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Detection of Paracetamol Concentration in Blood-, Saline- and Urine Samples With an Electrochemical Indicator in Healthy Volunteers - a Validation Study for a Novel Technique

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Backround:

Paracetamol is the most commonly overdosed drug, and paracetamol concentration measurement is a standard laboratory test. The ’gold standard’ for concentration determination is Mass-Spectrometry (MS), in our laboratory the High Performance Liquid Cromatography – Tandem Mass Spectrometry (HPLC-MS/MS).

In practice, other laboratory methods than MS are more feasible. Our clinical laboratory HUSLAB uses an immunological photometric method. The analysis is carried out in the laboratory, which, including the transport of the sample, causes a delay in receiving the results. Thus, Aalto University, University of Helsinki and Helsinki and Uusimaa Health care district (HUS) are collaborating to develop a fast, portable drug concentration measurement tool for patients at the point-of-care in the FEPOD project (Fast Electrochemical Point-Of-Care Diagnostics for Opioids and other pain medicine). The project has developed a prototype sensor that uses electrochemical analysis to analyse opioids and other analgesics. To date, the electrode has been shown to be capable of quantitative and selective measurement of certain drug concentrations in vitro. The aim is to develop a portable, fast-to-use drug analyzer for point-of-care use.

Paracetamol is a widely used drug used to treat pain and fever. It causes a significant proportion of intentional and unintentional drug poisonings. The therapeutic width of parcetamol is narrow, and relatively small overdoses can lead to significant liver failure and even death.
Paracetamol is rapidly absorbed orally and is quickly and evenly distributed in most tissues and fluid spaces, where the concentration of the drug can also be measured.

A small fraction of paracetamol is metabolised to highly reactive N-acetyl-p-benzoquinone imine (NAPQI), which is normally bound inactive by intracellular glutathione. In case of overdose, glutathione is insufficient and reactive NAPQI binds to other proteins in the liver, causing cell damage and destruction. As a treatment for overdose, N-acetylcysteine infusion is used. According to current therapeutic practice, a nomogram is used to

determine the initiation of N-acetylcysteine infusion, in which the measured serum paracetamol concentration is contrasted to the time elapsed since the time of drug administration. In practice, in a suspected overdose situation, the drug infusion is started before the drug concentration analysis is completed, so there is a clear clinical need for a rapid analysis method.

Paracetamol concentrations can be measured in plasma, saliva, urine and capillary blood samples by MS and UV methods (1,2,3). There appears to be a correlation between saliva, capillary blood and plasma concentrations, but the results are partly contradictory. Capillary blood and saliva samples are of interest due to their non-invasive nature, especially in paediatric and elderly patients. The electrochemical analysis method used in this study is also interesting because of the speed of the analysis. The analysis will be completed in a few minutes.

In addition to determining the concentration of paracetamol, this study aims to investigate the effect of paracetamol on serum lipidomics. The mechanism of action of paracetamol, despite its abundant use, is unclear. There are several suggestions: cyclo-oxygenase blockade, serotonin, opioid, adenosine, catecholamine, nitrate and endocannabinoid-mediated mechanisms. The site of action is likely to be in the central nervous system, especially activation of the descending pain regulation pathway is possible.

The central serotonin-mediated mechanism of action is one of the significant possible mechanisms of action of paracetamol. The endocannabinoid system is considered an important link between paracetamol and the serotonin system: AM404, the active metabolite of paracetamol, may strengthen the cannabinoid system via CB1 receptors, which strengthens the bulbospinal serotonergic brainstem inhibitory pain pathway. Antinociception would occur at the spinal cord level, where activation-dependent serotonin receptors would block the transmission of the pain impulse. In animal studies, inhibition of the serotonin descending pathway reduced pain relief induced by paracetamol (4). AM404 indirectly activated the cannabinoid receptor CB1 (5), and CB1 receptor blockade inhibited the analgesic effect of paracetamol in rats (6).

