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Efficacy of Potassium Citrate in the Treatment of Postmenopausal Osteopenia

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Study protocol and statistical analysis plan

1. Study Design and Participants

A randomized, double-blind, placebo-controlled, parallel-group study, with balanced randomization (1:1) was designed. The study was approved by the local research committee and registered at Clinical Trial Gov (ID: NCT02731820). The participants were recruited from a cohort of postmenopausal women treated at the Radiodiagnostic Unit of Rizzoli Orthopedic Institute (IOR) from January 2016 to June 2017; periodic measurements of lumbar (at the L2–L4 level) and femoral BMD were carried out using dual-energy X-ray absorptiometry (DXA) (Discovery DXA System, Hologic Inc., Bedford, MA, USA). Written informed consent was obtained from all participants. A code was assigned to each woman at the beginning of the trial which was used to track the data throughout the study. The inclusion criteria were women with osteopenia (T-score <−1 and >−2.5) and more than 5 years post-menopause. The risk of fracture was evaluated by using the FRAX® tool [1] based on an algorithm that takes into account demographic characteristics, medical history, some clinical risk factors as well as bone mineral density (BMD) at the femoral neck. The exclusion criteria were risk of fracture (FRAX: >20 major osteoporotic fractures; >3 hip fractures), hyperkalemia, renal insufficiency, urolithiasis, use of therapies influencing bone metabolism (e.g., corticosteroids, aromatase inhibitors and estrogens), use of drugs (such as diuretics and proton pump inhibitors), calcium and potassium supplements, vitamin D, current or recent (less than three years prior to the start of the study) use of bisphosphonates, gastrointestinal disorders which hamper nutrient absorption, and mental or psychiatric disorders which precluded the possibility of correctly adhering to the protocol. All the women were interviewed regarding food habits; they followed their usual diet throughout the duration of the study. Participants were assigned to the treatment or the placebo group using a randomization sequence created by independent personnel (IOR pharmacy) using a free online tool (http://randomization.com) and were stratified with a 1:1 allocation using a random block size of 4.

2. Supplements

The K citrate and the placebo were kindly provided by a manufacturer (Biohealth, Turin, Italy). They were in tablet form, identical in appearance, and were packaged in bottles which were rendered anonymous (A and B) until the end of the study. The products were stored at the IOR pharmacy and dispensed according to the computer-generated randomization list. Each woman received the supplements in the corresponding prepacked bottle. Both the subjects and the investigators who followed up the participants were blinded to group assignment. All subjects had daily supplementation with vitamin D3 cholecalciferol (400 IU day^{-1}) and calcium carbonate (500 mg

day^{-1}). The participants assigned to the experimental group received 30 mEq K citrate daily in two tablets for oral administration; those assigned to the placebo group received the same quantity of tablets containing only the excipients. The participants had clinic visits at the time of randomization (baseline), and at 3 and 6 months. At each time point, they underwent blood and urine evaluations. The laboratory staff performing the assays was unaware of the source of the samples which were identified by numeric codes.

3. Blood Analyses

Fasting venous blood samples were collected and analyzed for creatinine, sodium, potassium, total calcium, inorganic phosphate, parathyroid hormone (PTH), and 1,25-dihydroxyvitamin D. These analytes were measured by routine methods.

4. Urine Analyses

Urine samples were collected using a standardized protocol (Lithotest®, Biohealth, Turin, Italy), and the metabolic profile was examined in an external certified lab (Lithocenter, Turin, Italy). Briefly, during collection, 24-hour (24 h) urine was aliquoted into two fractions to which 10 mL hydrochloric acid (to prevent calcium/phosphate precipitation) and 1 mL Hibitane (to prevent bacterial contamination) were added. The acidified aliquot was used to quantify calcium (Ca), citrate, magnesium (Mg), oxalate, phosphate (PO4) and sulphate (SO_4); the Hibitane aliquot was tested for ammonium, chloride (Cl), creatinine, pH, potassium (K), sodium (Na), urea, and urate. In addition, a sample of fasting-morning urine was collected within 2 h after the 24 h collection ended, and was analyzed for citrate, calcium, urate (all expressed as creatinine ratios) and pH. Reference values were established on the basis of the assessment of mineral metabolic profile to evaluate the lithogenic risk [2,3,4]. The renal acid load was determined by calculating the 24 h urinary "potential renal acid load" (uPRAL) as follows [5]:

[Cl (mmol day^{-1}) + SO_4 (mmol day^{-1}) × 2 + PO_4 (mmol day^{-1}) × 1.8] − [Na (mmol day^{-1}) + K (mmol day^{-1}) + Mg (mmol day^{-1}) × 2 + Ca (mmol day^{-1}) × 2]

5. Immunoenzymatic Assay of BTM

The serum level of the bone turnover markers (BTMs) was evaluated at baseline and after 3 and 6 months of treatment. The BTM assay was carried out by following the recommended actions to minimize the pre-analytical variability [6]. The blood samples were collected after overnight fasting (between 08:00 and 10:00 a.m.), centrifuged within 2 h from collection, at 1000 g min^{-1} for 15 min and at room temperature. The serum was transferred into cryotubes (0.5 mL of serum per cryotube)

and frozen at −80 °C, within 1 h after centrifugation, until testing. The BTM serum levels were measured using commercially available reagents based on a sandwich enzyme immunoassay technique, following the manufacturer's protocols, with each sample tested in duplicate. The selected BTMs were related to (i) the number of osteoclasts (tartrate-resistant acid phosphatase 5b; TRACP5b); (ii) osteoblast function (bone alkaline phosphatase; BAP); (iii) type I collagen degradation (carboxy-terminal telopeptide; CTX); (iv) type I collagen precursor (procollagen type 1 N terminal propeptide; PINP). Source and technical notes of reagents used in this study are shown in Table 1.

