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

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Introduction

Biochemical markers of bone turnover are used to monitor the efficacy of bone-targeting therapies in clinical practice.[1] 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.[2] 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.[3] In non-clinical studies, rats are used as the major small species when studying the safety and efficacy of novel bone-targeting therapies.[4] 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 ethylenediaminetetra-acetic 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 337-421 g). The rats were randomized into morning group (body weight 382 ± 28 g) and afternoon group (body weight 382 ± 22 g) by stratification according to their body weight. In the morning group, fasting period was started at 8 pm and continued for 12 hours, followed by harvesting blood samples at 8 am. In the afternoon group, fasting period was started at 8 am and continued for 6 hours, followed by harvesting blood samples at 2 pm. In both groups, three blood samples with the volume of 0.6 mL per sample were collected from lateral tail vein, processed for serum, EDTA plasma and heparin plasma samples, and frozen and stored at -70ºC. This experimental protocol was approved by National Animal Experiment Board, Regional State Administrative Agency for Southern Finland, Hämeenlinna, Finland.

Biochemical markers of bone resorption: Commercial immunoassays and multilabel counter (VICTOR2™; PerkinElmer, Waltham, MA, USA) were applied to measure TRACP 5b activity [RatTRAP™ (TRAcP 5b) ELISA; IDS, Boldon, UK] and CTX-I levels [RatLaps™ (CTX-I) EIA; IDS] in rat serum, EDTA plasma and heparin plasma samples.

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

Study Design

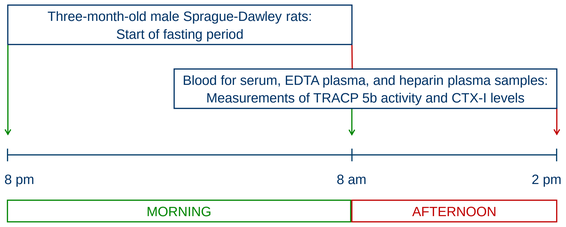

FIGURE 1. Experimental design of the study.

TRACP 5b in Rat Serum and Plasma

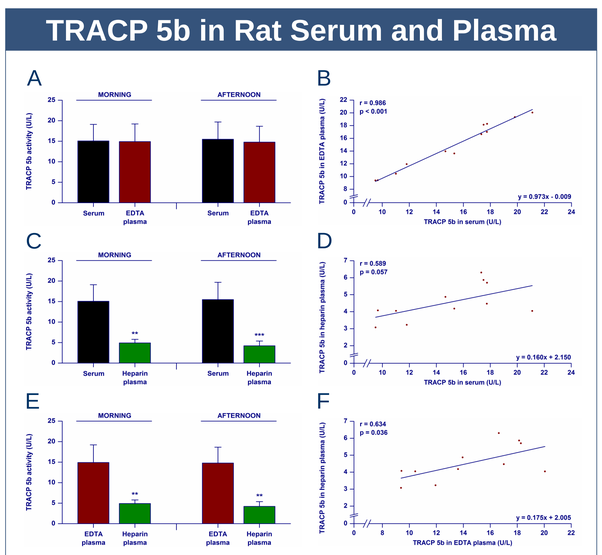

FIGURE 2. TRACP 5b activity (U/L) in the morning after overnight fasting and in the afternoon after six hours fasting. (A) Activity in rat serum and EDTA plasma and (B) their correlation. (C) Activity in rat serum and heparin plasma and (D) their correlation. (E) Activity in rat EDTA and heparin plasma and (F) their correlation. In panels B, D and F, r-letter indicates correlation coefficient and p-letter indicates statistical significance. ** $p < 0.01$; *** $p < 0.001$

CTX-I in Rat Serum and Plasma

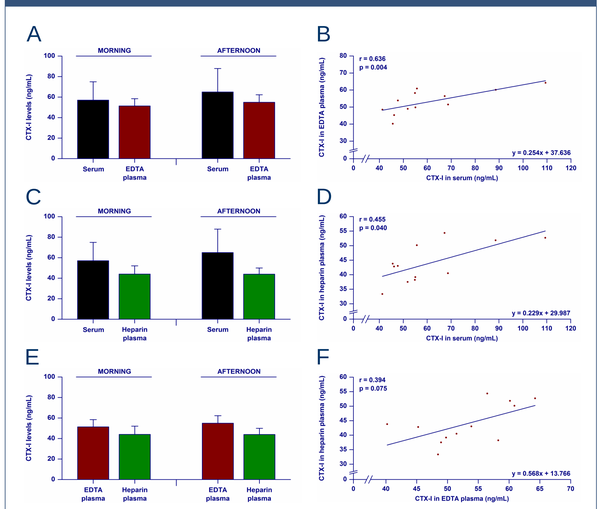

FIGURE 3. CTX-I levels (ng/mL) in the morning after overnight fasting and in the afternoon after six hours fasting. (A) Levels in rat serum and EDTA plasma and (B) their correlation. (C) Levels in rat serum and heparin plasma and (D) their correlation. (E) Levels in rat EDTA and heparin plasma and (F) their correlation. In panels B, D and F, r-letter indicates correlation coefficient and p-letter statistical significance.

Statistical Analysis

	TRACP 5b		CTX-I	
Morning vs Afternoon groups	**p-value**		**p-value**	
Serum	0.978		0.908	
EDTA plasma	0.800		0.992	
Heparin plasma	0.723		1.000	
Between sample type p-values	**Morning**	**Afternoon**	**Morning**	**Afternoon**
Serum vs EDTA plasma	1.000	0.778	0.987	0.882
Serum vs Heparin plasma	0.007	0.001	0.385	0.063
EDTA vs Heparin plasma	0.007	0.003	0.768	0.451
Correlations	**r-value**	**p-value**	**r-value**	**p-value**
Serum vs EDTA plasma	0.986	< 0.001	0.636	0.004
Serum vs Heparin plasma	0.589	0.057	0.455	0.040
EDTA vs Heparin plasma	0.634	0.036	0.394	0.075

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.

Acknowledgements

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References

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