The endocannabinoid system is a group of lipid mediators that regulate ion channel activity and neurotransmitter release. Physiological, pathological, or drug-induced changes affect the communication of the endocannabinoid system. Targeted lipidomics research can aim to understand the metabolism of endocannabinoids.

This study has two objectives: 1) Is the electrochemical sensor developed by the FEPOD project reliable for measuring paracetamol concentration in capillary, urine, venous blood and saliva samples compared to the result analysed with a Mass Spectrometry from a venous blood sample? 2) Does paracetamol affect serum lipidomics?

Course of the study

This is a one-day trial on healthy volunteers. The study will be carried out in the research laboratory of the University of Helsinki and HUS Clinical Pharmacology Unit at Biomedicum 2 in Helsinki (Tukholmankatu 8C).

Subjects

Test subjects are recruited by means of a recruitment advertisement sent to students of higher education institutions and/or universities and/or published on the Internet. 12 healthy volunteers between the ages of 18 and 45 representing both sexes will be selected for the study. Subjects referred to in sections 7 to 10 § of the "Medical Research Act" (incapacitated, minor, pregnant, breastfeeding or prisoner) are excluded. Before inclusion for the study, the subjects undergo a medical examination, which includes a health questionnaire.
The test subject's weight must be a minimum of 50 kg and the body mass index (BMI) must be between 18.5 and 30. Subjects should be healthy and non-smoking and should not take any continuous medication, including birth control pills or other hormonal contraceptives. During the medical examination, the test subjects are examined for blood counts and thrombocytes (B-PVKT), liver function tests (P-ALAT, P-AFOS and P-GT), creatinine (P-Krea), electrolytes P-K and P-Na, and a pregnancy test for women (P-hCG-tot). Haemoglobin should be at least at the lower limit of the reference range. For other results, minor deviations from normal estimates, which are considered clinically insignificant in the opinion of the study physician, are acceptable. Blood pressure should be within normal range. The participants fill in a questionnaire on substance use, Participation in other drug trials and blood donation is prohibited 3 months before the start of the study, within its period and 3 months after its completion. Subjects should not use any medication or dietary supplements for a week before the trial or the day of the trial. The consumption of alcohol is prohibited on the day before and during the day of the trial.

Admission criteria • signed informed consent • age 18-45 years • healthy • acceptable values in laboratory tests (determined in HUSLab): haemoglobin at least at the lower limit of the normal range (men 134 g/l, women 117 g/l), other results (B-PVKT, P-ALAT, P-AFOS, P-GT, P-Krea, P-K, P-Na) small deviations from normal values that are clinically insignificant according to the investigator's assessment are accepted. In women, the pregnancy test (P-hCG-tot) should be negative. • normal blood pressure • no evidence of substance abuse in screening

Exclusion criteria • major illness • alcohol consumption exceeding the risk threshold • smoking • birth control pills or other regular medication • pregnancy, its planning or breastfeeding • less than 3 months after the previous trial • less than 3 months after blood donation • veins that are considered difficult to cannulate • weight less than 50 kg, body mass index (BMI) less than 18.5 or over 30.

Medication

Subjects receive 1 g of paracetamol orally

Meals

The subjects fast from the evening before the trial day beginning at 24 o'clock. On the day of the trial, the subject is offered a standardized lunch after 4 hours and a standardized snack 8 hours after the dose of paracetamol.

Samples

On the day of the trial, the test subjects arrive at the examination facilities at about 7.30 in the morning. A cubital vein is cannulated, and a venous blood sample is taken (sample 0), followed by repetitious samples at 0,5h; 1h; 1,5h; 2h; 3h; 4h; 6h; 8h; and 12h. If the cannula becomes blocked, repetitive samples are collected by venipuncture. Simultaneously with venous sample, a capillary blood sample is collected from the fingertip. Saliva samples are collected at 0; 1h; 4h; 8h; and 12 h. The urine that accumulates during the day is collected in plastic canisters. A Salivette collection tube is used for saliva sampling. On the morning following the day of the examination, the subjects visit the laboratory to give the last blood sample from a vein.

