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ORIGINAL ARTICLE

Effective Increase of Serum Vitamin D3 by IV Application of a Cholecalciferol-N-Acetyl-Galactosamine-Stabilized Dimer: a Clinical Murine Trial Study

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SUMMARY

Background: **Pre-clinical toxicology studies of human Gc-protein (vitamin D binding protein) are of special interest as to the transport of vitamin D and its biological activities. We have demonstrated that the oral application of a special dimeric vitamin D complex reduces oxidative stress and increases the quality of life in autistic children. Therefore, safety and toxic effects of two dimeric cholecalciferol-N-acetyl-galactosamine-albumin complexes were evaluated in increasing intravenous (iv.) vitamin D levels administered in a pre-clinical trial in mice over a 5-week period.**

Methods: **Over a period of 5 weeks, two times a week, mice received iv. administration of one of the following: (a) 1.2 IE of vitamin D-N-acetyl-galactosamine-albumin (Vitamin D_3 NAGA, ImmunoD® group), (b) 1.2 IE of vitamin-D-poly-N-acetyl-galactosamine-albumin (Poly-Nac group), or (c) isotonic saline solution (sham group). Before and after the trial, red and white blood cell panels (RBS, WBC and platelets) were determined. Furthermore, vitamin D levels, electrolytes, and C-reactive protein levels were measured directly before sacrificing.**

Results: **No toxic effects were observed during iv. injection with dimeric vitamin D complexes, neither in the sham group, nor in the two treatment groups. Vitamin D levels increased significantly within 5 weeks in the Poly-Nac group (26.6 ± 8.8 ng/mL; p = 0.001) compared to the sham group (3.1 ± 0.9 ng/mL), and the Poly-Nac group to the ImmunoD group (7.0 ± 3.6 ng/mL; p = 0.003). A significant increase of vitamin D was also obtained in favor of the ImmunoD group compared to the sham (p = 0.03). Electrolytes (K, Na, Cl, Mg, Ca) and C-reactive protein showed no significant differences after administration in all three mice groups. Also, no significant differences were observed between these three groups in the WBC and RBC blood panels.**

Conclusions: **The two dimeric vitamin D complexes used in this pre-clinical study showed no side or toxic effects after iv. administration in mice, but a sole increase in vitamin D levels without any change in electrolytes or blood cells. Therefore, we assume this newly developed composition to be safe in oral or iv.-administration and further pre-clinical studies can be conducted to evaluate the value in treatment of various diseases related to vitamin D deficiencies.**

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KEY WORDS

Vitamin D, cholecalciferol, vitamin D binding protein (DBP), N-acetyl-galactosamine-albumin (NAGA), vitamin D - N-acetyl-galactosamine-albumin (ImmunoD®), poly-N-acetyl-galactosamine-albumin (Poly-Nac)

INTRODUCTION

Immunology and immunotherapy of cancer is an expanding field in oncology, with significant recent achievements obtained through the new successful approaches implemented to circumvent immune evasion, which is undoubtedly considered a novel hallmark of cancer [1,2].

Furthermore, 1,25-dihydroxyvitamin D(3) [1,25(OH)(2) D(3)], the bioactive form of vitamin D, has been shown to possess significant anti-tumor potential. While most studies so far have focused on the ability of this molecule to influence the proliferation and apoptosis of cancer cells, more recent data indicate that 1,25(OH)(2) D(3) also impacts energy utilization in tumor cells [3]. The protective effect of vitamin D on breast cancer risk may be subtype specific, being stronger for more aggressive tumors, which provides a new approach to prevent this disease [4].

Although vitamin D binding protein (DBP), a member of the albuminoid superfamily, was discovered as early as in 1959, the enormous number of published papers in recent years reveal that it is a hot research topic today. Besides the three major phenotypes (DBP1F, DBP1S and DBP2), more than 120 unique variants of this polymorphic protein have been described in the relevant literature. The presence of DBP has been demonstrated in different body fluids (serum, urine, breast milk, ascitic fluid, cerebrospinal fluid, saliva, and seminal fluid) and organs (brain, heart, lungs, kidneys, placenta, spleen, testes, and uterus). Although the major function is binding, solubilization, and transport of vitamin D and its metabolites, the name of this glycoprotein conceals numerous further important biological functions. Therefore, more focus on the analytical aspects, determination, and discussion in detail of its multifunctional capacity [actin scavenging, binding of fatty acids, chemotaxis, binding of endotoxins, influence on T cell response, and influence of vitamin D binding protein-macrophage activating factor] of this abundant plasma protein on bone metabolism, inflammation, and cancer is needed [5].

These results are consistent with the hypothesis that the known anticancer efficacy of vitamin D binding protein-macrophage activating factor can be ascribed to different biological properties of the molecule which include inhibition of tumor-induced angiogenesis and direct inhibition of cancer cell proliferation, migration, and metastatic potential [6].

ImmunoD and PolyNac are newly developed dimers of a recombinant modified VDBP combined with cholecalciferol (1,25-dihydroxyvitamin D(3)), which combines all the aforementioned immunological reactions. The aim of this trial was to evaluate the safety and effectiveness in vitamin D supplementation of these newly developed dimers during a 5 week iv. application.

