Additional impact on muscle function when treating active rheumatoid arthritis patients with high alfacalcidol doses

Dodatni uticaj visokih doza alfakalcidola na mišićnu funkciju prilikom lečenja bolesnika sa aktivnom formom reumatoidnog artritisa

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Abstract

Background/Aim. Hormone D (vitamin D) plays an important role in immunoregulation and musculoskeletal metabolism. The aim of this study was to assess the impact of alfacalcidol (ILD_3) or prednisone use on muscle function and disease activity in active rheumatoid arthritis (RA). **Methods**. The study included 67 RA patients with the active disease, disease activity score (DAS28) > 3.2, on the highest tolerable methotrexate (MTX) dose during last 3 months. Data collected were: DAS 28, muscle function tests [chair rising test (CRT) timed up and go (TUG), 6 minutes walk (6MWT), tandem walk (TW)], efficacy and safety laboratory tests. At enrollment, patients were randomly assigned to three-month supplementation with 1 µg (group A1) or 2 µg (group A2) or 3 µg (group A3) of 1αD3 daily or prednisone (group C) 20 mg daily, for the first month and 10 mg afterward, in addition to MTX. **Results**. After the treatment, we found highly significantly reduced disease activity in all four treatment arms (DAS28 $p < 0.01$). 1αD3 2 µg (A2 group, n = 19) treated patients significantly improved muscle function (TUG, 6MWT), while 1αD3 3 µg treated (A3, n = 16) improved 6MWT ($p < 0.05$), and CRT ($p < 0.01$). Serum 25(OH)D3 significantly decreased in the group C ($p < 0.01$), in contrast to its changes obtained in alfacalcidol treated ones. **Conclusion**. 1αD3 2 µg and 3 µg daily is as effective as prednisone (mean 13.3 mg daily) in RA activity control and also has the additional favorable impact on muscle function.

Key words:
arthritis, rheumatoid; alfacalcidol; muscles; disease progression.

Apstrakt

Uvod/Cilj. Hormon D (vitamin D) igra važnu ulogu u imunoregulaciji i koštano-mišićnom metabolizmu. Cilj rada bio je ispitivanje uticaja alfakalcidola (1αD3) analoga hormona D, i prednizona, na mišićnu funkciju i aktivnost bolesti kod obolelih od aktivnog reumatoidnog artritisa (RA). **Metode**. U istraživanje je bilo uključeno 67 bolesnika sa RA koji su imali aktivnu bolest (indeks aktivnosti bolesti – DAS28 > 3,2), uprkos maksimalno podnošljivoj dozi metotreksata (MTX), tokom prethodna tri meseca. Prikupljeni su podaci o aktivnosti bolesti (DAS28), mišićnoj funkciji [(test ustajanja sa stolice (CRT), test ustani i kreni (TUG), šestominutni test hodanja (6MWT), tandem hod (TW)], kao i zapaljenski i bezbednosni laboratorijski nalazi. Bolesnici su nasumično raspoređeni na tromesečno lečenje: 1 µg (grupa A1) 2 µg (grupa A2) ili 3 µg (grupa A3) 1αD3 dnevno ili prednizon (grupa C) 20 mg dnevno, prvih mesec dana, potom 10 mg, uz MTX. **Rezultati**. Na kraju lečenja u svim terapijskim grupama nađena je visoko statistički značajno snižena aktivnost bolesti ($p < 0,01$). U grupi A2, lečenih 1αD3 u dozi 2 µg/dan značajno su poboljšani TUG i 6MWT, a kod lečenih 1αD3 3 µg (A3 grupa) došlo je do visoko statistički značajnog poboljšanja CRT ($p < 0,01$) i značajnog poboljšanja 6MWT ($p < 0,05$). U grupi C došlo je do visoko statistički značajnog sniženja nivoa vitamina D (25(OH)D3) u serumu ($p < 0,01$), nasuprot njegovom povećanju kod lečenih alfakalcidolom. **Zaključak**. Alfakalcidol u dozi 2 µg i 3 µg dnevno podjednako je efikasan kao prednizon (13,3 mg dnevno) u kontroli aktivnosti RA, a ima i dodatni povoljan efekat na mišićnu funkciju.