Tube	**0-sample**	**30 min**	**1h**	**1,5h**	**2h**	**3h**	**4h**	**6h**	**8h**	**12h**	**24 h**
K2- EDTA-tube plasma 4 ml	1	1	2	1	1	2	1	1	1	1	
K_2- EDTA-tube 9 ml for lipidomics	1						1				1
Saliva	X		X				X		X	X	
Capillary for FEPOD	X	X	X	X	X	X	X	X	X	X	
Mitra dry blood sample for MS	X	X	X	X	X	X	X	X	X	X	
K2-EDTA-tube 4 ml whole blood	X	X	X	X	X	X	X	X	X	X	

K2-EDTA tubes are taken protected from light and placed in an ice bath immediately after sampling. The plasma is separated from the K2-EDTA samples by centrifugation at +4 °C immediately after sampling, divided into four tubes and frozen immediately. The 4 ml K2-EDTA tube is frozen as a whole blood sample to measure the whole blood content of drugs. The urine that accumulates on the study day is collected in plastic canisters. At the end of the study day, urine volume is measured and frozen into four 5 ml tubes to measure paracetamol concentrations. The lipidomics samples are divided into four tubes and frozen immediately at -80°C.
The reference samples are analysed at the Department of Clinical Pharmacology.

Capillary blood samples are analysed immediately after collection in the study facilities with FEPOD-sensor, and duplicate samples are analysed by Mass Spectrometry from fingertip Mitra dry blood samples later at the Department of Clinical Pharmacology laboratory. Saliva samples are immediately analyzed or frozen for later analysis with Mass Spectrometry and electrochemical methods with FEPOD-sensor.
From frozen whole blood samples, the concentration of paracetamol is determined using the FEPOD sensor, and the results are compared with the drug concentrations determined in the capillary blood sample.
Lipidomics samples are used to study changes in lipids. Lipidomics samples are analysed together with samples collected from other similar studies. If the samples are analysed together with samples from other studies, it may be possible that the statistical power is sufficient to demonstrate changes in lipidomics.

Pharmacokinetics

The paracetamol concentrations in plasma, saliva, whole blood and capillary blood are determined at different timepoints with FEPOD and MS methods.

Statistical methods

Pharmacokinetic characteristics (e.g. Cmax, tmax, AUC, t1/2) are calculated from paracetamol concentrations using standard methods. The pharmacokinetics of paracetamol measured with the FEPOD sensor and with Mass Spectrometry are compared using variance analysis of repeat measurements, the Bland-Altman method and other appropriate statistical methods.

Safety and potential harm

Paracetamol is a widely used drug and a single dose of 1 g cannot be considered to have adverse effects on subjects.
Study-related venous cannulation or repeated sampling from the vein and fingertip causes transient discomfort and pain in subjects. Venous cannulation may be accompanied by local inflammation of the cannulated vein (thrombophlebitis). The risk of local or bacteremic infection by the cannula can be estimated to be low. Repeated punctures of a vein may be accompanied by nerve damage or damage to the vein as a rare side effect. This is a rare complication. Blood volume to be collected during the examination is less than 200 ml, which is less than a normal blood donation, and cannot be considered to pose a health risk to healthy individuals.

Timetable

The experimental part of the study will be carried out during autumn 2020 and spring 2021.

Organization of the study

The study will be conducted at the Clinical Research Unit of the University of Helsinki and HUS Clinical Pharmacology Unit at Biomedicum 2 Helsinki (Tukholmankatu 8 C, 00290 Helsinki). The Clinical Pharmacology Unit has conducted similar pharmacokinetic studies for more than 20 years. On test days, investigators, a laboratory nurse responsible for sampling and processing and, if necessary, an assistant are present. The equipment needed for the study is ready. Researchers and the staff of the laboratory are responsible for processing the material and results collected in the study. The research plan is given to the staff in writing and its essential content is also explained orally.

Mode of release

The results of the research will be published in international scientific journals using expert judgement and possibly in a scientific meeting.

Financing

The FEPOD project is funded by Business Finland.
Lipidomics research is funded by funding from foundations, possibly also with research funding from Helsinki University Hospital.

