Determination of dihydropyrimidine dehydrogenase and thymidylate synthase activity in human peripheral blood mononuclear cells in healthy volunteers.

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Primary objective:• To determine the range and mean population DPD and TS enzyme activity in human peripheral blood mononuclear cells in 20 healthy volunteers. Secondary objectives:• To determine the circadian rhythm of DPD and TS enzyme activity in…

Ethical review	Approved WMO
Status	Recruitment stopped
Health condition type	Purine and pyrimidine metabolism disorders
Study type	Observational invasive

Summary

ID

NL-OMON36760

Source
ToetsingOnline

Brief title
DPD and TS enzyme activity determination

Condition

- Purine and pyrimidine metabolism disorders
- Miscellaneous and site unspecified neoplasms malignant and unspecified

Synonym
DPD and TS enzyme activity

Research involving
Human

Sponsors and support

Primary sponsor: Antoni van Leeuwenhoek Ziekenhuis
Source(s) of monetary or material Support: Nederlands Kanker Instituut - Antoni van Leeuwenhoek ziekenhuis

Intervention

Keyword: circadian rhythm, dihydropyrimidine dehydrogenase, enzyme activity, thymidylate synthase

Outcome measures

Primary outcome

ranges of and mean DPD resp. TS enzyme activity in healthy volunteers

Secondary outcome

Correlation of DPD and TS enzyme activity with DPD and TS gene expression and

observed polymorphisms in DPYD resp. TYMS.

Study description

Background summary

5-Fluorouracil (5-FU), the lead compound of the fluoropyrimidines, together with its prodrug capecitabine are two of the most frequently prescribed chemotherapeutic agents for the treatment of various malignant solid tumors, such as, breast and colorectal cancer. In order to exert its cytotoxic effect, 5-FU must first be metabolised to its active metabolite 5-fluoro-2*-deoxyuridine monophosphate (FdUMP). FdUMP inhibits thymidylate synthase which ultimately results in the inhibition of DNA synthesis. The degradation of 5-FU is initialized by the polymorphically expressed enzyme dihydropyrimidine dehydrogenase (DPD). This rate-limiting step in the catabolism pathway catalyses the conversion of 5-FU into 5,6-dihydro-5-fluorouracil (FUH2). Since DPD is responsible for catabolising more than 80% of administered 5-FU, patients with a complete or partial DPD deficiency are at high risk for developing severe and sometimes fatal fluoropyrimidine-induced toxicity. An estimated 3-5% of the Caucasian population has a decreased DPD enzyme activity, which may be caused by amongst others mutations in DPYD, the gene encoding for dihydropyrimidine dehydrogenase. Moreover, a known DPD-deficiency is a contraindication in the treatment with 5-FU and 5-FU analogues.

The enzyme thymidylate synthase (TS) is one of the key targets of fluoropyrimidine based chemotherapy, i.e. 5-fluorouracil (5-FU). The active metabolite 5-fluoro-2-deoxyuridine-5-monophosphate (5-FdUMP) inhibits TS, which is normally turns deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP). Consequently, dTMP is phosphorylated to deoxythymidine triphosphate (dTTP) which is used in DNA synthesis and repair. In other words, FdUMP inhibits TS and thereby de novo thymidine synthesis. The inhibition of thymidine synthesis will block synthesis of the DNA, thereby causing the cytotoxic effect of fluoropyrimidine based chemotherapy. It is believed that TS activity might serve as a predictive factor for 5-FU based chemotherapy. In several studies it was found that high TS activity or high expression of the TS protein was associated with 5-FU resistance. In addition, other studies found a significant improvement in survival in case of low TS activity in tumor tissue.

Next to detection of genetic mutations that may lead to a DPD-deficiency, several phenotypic assays have been described in the literature that determine the enzymatic DPD activity. Examples of these are measurement of 5-FU plasma clearance after a reduced test dose, [2-C13] uracil breath test and direct evaluation of DPD activity in peripheral blood mononuclear cells PBMCs using HPLC with on-line UV detection, tandem mass spectrometry or radioactivity detection.
Previous studies regarding TS enzyme activity have been conducted in surgical obtained tissue samples. These studies demonstrated a significant difference in TS activity between tumor tissue and normal surrounding tissue. Unfortunately, a correlation between TS activity in PBMCs and TS activity in tissue was not previously demonstrated. Nor was the difference in TS activity between PBMCs from healthy subjects compared to PBMCs from cancer patients.

These tests however, are currently not routinely assessed prior to start of therapy in clinical practice for several reasons. Besides lack of dosing guidelines for poor, intermediate and extensive 5-FU metabolizers, another major hurdle for using a phenotypic test prior to start of therapy is the lack of a rapid, inexpensive and non-invasive patient-friendly assay, that can be applied on a routine basis.
The TS enzyme activity radio assay was recently designed and has not been conducted in either healthy subjects or patients. As with the DPD assays, rapid, cheap, non-invasive and patient-friendly assays are lacking. Moreover, it is not known if TS activity in PBMCs and tumor tissue correlate. Additionally, the clinical translation from TS activity to dose- or regimen adjustments has not been made.