MATERIALS AND METHODS

Vitamin D3 - N-acetyl-galactosamine-albumin complex (ImmunoD®) and Vitamin D3 - N-acetyl-galactosamine-albumin complex (PolyNac; wn30031201) were obtained from HG Pharma (Vienna, Austria).

The Federal Ministry for Transport, Innovation and Technology, Republic of Austria approved this murine study and to conduct the animal trial at the Medical University of Graz, Austria (BMWFW-66.010/0317/WF/V/3b/2017). Seven mice were injected with iv. stabilized cholecalciferol/N-acetyl-galactosamine-albumin (12 IE, ImmunoD) in isotonic solution twice per week for 5 weeks, 7 mice with cholecalciferol/poly-acetyl-galactosamine-albumin (12 IE, PolyNac) in isotonic solution, and a sham group of 4 mice twice per week with only isotonic solution for 5 weeks. During the study, all mice received the same nutriment. Before starting the study, blood was collected for red and white panel estimation. After 5 weeks all mice were sacrificed and blood was collected again for the measurement for red and white blood panels and additional serum samples for the measurement of C-reactive protein, electrolytes, and vitamin D.

Measurement of Red and white blood panel and electrolytes

RBC and WBC blood panels were measured on a Becton Dickinson apparatus: platelets (PLT), mean platelet volume (MPV), plateletcrit (PCT), platelet volume distribution width (PDW), red blood cells (RBC), mean corpuscular volume (MCV), hematocrit (HCT), hemoglobin (HGP), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), white blood cells (WBC), lymphocytes (LYMF), granulocytes (GRAN), monocytes (MON), eosinophils (EOS). ICT Na, K, and Cl technique was used for the determination of sodium, potassium, and chloride (Abbott, Vienna, Austria), magnesium (Mg) and calcium (Ca) by the Alinity C Mg and Ca Reagent Kit (Abbott, Vienna, Austria).

Measurement of 25(OH)-Vitamin D

25(OH)-Vitamin D direct day was intended for the quantitative determination of the 25-OH-vitamin D in serum and fresh plasma (Immundiagnostik AG, Bensheim, Germany). The assay utilizes a competitive ELISA technique with a selected monoclonal antibody recognizing 25(OH)-vitamin D. For a reliable determination of 25(OH)-vitamin D, it was necessary to release it from the 25(OH)-vitamin D-VDBP-complex. Standards, controls, and mice samples, which were assayed

for 25(OH)-vitamin D, were pre-diluted with the releasing reagent and transferred to the microplate coated with 25(OH)-vitamin D. After an incubation to release the 25(OH) vitamin D, an anti-25(OH)-vitamin D antibody was added. After washing the microtiter plate, a peroxidase-conjugated antibody was added into each microplate well. A complex of 25(OH)-vitamin D/anti-25(OH)-vitamin D antibody/peroxidase conjugate was formed. Tetramethylbenzidine (TMB) was used as a peroxidase substrate. Finally, an acidic stop solution was added to terminate the reaction, whereby the color changes from blue to yellow. The intensity of the yellow color was inversely proportional to the concentration of 25(OH)-vitamin D. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration was generated using the values obtained from the standard. 25(OH)-vitamin D in the samples was determined from this curve.

Measurement of CRP

CRP ELISA Kit was intended for the quantitative determination of the CRP in serum (Immundiagnostik AG, Bensheim, Germany).

This enzymatic immunoassay is a sandwich assay for the determination of CRP in serum, plasma, urine, and stool samples. The wells of the microtiter plate were coated with polyclonal antibodies directed against C-reactive protein. In a first incubation step, the CRP in the mice sera were bound to the coated polyclonal rabbit antibodies. To remove all unbound substances, a washing step was carried out. In a second incubation step, a peroxidase-labeled antibody (polyclonal, rabbit-anti-CRP) was added. After another washing step the solid phase was incubated with the substrate, tetramethylbenzidine. An acidic stopping solution was then added. The color converted to yellow. The intensity of the yellow color was directly proportional to the concentration of CRP in the sample. A dose response curve of the absorbance (at 450 nm) unit vs. concentration was generated. CRP, present in the mice samples, was determined directly from this calibration curve.

The combination of two specific antibodies in the CRP ELISA drastically reduced the possibility of wrong-negative results and offers a secure diagnostic system to the user.

Statistics

Statistical analyses were performed using IBM SPSS Statistics 24.0. Data are presented as means with standard deviation. Wilcoxon-Mann-Whitney-Test ("*U* test") was used for intra-group comparison. The non-parametric Kruskal-Wallis test ("H-test") was used to compare the differences between the treatment groups.

RESULTS

Comparison of red and white blood panels between the sham, ImmunoD, and PolyNac groups, prior to and following the 5-week period of injections.

