Study protocol and statistical analysis plan

Metabolites of Tramadol in the Postoperative Surgical Patients Admitted in the ICU

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Background

Tramadol is a commonly used opioid analgesic for the treatment of moderate to severe acute and chronic pain. It is metabolized in the liver by O- and N-demethylation (phase I) and conjugation (phase II). O-demethylation of tramadol to the active metabolite O-demethyltramadol (ODT) is catalyzed by cytochrome CYP2D6, and N-demethylation to N-demethyltramadol (NDT) occurs via CYP2B6 and CYP3A4. The ODT metabolite of tramadol is an active metabolite that has 200-fold higher affinity for opioid receptors than tramadol itself and is thought to be responsible for the bulk of the analgesic effect of tramadol. CYP2D6 is a non-inducible enzyme, and its gene shows marked polymorphism due to which there are large individual and population differences in metabolism, analgesic action, and side effects of tramadol. Individuals carrying two inactive copies of CYP2D6 are poor metabolizers (PM) of tramadol compared to those carrying two copies of the extensive wild allele (EM). Individuals carrying one or more copies of inactive alleles or alleles of reduced CYP2D6 activity are intermediate metabolizers (IM), and those carrying more than two copies of wild alleles for CYP2D6 are ultrarapid metabolizers (UM). In addition to pharmacogenetics, the functional status of the liver and kidneys plays an important role in the pharmacokinetics of tramadol. Tramadol and the ODT metabolite are excreted by the kidneys, therefore renal failure, which is common in intensive care units (ICU), leads to the accumulation of tramadol and the ODT metabolite in the blood. More than 80% of tramadol is metabolized in the liver. There are known to be significant differences in the pharmacokinetics of tramadol between healthy subjects and patients with hepatic failure. In patients with hepatic failure, tramadol plasma concentrations are higher and the elimination half-life is longer. These changes are primarily due to decreased hepatic elimination. Patients in ICU often have mildly reduced liver function as part of multiorgan failure due to sepsis or Systemic Inflammatory Response Syndrome (SIRS), while major abdominal surgery and anaesthesia reduce liver perfusion and contribute to hypoxic liver damage. Drug distribution changes as a result of decreased protein production and changes in extracellular volume, and the activity and metabolic capacity of liver enzymes decreases resulting in increased concentration and decreased plasma elimination of drugs, which is often very difficult to predict. The CYP2D6 genotype-phenotype has been shown to be relatively effective in clinical practice, but it should be noted that a number of genotype-phenotype discrepancies have been documented, therefore the absorption, distribution, metabolism and excretion of tramadol cannot be determined by a single protein genotype. The expression and activity of CYP2D6 is regulated by a number of physiological, pathological and environmental factors. CYP2D6 expression is susceptible to changes in infection states or systemic inflammatory response associated with elevated cytokine values. Expression of CYP2D mRNA and protein is reduced by proinflammatory cytokines in the liver of mice and rats, while administration of anti-inflammatory cytokines such as IL-10 reduces CYP3A activity. This decrease was not observed for CYP2D. At low CYP2D6 activity or when O-demethylation of tramadol is reduced, tramadol is primarily metabolized by N-demethylation. A study in cancer patients known to have a chronic inflammatory condition accompanied by elevated values of proinflammatory mediators showed that elevated IL-6 values correlated with N-demethylation of tramadol and patient tolerance to the analgesic effect of tramadol. Surgical stress, anaesthesia, starvation, postoperative pain, emergency surgery, and surgery for malignant diseases contribute to the development of a systemic inflammatory response. Hypothermia or hyperthermia, leucocytosis or leukopenia, tachycardia and tachypnoea, are clinical components of SIRS which is often difficult to distinguish clinically from acute inflammation, but in both conditions

there is an increase in proinflammatory cytokines, CRP, and PCT. During the first 24 hours, SIRS is common and is not a good predictor of a patient's final outcome, but if the proinflammatory response lasts longer than 24 hours, then it is associated with higher mortality. In addition to elevated values of proinflammatory cytokines, CRP, and PCT, low cholinesterase values in elderly patients are a good indicator of a systemic inflammatory response due to infection, SIRS, and trauma and are an independent risk factor for poor hospital outcome. Decreased serum cholinesterase in the development of SIRS occurs before the rise in CRP, and it is a good marker of early detection of the systemic inflammatory response. Mild or subtle hepatic dysfunction is often seen in patients in intensive care units due to hepatocellular damage caused by hypoxemia due to decreased hepatic flow and degranulation of neutrophils into hepatic sinusoids in sepsis or SIRS. Therefore, the metabolism and analgesic effect of tramadol in ICU patients after major surgeries will depend on a number of factors. On the one hand of the polymorphism of the CYP2D6 gene, and on the other of the state of important organ systems involved in the metabolism and elimination of tramadol, as well as the development of a systemic inflammatory response that alters cytochrome activity.

