Tartrate-Resistant Acid Phosphatase 5b in Rat Serum and Plasma

Jussi M Halleen, Jukka Vääräniemi, Jenni HE Mäki-Jouppila, Katja M Fagerlund, and Jukka Morko
Pharmacent Services, Turku, Finland
E-mail correspondence to Jussi Halleen (jussi.halleen@pharmatest.com)

Introduction

Biochemical markers of bone turnover are used to monitor the efficacy of bone-targeting therapies in clinical practice.[5] Tartrate-resistant acid phosphatase isoform 5b (TRACP 5b) is expressed by osteoclasts and secreted as an active enzyme into the blood circulation by mature osteoclasts, and serum TRACP 5b activity is a marker of osteoclast number.[6] C-terminal cross-linked telopeptides of type I collagen (CTX-I) is released from organic bone matrix during osteoclastic bone resorption, and serum CTX-I is a marker of bone resorption.[5] In non-clinical studies, rats are used as the major small species when studying the safety and efficacy of novel bone-targeting therapies.[7] Serum TRACP 5b activity and CTX-I levels can be used for monitoring changes in osteoclast number and bone resorption in rat studies.

Aim of the Study

We evaluated the suitability of ethylenediaminetetraacetic acid (EDTA) plasma and heparin plasma to determine TRACP 5b activity and CTX-I levels in rats.

Materials and Methods

Animal experimentation: The study was conducted using three-month-old male Sprague-Dawley rats (body weight 380 ± 20 g) and afternoon group (body weight 380 ± 22 g) by stratification according to their body weight in the morning group. Bading period was started at 8 pm and continued for 12 hours, followed by harvesting blood samples at 8 am. In the afternoon group, bading period was started at 8 pm and continued for 4 hours, followed by harvesting blood samples at 2 pm. Both groups were fasted overnight. Blood was harvested in EDTA and heparin plasma samples, and serum was prepared from EDTA plasma and heparin plasma samples, and frozen and stored at -70°C. This experimental protocol was approved by the National Animal Experiment Board, Regional State Administrative Agency for Southern Finland, Helsinginmaa, Finland.

Biochemical markers of bone resorption: Commercial immunoassays and multiplexing course (VICTORS)® (PerkinElmer, Waltham, MA, USA) were applied to measure TRACP 5b activity (TRACP™ 254-FL; EUA-IDS, Boston, MA, USA) and CTX-I levels (PharmacoTM 210-IDS; EUA-IDS, Boston, MA, USA) in serum, EDTA plasma, and heparin plasma samples.

Statistical analysis: All data is presented as mean ± standard deviation. If the assumptions of parametric test were fulfilled as such or after data transformations, one-way analysis of variance (ANOVA) and multiple comparisons tests (Dunn’s test or Bonferroni–Dunn’s test) were used for evaluating statistical significance. Otherwise, statistical significance was evaluated using non-parametric Kruskal-Wallis and Dunn’s test. Correlation between results from serum, EDTA plasma, and heparin plasma samples was evaluated using Pearson r correlation or Spearman rank correlation accordingly.

Study Design

Three-month-old male Sprague-Dawley rats

Blood for serum, EDTA plasma, and heparin plasma samples

Measurements of TRACP 5b activity and CTX-I levels

Statistical Analysis

TRACP 5b activity (IU/L) in the morning after overnight fasting and in the afternoon after six hours fasting:

- Activity in rat serum and EDTA plasma and (B) their correlation. (C) Activity in rat serum and heparin plasma and (D) their correlation. (E) Activity in rat EDTA and heparin plasma and (F) their correlation. In panels B, D and F, r indicates correlation coefficient and *r*-value indicates statistical significance. *p < 0.05, **p < 0.001

Summary

TRACP 5b in serum, EDTA plasma, and heparin plasma:

- No differences and a very strong correlation between serum and EDTA plasma samples.
- Lower values in heparin plasma, minor correlation with EDTA plasma, and no correlation with serum samples.
- No differences between samples harvested in the morning after overnight fasting and in the afternoon after six hours fasting

CTX-I in serum, EDTA plasma, and heparin plasma:

- No differences between serum, EDTA plasma and heparin plasma samples.
- Minor correlation between serum and EDTA plasma, and serum and heparin plasma samples.
- No differences between samples harvested in the morning after overnight fasting and in the afternoon after six hours fasting

Conclusions

This study demonstrates that TRACP 5b activity can be measured both in serum and EDTA plasma in rats, but heparin plasma is not recommended. Diurnal variability of TRACP 5b activity and CTX-I levels is very low in rat serum and plasma after overnight and six hours fasting periods.

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References


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