenazone) and ibuprofen are well-known drugs which are frequently used for the treatment of fever and minor pain and are available without prescription (Heitmeier & Blaschke, 1999).

Aspirin also has a relatively high risk of inducing poisoning following improper use, especially in young children. Monitoring of the serum salicylic acid level may be essential in decisions regarding the treatment of alkaline diuresis or hemodialysis in case of severe aspirin intoxication (Goto et al., 1998).

Besides their usual therapeutic use, chronic abuse, accidental intoxications and the intake of high doses especially of paracetamol and acetylsalicylic acid for suicide purposes have been described. In all these cases simple and fast assays of the drugs and their metabolites in body fluids are needed for rapid and certain diagnoses and interpretations of studies (Heitmeier & Blaschke, 1999).

1.3 Objective

The objective of this study is:

- To analyze the acetylsalicylic acid and their metabolites in human urine.
1.4 Scope of study

In this study, aspirin and polycation hexadimethrine bromide (HDB) are obtained from Sigma-Aldrich in the highest grade available. The CE use is Beckman P/ACE MDQ (Beckman Coulter, Inc., Fullerton, California) equipped with an ultraviolet absorbance detector.
CHAPTER 2

LITERATURE REVIEW

2.1 Non-opioid

Non-opioid (non-narcotic) analgesics are drugs that have principally analgesic, antipyretic, and anti-inflammatory actions. They are milder forms of the painkiller. Non-narcotic drugs include acetaminophen are the most commonly used over-the-counter non-narcotic analgesic. Other drugs are not technically part of the analgesic family, but are nonetheless considered analgesics in practice. These include nonsteroidal anti-inflammatory drugs (NSAIDs). Aspirin and acetaminophen are two of the most widely used analgesics. Non-opioid agents are different from opioid analgesics in several ways.

The effects of opioid analgesics are; non-opioids have a ceiling effect in analgesia (a maximum dose beyond which analgesic effect does not increase), do not produce tolerance or physical dependence and are not associated with abuse or addiction,
they are antipyretic and all except acetaminophen are anti-inflammatory agents, the primary mechanism of action of non-opioid analgesics is inhibition of prostaglandin formation.

They are useful for everyday aches and pains, such as headaches, joint and muscle pain, which work more directly on injured body tissues. The non-opioids affect some of the chemical changes that normally take place wherever body tissues are injured or damaged. These chemical changes at the site of the injury typically result in inflammation and increased pain sensitivity.

The drawbacks of the non-opioids are their side effects. Although most non-opioids are quite safe when used for temporary acute pain, problems may arise when people take them over a long period of time (for chronic pain). This is especially true when large quantities of non-opioids are taken. Most are aware of the adverse effects of these drugs on the gastrointestinal system. However, excessive use of the non-opioids can also damage your liver or your kidneys. Besides that, chronic abuse, accidental intoxications and the intake of high doses especially of paracetamol and aspirin for suicide purposes have been described (Heitmeier and Blaschke, 1999).
2.1.1 Aspirin

Aspirin (acetylsalicylic acid, ASA) was introduced to medicine by Bayer in 1899. Originally used as an analgesic, antipyretic and anti-inflammatory drug, nowadays ASA is frequently prescribed for secondary prevention to relieve the symptoms of rheumatoid arthritis (arthritis caused by swelling of the lining of the joints), osteoarthritis (arthritis caused by breakdown of the lining of the joints), systemic lupus erythematosus (condition in which the immune system attacks the joints and organs and causes pain and swelling) and certain other rheumatologic conditions (Gaciong, 2003; Zaugg et al., 2001). It has analgetic, antipyretic, antiinflammatory and anticoagulant properties and is used as free acid, calcium or magnesium salt, or lysine conjugate (Zaugg et al., 2001).

Aspirin’s major therapeutic and adverse effects could be explained by inhibition of prostaglandin synthesis by inactivation of the key enzyme-cyclo-oxygenase (COX) which exists in at least three isoforms. COX-1 is the constitutive enzyme producing prostaglandin and thromboxanes involved in physiologic activities like cytoprotection and platelet aggregation, whereas COX-2 appears to be preferentially, if not exclusively, expressed in the inflamed tissues. Recently, a COX-1 splicing variant, named COX-3, has been cloned and found to be abundantly expressed in mature brain and spinal cord. ASA inhibits all three COX isoforms, coxibs selectively inhibit COX-2.
ASA is unique since it inactivates COX by irreversible acetylation of serine residue in the active site of the enzyme, whereas other NSAIDs are competitive reversible inhibitors of COX. Prostaglandins sensitize free nerve endings (pain receptors) to numerous inflammatory mediators and injected directly into the brain induce fever and pain. ASA, by inhibiting all isoforms of COX, can be considered both peripherally and centrally acting analgesic. The central mechanism of action is supported by autoradiographic studies that showed high affinity binding of ASA to nociceptive structures in the brain.

Also, in animal studies, ASA inhibits activity of central brain stem nuclei after the stimulation of the sagittal superior sinus. ASA may act on the molecular level, i.e., by inhibiting gene expression of COX, and interfering with intracellular signal transduction by transcription factors. Furthermore, in vitro ASA blocks formation of nitric oxide which is implicated in the pathophysiology of certain forms of pain like migraine (Gaciong, 2003).

Orally administered aspirin is absorbed and rapidly hydrolyzed to salicylic acid (SA); the serum half-life of aspirin is only about 15 min. The serum half-life of SA is 2 to 3 hour following low-dose consumption of aspirin, about 12 hour for the therapeutic doses commonly used for inflammation, and 15 to 30 hour following high doses or intoxication. Serum SA concentrations of 100 µg/mL and 150 to 300 µg/mL are required for analgesic and anti-inflammatory effects, respectively. When aspirin is frequently used to manage acute rheumatic fever, the serum SA concentration is increased to 300 to 400
μg/mL. Therapeutic drug monitoring is therefore necessary for the treatment of rheumatoid arthritis with aspirin. Aspirin also has a relatively high risk of inducing poisoning following improper use, especially in young children. Monitoring of the serum SA level may be essential in decisions regarding the treatment of alkaline diuresis or hemodialysis in case of severe aspirin intoxication (Goto et al., 1998). Figure 2.1 shows the chemical structure of acetylsalicylic acid and its metabolites.
REFERENCES