Design of a research

The research will be designed as a prospective observational research. The research will include patients hospitalized at the Department anaesthesiology and intensive care unit (ICU) of the Clinical Hospital Osijek, and after the signed informed consent. Informed consent will be signed by the legal guardian if the patient is unable to do so alone for various reasons.

Objectives

1. measure the plasma concentrations of tramadol, O-demethyltramadol and N-demethyltramadol in a patient admitted to the intensive care unit (ICU) after major abdominal surgery;
2. examine differences in tramadol, ODT and NDT concentrations with respect to CYP2D6 gene polymorphism;
3. examine the effect of systemic inflammation on the concentrations of tramadol, ODT and NDT in the early postoperative period;
4. examine the analgesic effect of tramadol with respect to systemic inflammation and organic dysfunction.

Patients and methods:

Patients undergoing major abdominal surgery will be included in the study. A major surgical procedure in the abdomen will be considered all procedures that require a surgical approach by laparotomy, which include resections of parts of the organs of the digestive system. Organic dysfunction will be considered laboratory or clinical signs of decreased renal function measured by diuresis with laboratory increase in serum nitrogen metabolites (increase by ≥30% of normal values), and decrease in liver function measured by increase in liver enzymes (increase by ≥30% of reference values). , by a decrease in serum cholinesterase levels (≤20% of normal), an increase in bilirubin by 30% of normal, or a decrease in synthetic liver function as measured by prolongation of PV to ≤60% of INR. A systemic inflammatory response will involve the perioperative presence of two or more of the following factors: body temperature> 38 oC or <36 oC, leukocytes> 12,000 or <4,000 mm3, pCO2 <4.3 kPa, and pulse> 90 / min. In addition, a perioperative increase in CRP, PCT, and lactate above reference values,

as well as a decrease in serum cholinesterase levels, will be considered a systemic inflammatory response. Patients who have a known allergic reaction to tramadol, and patients who have received tramadol for the past 7 days, patients younger than 18 years, BMI <18 kg / m2> 40 kg / m2, and patients who do not sign the informed consent will be excluded from the study. Also excluded from the study will be patients who have had laparoscopic surgery, as well as those on chronic therapy with cimetidine, paroxetine, pimozide, metoclopramide, amiodarone, olanzapine, chlorpromazine, fluphenazine, haloperidol, thioridazone, risoridazone, risoridazone drugs inhibit the activity of CYP2D6 enzyme important in tramadol metabolism.

After signed informed consent, all patients included in the study will have a blood sample taken for analysis of the CYP2D6 polymorphism, and ultrafast and slow metabolizers will be excluded from further analysis. A commercial set of "High Pure PCR Template Preparation Kit" according to the manufacturer's instructions (Roche Life Science) will be used to isolate the genomic DNA of the subjects. DNA isolation will be performed from whole blood samples with the anticoagulant EDTA. The protocol consists of lysis of blood cells using lysis buffer, degradation of protein by proteinase K, separation of DNA molecules from the mixture, and purification of DNA using glass mesh columns in a series of washing and centrifugation steps. Measurement of the isolated DNA concentration will be performed using a UV / VIS nanospectrophotometer. DNA samples will be stored at -20 oC until the testing procedure. To determine the allelic variants CYP2D6 * 3 (rs35742686), CYP2D6 * 4 (rs3892097) and CYP2D6 * 5 (whole gene deletion), the commercial set LightMix Kit CYP2D6 * 3 * 4 * 5 / * 5 will be used according to the manufacturer's instructions (TibMolbiol). The method is real-time PCR (real-time polymerase chain reaction). The test is based on the use of a certain type of fluorescently labeled hybridization assays, the so-called "SimpleProbe" that specifically binds to a sequence that includes a targeted change in DNA. Commercial sets "CYP2D6 TaqMan Copy Number Assay" and "TaqMan Copy Number Reference Assay" according to the manufacturer's instructions (Applied Biosystems) will be used to determine duplication / amplification and the number of copies of the CYP2D6 gene (CYP2D6 * 1xN). The method is real-time PCR, and the assay is based on the use of fluorescently labeled specific TaqMan probes that bind to the target gene sequence and reference TaqMan probes that bind to a reference sequence that is known to be present in the diploid genome. Relative quantification of the number of copies of the CYP2D6 gene will be possible by analyzing the results using CopyCaller (Applied Biosystems).