Ključne reči:
artritis, reumatoidni; alfakalcidol; mišići; bolest, progresija.

Introduction

Rheumatoid arthritis (RA) is a chronic, systemic auto-immune disease, affecting 0,5-1% of the population [1].

The most prominent feature of the disease is synovitis of diarthrotic joints resulting in severe joint pain, reduced muscle strength and impaired physical function [2, 3]. Autoimmune driven synovium and systemic inflammation lead to cytokine

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dysregulation, elevation (3–100 times) of inflammatory cytokines in both beds [interleukin (IL) 6, interleukin 1 (IL1), tumor necrosis factor alpha (TNFα), etc], even during inactive phases of the disease [4–6]. Active RA also results in downregulation of sex steroids, growth hormone, anabolic factors such as insulin-like growth factor1 (IGF-1), insulin resistance and impairment of lipids metabolism, resulting in deleterious co-morbidities of the disease such as advanced atherosclerosis, muscle wasting-rheumatoid cachexia, osteoporosis [4–7]. Rheumatoid cachexia is reported in two-thirds of all RA patients, as cytokine imbalance, hypermetabolism and inactivity driven accelerated loss of skeletal muscle mass with the elevation of fat mass contribute to functional disability and lowering of the quality of life [6]. The course of RA is typically one of exacerbations and remissions. Aggressive treatment of RA also treats co-morbidities [8]. All RA patients at some point of the disease course require adaptation of disease-modifying antirheumatic drug (DMARD) or corticosteroid (CS) therapy, and combined strength and endurance training. Despite anti-inflammatory, symptomatic and structural efficacy in RA, a CS use is suggested to be as short as possible, due to its adverse effects [8, 9].

The discovery of the immunomodulatory and antitumor properties of D hormone prompted researchers to investigate the possibility of its use as a therapeutic agent for autoimmune and malignant diseases [10–18]. During the last several decades, there were many investigations with the aim to synthesize hormone (vitamin) D analogs [vitamin D receptor (VDR) agonists] with the same biologic activity and even stronger anti-inflammatory properties, but with lower blood calcium-increasing capacity [13, 18]. One of steroidal VDR agonists, alfacalcidol (1αD3) differs from hormone D only by lacking the 25(OH) group. Agonists with the OH group in 1α position having the highest specificity of binding to VDR were developed based on the knowledge about the specificity of biochemical mechanisms of VDR binding with natural D hormone or its analogs[16]. There are numerous preclinical and clinical data on preventive and therapeutic potential of alfacalcidol in the primary and secondary osteoporosis, osteoporotic fractures, neuromuscular functioning, transplantation, displaying its exclusive pleiotropic capacity [11, 14, 19–26]. Recently, greater intake of vitamin D is associated with a lower risk of RA, as well as the lower serum levels of vitamin D with higher disease activity [27, 28]. Hormone D deficiency in tissues, which is present in chronic inflammation, caused by inhibition of α-hydroxylase, can be corrected by alfacalcidol use, due to its activation in the liver or other target organs, bypassing body`s own feedback regulation [29, 30]. It has shown beneficial effects in autoimmune diseases such as RA and Psoriatic arthritis [11, 19]. Positive effect of alfacalcidol on regulating the cytokine homeostasis and increasing suppressor cells has been recognized [11, 19, 31, 32]. Recently, it has been shown that despite previous beliefs, it acts directly on inflammatory cytokine production without being additionally hydroxylated at the 25 position [33]. Due to the multifaceted potential of alfacalcidol in the autoimmune diseases, we wanted to assess its performance on joints and muscular features in clinical setting of active RA, at the point of current therapy adaptation. The aim of this study was to assess the impact of alfacalcidol or prednisone use on the muscle function and disease activity in active RA.

Methods

Study population and protocol

This was the open label prospective study approved by the Institutional Review Board/Ethics Committee (Decision No 29/1-7, February 19th, 2010) and the national Medicine and Medical Devices Agency of Serbia (Decision No 515-04-0544-12-2, September 28th, 2012). Written informed consent was obtained from all patients prior to enrollment.