Within the Department of Experimental Therapy of The Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital (NKI-AVL) a rapid and sensitive method was developed to determine the DPD-activity in PBMCs by HPLC with on-line radioactivity detection. Subsequently a radio assay was designed to determine TS enzyme activity in PBMCs,
To gain insight into the DPD and TS enzyme activity in the healthy population

measured by both assays, we propose to measure the DPD and TS enzyme activity in 20 healthy volunteers. In addition, DPYD and TYMS gene expression will be measured from blood obtained at all time points, and observed polymorphisms in DPYD and TYMS will be associated with the observed DPD resp. TS enzyme activity. Also, the uracil/dihydrouracil ratio in plasma will be determined and related to DPD enzyme activity. Furthermore, to gain insight into the circadian rhythm of DPD and TS, we propose to perform our method with blood drawings on 7 different time points during one day.

Study objective

Primary objective:
• To determine the range and mean population DPD and TS enzyme activity in human peripheral blood mononuclear cells in 20 healthy volunteers.

Secondary objectives:
• To determine the circadian rhythm of DPD and TS enzyme activity in PBMCs of 12 healthy volunteers (male:female 1:1);
• To correlate DPD and TS gene expression in PBMCs with DPD and TS enzyme activity;
• To associate any observed polymorphisms in DPYD or TYMS with the observed DPD and TS enzyme activity.

Study design

To determine the DPD and TS enzyme activity in a normal population measured by enzyme activity assays, healthy volunteers are asked for participation in this study. Written informed consent will be obtained from all healthy volunteers prior to any study procedure. From every volunteer (male:female = 1:1), 48 ml of whole blood will be drawn at 9:00 h ± 30 minutes in the morning. A volume of 2 x 16 ml will be used for the DPD and TS enzyme activity determination. A volume of 8 ml will be used for mRNA isolation. A volume of 4 ml will be used to test for polymorphisms in DPYD and TYMS. A volume of 4 ml is used to isolate plasma for uracil and dihydrouracil analysis. Volunteer demographics (initials, date of birth, gender and wake up time) will be obtained after blood donation. In 12 volunteers (male:female = 1:1) the circadian rhythm of DPD and TS will be determined. These subjects will undergo blood sampling 7 times within 24 hours at 09:00 h, 13:00 h, 17:00 h, 21:00 h, 01:00 h, 05:00 h and 9:00 h the day after (all ± 30 minutes). In order to take blood samples at night, volunteers will be hospitalized in the Slotervaart hospital. Nurses will draw blood samples using a venflon while the volunteers are asleep in order to limit the disturbance of the normal day/night rhythm.

Study burden and risks

To isolate human PBMCs, 32 ml of peripheral blood will be collected in heparin

tubes for the DPD and TS activity determination, 8 ml of blood will be collected in CPT tubes for the gene expression analysis, 4 ml of blood will be obtained in heparing tubes for uracil/dihydrouracil analysis and 4 ml of blood will be obtained in EDTA tubes for pharmacogenetic analysis. In 8 patients this will be performed once and in 12 volunteers, 48 ml of blood will be collected at 1 time point and 44 mL of blood will be collected at 6 other time points within 24 hours by the use of one venipunture and a venflon.
The burden of this sampling includes one venipuncture which could consist of the following side-effects: discomfort, bruising and hematoma and very rarely infection.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

1. Healthy volunteer; not known with cancer or current treatment for cancer, and without any surgical operation in the past 6 months
2. Age > 18 years
3. Able and willing to give written informed consent
4. Able and willing to undergo blood sampling for DPD enzyme activity analysis
5. Able and willing to undergo blood sampling for pharmacogenetic analysis

Exclusion criteria

1. Any treatment with investigational drugs within 30 days before the start of the study
2. History of cancer
3. Any treatment with anti-cancer drugs
4. Legal incapacity
5. Any condition that may interfere with the study protocol

Study design

Design

Study type: Observational invasive

Masking:	Open (masking not used)
Control:	Uncontrolled
Primary purpose:	Treatment

Recruitment

NL

Recruitment status:	Recruitment stopped
Start date (anticipated):	29-03-2010
Enrollment:	40
Type:	Actual

Ethics review

Approved WMO

Date:	10-03-2010
Application type:	First submission
Review commission:	METC Slotervaartziekenhuis en Reade (Amsterdam)
Approved WMO Date:	26-11-2010
Application type:	Amendment
Review commission:	METC Slotervaartziekenhuis en Reade (Amsterdam)
Approved WMO Date:	28-10-2011
Application type:	Amendment
Review commission:	METC Slotervaartziekenhuis en Reade (Amsterdam)
Approved WMO Date:	05-12-2011
Application type:	Amendment
Review commission:	METC Slotervaartziekenhuis en Reade (Amsterdam)

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL30880.048.10