After induction under general anaesthesia, in addition to standard laboratory findings, plasma concentrations of albumin, cholinesterase, gamma-glutamyltransferase, bilirubin, C-reactive protein, procalcitonin will be measured, and pH, pO2, pCO2 and lactate values will be determined from an arterial blood sample. During the procedure, patients will be anesthetized by the method of general balanced inhalation anaesthesia with sevoflurane. Postoperatively, vital parameters, such as temperature, pressure, pulse and diuresis, will be continuously monitored in the ICU, and postoperative values of arterial blood gases, lactate and routine laboratory findings - complete blood count - will be determined. In the ICU patients will receive a total of 500 mg of tramadol intravenously divided into 5 doses during the first 24 hours of admission to ICU.

Plasma concentrations of tramadol, O-demethyltramadol and N-demethyltramadol will be measured 1, 2 and 4 hours after the first dose, and immediately before the 2nd, 3rd and 5th doses. In total, therefore, there will be 6 measurements within 24 hours. Determination of the concentration of tramadol and ODT and NDT metabolites from patient

plasma samples will be performed by high performance liquid chromatography (HPLC) on a Shimadzu Nexera XR (Shimadzu Corporation, Kyoto, Japan) on a reverse phase chromatographic system with fluorescence detection (excitation wavelength 200 a emission 301 nm). Separation will be performed on an Agilent Zorbax SB-C8 column, 3.5 µm, 4.6x150 mm (Agilent Technologies, Santa Clara, CA, USA) using a mobile phase consisting of methanol and 1.5 mM H3PO4 in a ratio of 19:81), pH 2.5.

The analgesic effect of tramadol will be measured in patients who are conscious on the NRS scale (0 - no pain, 10 - most severe pain) 30 minutes before and 30 minutes after tramadol administration. An NRS value of 3 or less will be considered adequate analgesia, and in case of inadequate analgesia, an additional analgesic will be used - morphine 2 mg. In patients with impaired consciousness, the analgesic effect of tramadol will be examined by the Behavioral Pain Scale (BPS) (3 - no pain, 12 - most severe pain) and the Critical Care Pain Observation Tool (CPOT). A BPS value of 5 or less will be considered adequate analgesia, as well as CPOT values less than 2. Consumption of additional analgesic will be recorded regularly. Nausea, vomiting, and newly developed respiratory depression will be reported within 24 hours. In all patients, they will record the total time of hospitalization in ZIM, hospitalization and the patient's outcome - death or survival until discharge from the hospital.

Statistical analysis plan:

Category data will be presented in absolute and relative frequencies. Numerical data will be described by the arithmetic mean and standard deviation in the case of distributions following the normal, and in other cases by the median and limits of the interquartile range. Differences in category variables will be tested by the $\chi 2$ test and, if necessary, the Fisher exact test. The normality of the distribution of numerical variables will be tested by the Shapiro-Wilk test. Differences of normally distributed numerical variables between two independent groups will be tested by Student's t test, and in case of deviation from normal distribution by Mann-Whitney U test, while in case of 3 or more groups will be tested by analysis of variance (ANOVA, PostHoc Bonferonni, Sheffe), and in case of deviation from the normal distribution by the Kruskal-Wallis test (PostHoc Conover). The correlation of continuous numerical variables will be evaluated by Pearson's correlation coefficient r, and in the case of ordinal distribution variables by Spearman's correlation coefficient ρ (rho). Differences in plasma concentrations of tramadol, O-demethyltramadol and N-demethyltramadol according to measurements (6 measurements) will be tested by analysis of variance for repeated measurements (normal distribution) or by Friedman test (Post-hoc Conover) in case of distributions that do not follow normal. The significance level will be set to Alpha = 0.05. MedCalc Statistical Software version 19.0.5 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2019) and SPSS (version 16.0, SPSS Inc., Chicago, IL, USA) will be used for statistical analysis.