The study population consisted of 67 active RA patients (46 females) on stable prior methotrexate (MTX) (10–25 mg/weekly) therapy and no steroids use for more than 3 months before enrollment. Patients were randomly assigned to administration of 1 μgr/daily 1αD3 (group A1, n = 17), 2 μg/daily 1αD3 (group A2, n = 19), 3 μg/daily 1αD3 (group A3, n = 16) treatment for 3 months or 20 mg of prednisone/daily for 1 month, followed by 10 mg prednisone/daily for 2 months (group C, n = 15), added to background stable MTX treatment. Alfacalcidol was provided by the investigator as gelatin capsules for oral administration (Alpha D3[R], TEVA, Serbia). Alfacalcidol dosing was modified only in case of toxicity, i.e. disturbances in calcium levels in blood or urine [more than 2.65 mmol/L in blood or more than 0.3 g daily in urine (DU) measured in two consecutive samples], drug dose half lowered during 1 week, until calcium and phosphorus levels normalized, then supplementation was re-started with the assigned treatment dose, if not, a patient was excluded from the study.

RA diagnosis was established at least 6 months prior the study started, using American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) 2010 criteria [34, 35], and patients were eligible for participation if ESR-DAS28 was > 3.2 [36]. Neither previous history of calcium metabolism disturbances or renal or bladder stones, nor any of following was allowed: psychiatric illness and/or social situation that would limit compliance with study medication and protocol requirements, signs and symptoms of severe, progressive or uncontrolled renal, hepatic, hematologic, endocrine, pulmonary, cardiac, neurologic or cerebral disease, inherited metabolic disease, pregnancy or lactation, presence of malignancy, or history of any malignancy in previous 10 years. Drugs which effect could be influenced or known to influence calcium or phosphorus metabolism, were not allowed. Any of the nonsteroidal anti-inflammatory drugs (NSAIDs), the additional DMARDs or steroid drugs, not present at the enrollment, could not be used during the study. Patients were advised to maintain a daily fluid intake of at least 1.5 L throughout the duration of the study and to intake only dietary calcium.

Study outcomes and safety parameters

Demographic and clinical data were collected, blood samples were taken, muscle function tests and disease activity were assessed at the study entry and after 3 months

after the treatment was assigned per protocol. Disease activity was assessed by the ESR DAS 28, calculated from the erythrocyte sedimentation rate (ESR), number of tender (TJC) and swollen joints (SJC) and the patients` assessment of disease activity (Patient Visual Analogue Scale – PVAS) based on the answer to the question "Given the overall impact of your arthritis to you, how are you feeling today?" Rated on a scale of 100 mm, answer may vary from 0 mm - very good to 100 mm - very poor, i.e. lower score reflected better state [37]. Muscle function was assessed by a battery of 4 tests usually used in the clinical setting:1) chair rising test (CRT), time to rise five times from a standard chair i.e. 46 cm high, with arms folded, shorter time (seconds) represent better muscle power [38], 2) timed up and go (TUG) test, time taken to rise from a chair, walk 3 meters, turn around, walk back and sit down, shorter time (seconds) represent better muscle coordination [39, 40], 3) 6-minutes walk test (6MWT) average distance (meters) of two attempts to walk during six minutes, longer distance represent better functional mobility [41], and 4) tandem walk (TW), time (seconds) of walking heel-to-toe in a 2 meters straight line, shorter time represent better muscle balance [40].

Safety follow-up visits monitoring calcium metabolism were performed 2 weeks apart from the start and then they were followed monthly for the clinical and laboratory findings, as well as adverse events (AE). At each of two follow-up visits, the patients were assessed for any relevant change in the clinical status, weight, arterial pressure, electrocardiogram, ESR (Westergren method), routine hematology (Coulter HmX haematology analyzer), biochemistry such as C-reactive protein (turbidimetry Gilford), glucose, albumin, liver enzymes, creatinine, uric acid, alkaline phosphatase, serum calcium, ionized calcium and 24 h calciuria were tested, while serum 25(OH)D3, (ECL electrochemiluminiscence-Elecsys 2010) and parathormone (PTH) (FPIA- fluorescent polarisation imunoassay–AxSym Abbot) were determined only at the start and at the end of the treatment period.