Table 1. Source of the immunoenzymatic assay kits, sensitivity, precision, least significant change (LSC) and references values from healthy individuals.

Marker (Unit) Commercial name (Source)	**Detection limit**	**Min - Max intraassay CV (%)**	**Min - Max interassay CV (%)**	**LSC (%)**	**Mean ± SEM Median Min - Max**		
					Female (postmenopausal)	Female (premenopausal)	Male
CTX (μg · L^{-1}) Serum Crosslaps - (Immunodiagnostic systems Limited, Boldon, UK)	0.02	1.8 - 3.0	2.5 - 10.9	16.6	0.64 ± 0.36 0.44 0.12-1.35	0.38 ± 0.19 0.29 0.11-0.74	0.39 ±0.19 0.29 0.12-0.75
BAP (μg · L^{-1}) Ostase BAP - (Immunodiagnostic systems Limited)	0.7	2.6 - 6.5	3.7 - 6.1	15.6	14.53 ± 4.21 13.2 8.0-22.4	9.0±2.98 8.78 4.0-14.30	13.13±3.8 12.3 7.0-20.1
PINP (pg · L^{-1}) Human Procollagen I N-terminal Peptide (Cusabio Technology LLC, Aachen, Germany)	18.75	8	10	29.8		22.6 ± 1.7 [1] 20.1 15.4–40.8	
TRACP5b (U · L^{-1}) Bone TRAP (Immunodiagnostic systems Limited)	0.5	6.0-6.6	5.8-7.2	21.1	3.19±0.85	2.59±0.78	3.06±0.88

[1] Reference values have not been differentiated by gender and age.

6. Calculations and Statistical Analysis

All calculations and analyses were carried out using StatView 5.01 for Windows (SAS Institute Inc., Cary, NC, USA) and MedCalc Statistical Software version 18.2.1 (MedCalc Software bvba, Ostend, Belgium). The sample size calculation was based on a previous randomized clinical trial (RCT) aimed to evaluate the effect of K citrate on calcium metabolism and BTM in a cohort of individuals (males and females, age >55 years) treated for 6 months [7]. The present RCT was powered considering a Type I error (α) = 0.05 (two-sided), and Type II error (β) = 0.20 (power is 80%). Fifteen patients per group were sufficient, but the expected proportion of dropouts was 20%; therefore, as a precaution, 20 women in each group were recruited. Quantitative data were expressed as the arithmetic mean plus or minus the standard error of the mean (mean ± SEM). The D'Agostino-Pearson method was used

to test the normality assumption of continuous variables, and a *log* transformation was applied when the data distribution was non-normal. The data analysis was carried out following intention-to-treat principles. All follow-up parameters were analyzed using a "last value carried forward" approach under the intention-to-treat principle in order to address missing data [8]. The effect of K citrate on BTMs and blood/urine analytes was evaluated by applying the *t*-test, with comparison within the groups (paired analysis of the effect of treatment over time), and between the groups (independent comparison between K citrate and the placebo at the same time point). In order to highlight the changes over time, the results were expressed in line-graphs as deviations from the value observed before treatment, i.e., $[(\text{BTM concentration at time point} \times \text{BTM concentration at baseline}^{-1}) - 1]$ Thus, obtaining a baseline value equal to zero. The degree of association between the continuous variables was calculated by measuring the Pearson correlation coefficient. Differences and correlations were considered to be statistically significant when the p value was <0.05.

References

1. Kanis J.A., Johnell O., Oden A., Johansson H., McCloskey E. FRAX and the assessment of fracture probability in men and women from the UK. Osteoporos. Int. 2008;19:385–397. doi: 10.1007/s00198-007-0543-5.
2. Rivers K., Shetty S., Menon M. When and how to evaluate a patient with nephrolithiasis. Urol. Clin. N. Am. 2000;27:203–213. doi: 10.1016/S0094-0143(05)70251-2.
3. Marangella M., Di Stefano M., Casalis S., Berutti S., D'Amelio P., Isaia G.C. Effects of potassium citrate supplementation on bone metabolism. Calcif. Tissue Int. 2004;74:330–335. doi: 10.1007/s00223-003-0091-8.
4. Mayo Clinic Medical Laboratories Endocrinology Catalog Bone/Minerals. [(accessed on 26 July 2018)]; Available online: https://Endocrinology.Testcatalog.Org/Search?Q=Mml-Bone-Minerals&Sort=Alpha.
5. Krupp D., Shi L., Remer T. Longitudinal relationships between diet-dependent renal acid load and blood pressure development in healthy children. Kidney Int. 2014;85:204–210. doi: 10.1038/ki.2013.331.
6. Eastell R., Szulc P. Use of bone turnover markers in postmenopausal osteoporosis. Lancet Diabetes Endocrinol. 2017;5:908–923. doi: 10.1016/S2213-8587(17)30184-5.
7. Moseley K.F., Weaver C.M., Appel L., Sebastian A., Sellmeyer D.E. Potassium citrate supplementation results in sustained improvement in calcium balance in older men and women. J. Bone Miner. Res. 2013;28:497–504. doi: 10.1002/jbmr.1764.
8. Shao J., Zhong B. Last observation carry-forward and last observation analysis. Stat. Med. 2003;22:2429–2441. doi: 10.1002/sim.1519.