Processing of research data

Researchers are responsible for processing the data collected in the study. A register is formed from the collected data. Research data is processed in such a way that the identity of study subjects cannot be identified. The data is stored in a locked space for 15 years from the publication of the research results or the end of the study. After obtaining the necessary permits, a research register will be established.
If a subject withdraws consent, the data collected about him or her will be processed as part of the research data as referred to in the research plan, but no new data will be collected.
Before giving consent, research participants are informed that the data collected by the time their consent is withdrawn will be processed as part of the research data.

Researchers

Eija Kalso, PhD, Professor of Pain Medicine UH, Principal investigator. Responsibilities: study design, participation in conducting research and analyzing and reporting results, supervision of research.

Tuomas Lilius PhD, Chief Physician HY/HUCH Poison Information Centre Responsibilities: study planning, participation in conducting the study, analysing and reporting the results

Janne Backman, PhD, Professor, UH Clinical Pharmacology Responsibilities: study planning, participation in conducting the study, analysing and reporting the results

Johanna Kujala, M.D., Doctoral Student UH Responsibilities: Participation in research planning, participation in conducting research and analysing and reporting results

Mikko Neuvonen, chemist, UH Clinical pharmacology Responsibilities: Participation in study planning, participation in conducting the study, analysing and reporting the results

Niklas Wester, Doctoral Student, Aalto University Responsibilities: Research planning, participation in research conduct and analysis and reporting of results

Elsi Mynttinen, Doctoral Student, Aalto University Responsibilities: Research planning, participation in research conduct, analysis and reporting of results

Mikko Niemi, Professor at UH Clinical Pharmacology Responsibilities: Participation in study design, participation in conducting the study, analysis of results and reporting

Terhi Lohela, MD, Specialist in HUS ATeK Responsibilities: Participation in research planning, participation in conducting research and analysing results, as well as reporting

Assessment of the ethics of the research, consent procedure

The purpose and course of the study are explained to the study participants both orally and in writing. After a sufficient period of reflection, the participants sign their consent. Research subjects may withdraw from the study at any time, without giving a reason.

Research participants will be compensated (EUR 170.00 + daily allowance – taxes) for participating in this study (detailed grounds for compensation are presented below).

The research plan is reviewed by a **regional ethics committee** by decision of TUKIJA and notified to the Finnish Medicines Agency **FIMEA**. Research subjects are covered by Pharmaceutical Injury Insurance and Patient Injury Insurance.

The study will investigate the 1) new electrochemical paracetamol concentration sensor reliability, 2) usefulness of the saliva sample in determining paracetamol concentration, and 3) the effect of paracetamol administration on serum lipidomics.

Given the benefits of the research and the fact that the harm caused to research subjects is likely to be minimal, we consider the research medically justified and ethically acceptable.

Remuneration for participation in the study

In accordance with the Finnish Social and Health Ministry (FSHM) Decree, research participants are paid EUR 170.00 as reasonable compensation for inconvenience and compensation corresponding to loss of earnings (from which tax is withheld according to the normal tax rate) for participating in this study, which includes 2 visits and includes one day spent entirely in laboratory facilities. The participants are first thoroughly explained the purpose and course of the study, after which they are asked to carefully read the written notice and consent document received. Only then, after a sufficient period of reflection, do the subjects sign a consent document and undergo a medical examination, blood samples are taken to confirm their state of health and the results of laboratory tests are clarified when they are completed. There is one actual research day. The examination day lasts approximately 14 hours and the subjects are not allowed to leave the examination facilities during the research day. The morning after the examination day, the test subjects should visit the laboratory for a blood test. On the actual day of the examination, the subjects have an intravenous cannula inserted into the bend of the elbow throughout the examination day, and if the cannula becomes blocked, samples are taken by puncture from a vein, if necessary. It should be noted that, in addition to visits to the research laboratory, the research limits the normal life of the research subjects. If, regardless of the reason, the research subjects are not completed, the research subjects will be paid a reasonable share of the total compensation based on the FSHM Decree.

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