Statistical analysis

Statistical analysis was performed using the SPSS 20 package [data are presented as mean±SD (min-max)]. Subgroup changes of variables pre and post treatment were analyzed by paired *t*-test or Wilcoxon's test. The subgroup differences were assessed by independent *t*-test, ANOVA or χ^2 test, as appropriate and least significant difference (LSD) method, *post hoc*; $p < 0.05$ was considered statistically significant in all analyses.

Results

Demographic and clinical characteristics of patient population

All patients completed 3 months study period. Out of 67 RA patients included, 46 (68.65%) were females, average age was 56.24 ± 12.423 (23–83), disease duration 7.71 ± 6.68 (1-33) years, MTX dose 15.41 ± 3.28 (10–25) mg/weekly and MTX use of 5.67 ± 5.9 (0.5–15) years, mean disease activity (ESR DAS28) was 5.58 ± 0.905 (3.22–7.55) at the baseline. Baseline DAS28 was > 5.1 in 47 (70.14%) and DAS28 > 3.2 in 20 (29.85%) patients. The patients, randomized in 4 different treatment arms for 3 months, were fully comparable and their demographic and clinical characteristics are presented in Table 1. At enrollment, randomized groups were mutually comparable with respect to the

Table 1

Demographic, disease and muscle function characteristics in study groups

Demographic characteristics	A1 (n = 17)	A2 (n = 19)	A3 (n = 16)	C (n = 15)
M/F ratio, n	5/12	7/12	5/11	4/11
Age (years), $\bar{x} \pm SD$	57.94 ± 12.28	53.79 ± 12.012	53.06 ± 10.91	60.67 ± 13.75
BMI (kg/m^2), $\bar{x} \pm SD$	22.2 ± 1.9	21.9 ± 2.34	23.1 ± 2.76	22.7 ± 2.98
RA duration (y), $\bar{x} \pm SD$	9.82 ± 8.15	7.63 ± 7.12	5.63 ± 4.33	7.53 ± 6.79
Comorbidity, n (%)	6 (35.29)	9 (47.36)	6 (37.5)	7(46.66)
MTX duration (years)	7.82 ± 2.112	6.71 ± 1.567	5.31 ± 2.22	6.89 ± 4.11
MTX average (mg/w)	14.09 ± 1.988	14.45 ± 3.533	16.87 ± 3.48	16.66 ± 2.94
PsDMARD, n (%)	8 (47.05)	12 (63.15)	11 (68.75)	9 (60)
PbDMARD, n (%)	1 (5.88)	1 (5.26)	1 (6.25)	1 (6.66)
Disease activity, $\bar{x} \pm SD$				
DAS28	5.28 ± 0.874	5.81 ± 0.891	5.73 ± 0.87	5.86 ± 0.789
TJC	6.55 ± 2.999	9.21 ± 3.735	8.02 ± 4.33	9 ± 4.309
PVAS (mm)	52.51 ± 16.070	51.89 ± 17.129	51.73 ± 14.99	50.40 ± 15.624
ESR (mm/h)	28.18 ± 20	39.84 ± 23.735	42.88 ± 28.98	43.73 ± 15.895
RF, n (%)	15 (88.23)	13 (68.42)/19	14 (87.5)/16	12 (80)/15
ACPA, n (%)	3 (17.64)	7 (36.84)/19	4 (25)/16	4 (26.66)/15
25(OH)D3 (ng/mL), $\bar{x} \pm SD$	31.88 ± 13.57	28.97 ± 9.914	28.02 ± 14.12	34.02 ± 15.741
PTH (pg/mL), $\bar{x} \pm SD$	41.49 ± 19.64	31.2 ± 9.382	42.74 ± 11.66	40.49 ± 15.49
Muscle function, $\bar{x} \pm SD$				
CRT (s)	11.02 ± 2.91	12.02 ± 5.20	12.28 ± 3.46	12.72 ± 3.75
TUG (s)	6.92 ± 1.17	7.68 ± 2.91	7.22 ± 3.31	8.8 ± 3.138
6MWT (m)	437.3 ± 63.93	411.58 ± 127.35	423 ± 97.18	347.8 ± 15.741
TW (s)	7.93 ± 3.65	8.24 ± 4.05	9.7 ± 2.25	8.12 ± 5.343

n – number of patients; M – men; F – women; BMI – body mass index; RA – rheumatoid arthritis; MTX – methotrexate; W – week; VDMARD – disease modifying antirheumatic drugs; psDMARD – previous syntetic DMARD treatment; PbDMARD – previous biologic DMARD treatment; DAS28 – disease activity score; 25(OH)D3 – serum level of vitamin D; PTH – serum level of parathormone; ESR – erytrocite sedimentation rate; RF – rheumatoid factor positive; ACPA – anti-citrullinated protein atibodies positive; TJC – tender joint count; SJC – swollen joint count; pVAS – patient visual analogue scale; CRT – chair rising test; TUG – timed up and down test; 6MWT – six minute walk test; TW – tandem walk.

total number of the participants per group ($p = 0.881$, χ2), gender distribution ($p = 0.926$, χ2), age ($p = 0.257$, ANOVA), body mass index (BMI) ($p = 0.542$, ANOVA), duration of RA ($p = 0.374$, ANOVA), dosage of MTX 12 weeks before the study ($p = 0.061$, ANOVA), activity of the disease ($p = 0.058$, ANOVA), duration of MTX treatment ($p = 0.543$, ANOVA), rheumatoid factor (RF) positivity ($p = 0.567$, χ2), anticitrullinated protein antibodies (ACPA) positivity ($p = 0.588$, χ2), the serum levels of 25(OH)D3 ($p = 0.237$, ANOVA), PTH ($p = 0.075$), ESR ($p = 0.188$, ANO-VA), comorbidity ($p = 0.690$, χ2), previous treatment with synthetic DMARD, (sDMARD) ($p = 0.978$, χ2) and biologic DMARD, (bDMARD) ($p = 0.998$, χ2), CRT ($p = 0.659$, ANO-VA), TUG ($p = 0.173$, ANOVA), 6MWT ($p = 0.074$, ANOVA), TW and ($p = 0.062$, ANOVA).

Influence on muscle function

Assessment of muscle function revealed improvement in the time needed to perform CRT in all therapeutic regimens, while in one group treated with 3 µg of 1αD3, the improvement was highly statistically significant ($12.28 \pm 3,464$ *vs* 8.9 ± 2.33, $p < 0.01$, paired *t*-test) (Figure 1a). Walking distance during 6 minutes (MWT) improved significantly in all treatment groups, in the A1 (437.29 ± 63.933 *vs* 486.35 ± 118.958, $p < 0.05$, pai-

red *t*-test), in the A2, (411.58 ± 12.347 *vs* 483.95 ± 58.228, $p < 0,05$, paired *t*-test), in the A3 (439.3 ± 74.47 *vs* 483.13 ± 51.64, $p < 0.05$, *t*-test), as well as in the C group (347.73 ± 97.17 *vs* 398.73 ± 61.002, $p < 0.05$, paired *t*-test), as shown in Figure 1b. Timed up and go test (TUG), improved in 2 µg, 3 µg 1αD3 and prednisone treated patients, yet only in the A2 group the difference pre-post treatment was statistically significant (7.67 ± 2.91 *vs* 6.02 ± 1.15, $p < 0.05$, paired *t*-test) (Figure 1c). Tandem walk test (TW) slightly improved in all treatment groups, except in the A1 one, yet neither change was statistically significant. Subgroup analysis showed no difference in muscle tests changes pre-post treatment, except for ΔTUG (A2 *vs* C, $p < 0.05$, ANOVA).

Influence on disease activity

Clinical efficacy indices showed marked improvement in term of ESR DAS28 in all therapeutic regimens, with highly statistically significant reduction achieved in the group A1 (5.28 ± 0.874 *vs* 4.16 ± 1.03, $p < 0.01$, paired *t*-test), A2 (5.81 ± 0.89 *vs* 4.32 ± 1.03, $p < 0.01$, paired *t*-test), A3 (5.74 ± 0.87 vs 4.44 ± 0.86, $p < 0.01$, paired *t*-test), as well as in the group C (5.86 ± 0.79 *vs* 4.13 ± 0.913, $p < 0.01$, paired *t*-test). In order to further explore the efficacy of different
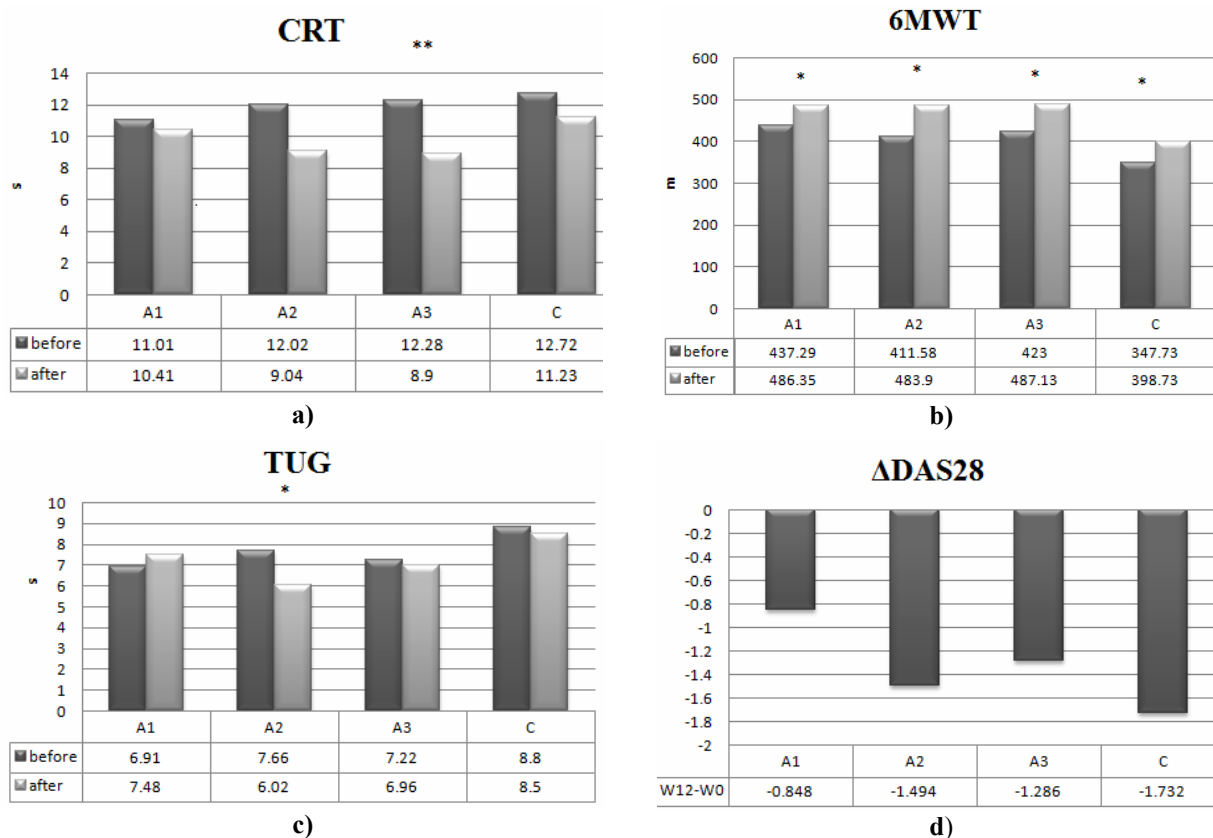
Fig. 1 – Changes of muscle function tests and the disease activity over time; a) Chair rising test (CRT) pre-post treatment; b) Six minutes walk test (6MWT) pre-post treatment; (c) Timed up and go test (TUG) pre-post treatment; d) Difference of disease activity score (ΔDAS28) post-pre treatment; A1 – group supplemented with 1µg 1αD3; A2 – group supplemented with 2 µg 1αD3; A3 – group supplemented with 3 µg 1αD3; C – group treated with prednisone; * – statistically significant difference ($p < 0.05$); ** – highly statistically significant difference ($p < 0.01$).

treatment regimens in term of the disease activity, we used EULAR DAS28 response model [37]. Changes of the disease activity (ΔDAS28) during 12 week study in four different treatment regimens were A1 *vs* A2 *vs* A3 *vs* C, $p < 0.05$, ANOVA, while there was no difference in A2, the A3 groups *vs* C group ($p = 0.437$, post-hoc Fisher's Least Significant Difference – LSD), (Figure 1d). Also, there was no significant difference in the number of patients with the good DAS28 EULAR response, which means the reduction of DAS28 > 1.2, in the groups A2, A3 and C ($p = 0.532$, χ2).

Influence on calcium metabolism parameters and safety issues

The patients reported overall good tolerability of study treatments, no serious adverse events or any laboratory or clinical adverse events were observed.

Compared to baseline levels, the serum 25(OH)D3 levels raised in all alfacalcidol treated patients, at the end of the study, while in patients treated with prednisone significantly decreased (34.02 ± 15.74 *vs* 21.93 ± 10.90, $p < 0.01$, paired *t*-test). PTH levels significantly decreased in the A2 group (31.2 ± 9.39 *vs* 26.42 ± 10.29, $p < 0.05$, paired *t*-test) and the A3 group (42.88 ± 11.66 *vs* 26.45 ± 13.401, $p < 0,01$, paired *t*-test) at the end of the treatment period.

The serum calcium and ionized calcium follow-up were particularly of interest, both remained unchanged in all treatment groups, while calciuria was significantly raised in the groups treated with alfacalcidol 2 μg and 3 μg, only in the latter group, the upper limit of normal exceeded slightly (ULN) of 0.3 g/DU (in the A2 0.18 ± 0.077 *vs* 0.27 ± 0.09, $p < 0.01$, in the A3 group 0.13 ± 0.047 *vs* 0.32 ± 0.11, $p < 0.01$, paired *t*-test). Alfacalcidol daily dose was corrected from 3 μg to 1 μg daily for 1 week in 4 patients, due to an increase in calciuria registered in 2 patients in the A3 group, 2 weeks, and 8 weeks apart from the start of the study, respectively. After daily dose reduction, calciuria normalized, and study treatment was continued as before.

The serum glucose levels raised pre-post treatment in the C group by 1.7 mmol/L (about 40% compared to baseline), yet not above ULN, in contrast to 1αD3 treated patients, who had about 2% lower glucose levels.

Discussion

As RA is associated with the significant physical disability, which has a negative impact on employment and health related quality of life. A primary goal for many patients is to maintain and improve physical function [42]. In the mutifactorial model of disability, skeletal muscle wasting and weakness in RA patients have a large influence [2, 3, 6]. Data on the impact of RA therapy on the skeletal muscle function are lacking. Contemporary aggressive sDMARD and bDMARD RA treatment, accompanied with exercises is effective, as for other metabolic co-morbidities, yet the challenge is to identify and treat any modifiable factors that contribute to physical disability [8, 36, 43]. Vitamin D3 (hormone D) deficiency may be one of it [20, 25].

Evidence from recent meta-analysis of 16 randomized controlled trials (RCTs) of vitamin D treatment influence on muscle function, do support the beneficial effect of vitamin D supplementation on muscle strength and function in the elderly, vitamin D insufficient subjects, with major lack of data on the possible effect in younger people [43]. Identified studies were heterogeneous with regard to most aspects including the indices measured. Yet, some analogy can be made with the study of Lips et al. [44] who used a battery of physical performance tests including TUG, CRT, 6MWT, in 226 ambulatory patients, in which 8400 IU vitamin D3/weekly or placebo were applied for 4 months. They observed a rise in baseline vitamin D3 serum levels, yet no significant positive effect on muscle function was observed, compared to placebo. The average age of their study population was 78 years, with no data about co-morbidities. We had much younger (56 years), ill (RA) and vitamin D replete population (31.56 ng/mL) in our study. We observed better muscle functioning in all of our patients, except for TUG test in the 1 μg αD3 subgroup (0.57 s), with the significant improvement in CRT, 6MWT (in 3 μg αD3 treated patients) and 6MWT and TUG (in 2 μg αD3 treated ones), accompanied with statistically significant serum 25(OH)D3 elevation and decrease of PTH in the same subgroups and highly statistically significant reduction of RA activity, also. Alfacalcidol 1 μg treated ones had significant reduction of disease activity (ΔDAS28 -0.84) accompanied with improvement in walking distance as measured by 6MWT, similar to findings of some improvement in muscle function noticed in prednisone treated ones, yet, only walking distance in 6MWT significantly increased, even they decreased the disease activity the most (ΔDAS28 -1.73). Our data additionally support the evidence of 1αD3 efficacy in RA, as we got highly significant improvement of the disease activity assessed by DAS28, in all alfacalcidol treated patients, as one of the most cited open labeled trial of alfacalcidol 2 μg use for twelve weeks in the active RA patients (n = 19), that showed significantly fewer swollen joints and improvement in two symptom scale scores, the Ritchie articular and Lee indices, with *in vitro* immunomodulatory effects on their lymphocytes [11].

Scharla et al. [26] showed that 1 μg alfacalcidol use for 3 months in RA vitamin D replete patients resulted in increase of lower extremity muscle power by isometric knee extension measurement (60%) compared to native vitamin D 1000 IU use (18%). We also observed an increase in CRT as a measure of muscle power in all alfacalcidol treated patients. It is of importance that no endurance nor strength training in any of our subjects was applied.

Study of muscle biopsies in osteoporotic patients showed that 1 μg alfacalcidol treatment for 3–6 months, induced an increase in the relative number and cross-sectional area of fast-twitch type IIA muscle fibers, accompanied by the reduction of fast-switch type IIB fibers, altogether with the improvement in activities of daily living [45]. We did not perform any imaging or morphological analysis of muscle tissue, while the clinical impression of the reduced disease activity index and increased mobility in our patients was im-

pressive. This represents the starting point for future investigations of $1\alpha D_3$ in RA patients in a placebo-controlled manner, including whole body densitometry scan with an estimation of lean/fat mass pre-post treatment.

We can say that the most gain of three-month study treatment in active RA patients was observed when 2 µg and 3 µg 1αD3 were used, as a dual action on inflammation and muscle performance was documented. Dual effect of alfacalcidol treatment is observed in osteoporotic subjects, both in primary and corticosteroid induced osteoporosis [23, 24, 46–48]. Meta-analysis of those studies showed a reduction of fracture rates as the result of improved bone mass, quality and a reduction of falls by improvement of muscle function, which was the exclusive action of alfacalcidol [24, 48]. Hypercalcemia as the adverse effect is rare in alfacalcidol use, as shown in post-marketing surveillance of 13550 osteoporotic patients [49]. We used high doses of alfacalcidol, but also found no hypercalcemia, yet, reversible calciuria occurred in 2 µg and 3 µg treated subjects, not of clinical importance.

Prednisone treatment (average 13.3 mg daily, for 3 months) in our study resulted in a highly significant reduction of serum level of 25(OH)D3, which was definitely not a desirable state, as it was closely negatively related to at least RA disease activity [28, 50]. On the contrary, anti-inflammatory effects of all three alfacalcidol treatment regimens in our study resulted in increased levels of serum 25(OH)D3, lower serum PTH, the state which is known to be related to better muscle mass and strength, reduced falls rate, better physical performance as was shown in systematic review of Schacht and Richy [51] and in 3 year`s prospective study of Visser et al. [52]. All the benefits of the disease activity, PTH metabolism and muscle function of 1αD3 supplementation in active RA, did not result from correcting vitamin D deficiency since the patients were already vitamin D replete. We assume that direct effect of high doses of alfacalcidol on the nuclear VDRs in different target tissues produced such effects. Corticosteroids are widely used due to their symptomatic, anti-inflammatory and structural effects in active RA [8, 9].

Conclusion

Based on our findings, alfacalcidol might find its place as bridging therapy instead of corticosteroids, in at least selected group of RA patients. Three-month alfacalcidol treatment is as effective as prednisone in the disease control and has the dose dependent positive effect on muscle function in vitamin D replete active RA patients with a good safety profile.

Acknowledgements

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