Red and white cell panels were measured (Table 1) before and after the 5 weeks of iv. injections of isotonic (sham group), PolyNac (group A), and ImmunoD (group B). There was no significant difference between all three groups at the beginning of the study in PLT, MPV, PCT, PDW, RBS, MCV, HCT, HGB, MCH, MCHC, RDW, WBC, LYMF, GRAN, MON, LYN, GRA, MON, and EOS. Furthermore, after 5 weeks, no significant differences were observed between all three groups in PLT, MPV, PCT, PDW, RBS, MCV, HCT, HGB, MCH, MCHC, RDW, WBC, LYMF, GRAN, MON, LYN, GRA, MON, and EOS.

Comparing time-dependent effects in each group before and after therapies, we could see significant differences in WBC, LYN, and GRA of ImmunoD and PolyNac treated mice, but not in the sham group: Figure 1A shows a significant reduction between WBCs before and after PolyNac treated mice ($8.4 \pm 1.9 \times 10^9$/L vs. $2.8 \pm 1.2 \cdot \times 10^9$/L; $p = 0.018$) and ImmunoD® treated mice ($8.4 \pm 2.3 \times 10^9$/L vs. $3.0 \pm 1.2 \cdot \times 10^9$/L; $p = 0.018$). Lymphocytes (LYMF) decreased significantly before and after PolyNac ($6.6 \pm 1.6 \times 10^9$/L vs. $2.0 \pm 0.9 \times 10^9$/L; $p = 0.018$) and ImmunoD treatment ($6.6 \pm 1.7 \times 10^9$/L vs. $2.1 \pm 0.7 \times 10^9$/L; $p = 0.018$). Figure 1C shows a significant reduction between RBC before and after PolyNac treated mice ($9.8 \pm 0.7 \times 10^{12}$/L vs. $9.1 \pm 0.6 \times 10^{12}$/L; $p = 0.018$) and ImmunoD® treated mice ($9.8 \pm 0.5 \times 10^{12}$/L vs. $9.2 \pm 0.4 \times 10^{12}$/L; $p = 0.018$). Granulocytes (GRAN) decreased significantly before and after PolyNac ($1.6 \pm 0.4 \times 10^9$/L vs. $0.7 \pm 0.2 \times 10^9$/L; $p = 0.043$) and before and after ImmunoD treatment ($1.6 \pm 0.5 \times 10^9$/L vs. $0.8 \pm 0.3 \times 10^9$/L; $p = 0.028$). It should be pointed out that all reductions were within the normal range.

Vitamin D measurements

The vitamin D_3 level of the PolyNac injected mice was significantly increased (26.6 ± 8.8 ng/mL) as compared to the sham group (3.1 ± 0.9 ng/mL; $p = 0.001$) and the ImmunoD group (7.0 ± 3.6 ng/mL; $p = 0.003$) (Figure 2). Additionally, there was a significant difference in favor of the vitamin D_3 complex (7.0 ± 3.6 ng/mL) as compared to the sham group (3.1 ± 0.9 ng/mL; $p = 0.03$).

Electrolytes and CRP measurements

To see if vitamin D complexes or isotonic saline influence the amount of plasma electrolytes or CRP in any one of the groups, we measured them in plasma following 5 weeks of injections. There were no significant differences between all three groups in the content of sodium, potassium, magnesium, calcium, and chloride (Table 2). Additionally, no significant differences in the CRP concentration were found between the sham, Poly-

Table 1. Measurement of red and white blood cell panels before and after 5 weeks iv. injection of isotonic saline (sham group, n = 4), PolyNac (n = 7), and cholecalciferol-dimer (ImmunoD®), (n = 7). PLT = platelets, MPV = mean platelet volume, PCT = platelectric, PDW = platelet volume distribution width, RBC = red blood cells, MCV = mean corpuscular volume, HCT = hematocrit, HGB = hemoglobin, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, RDW = red distribution with, WBC = white blood cells, LYMF = lymphocytes, GRAN = granulocytes, MON = monocytes, EOS = eosinophils.

	Study begin			Study end (5 weeks)			Reference range
	Sham (n = 4)	PolyNac (n = 7)	Immuno D (n = 7)	Sham (n = 4)	PolyNac (n = 7)	Immuno D (n = 7)	
PLT	1,274 +/- 397	1,290.2 +/- 274	1,310.1 +/- 399	833 +/- 271	866.2 +/- 423	1,165.5 +/- 188	450 - 1,590 G/L
MPV	5.8 +/- 1.0	5.4 +/- 0.7	5.4 +/- 0.5	4.7 +/- 0.4	4.7 +/- 0.8	4.4 +/- 0.2	3.8 - 6.0 %
PCT	0.3 +/- 0.4	0.4 +/- 0.3	0.4 +/- 0.3	0.4 +/- 0.1	0.4 +/- 0.2	0.5 +/- 0.1	0.2 - 0.25 %
PDW	17.8 +/- 0.9	17.5 +/- 0.9	17.5 +/- 0.7	16.6 +/- 0.3	16.5 +/- 0.6	16.5 +/- 0.2	
RBC	10.2 +/- 0.4	9.8 +/- 0.7	9.8 +/- 0.5	8.8 +/- 0.2	9.1 +/- 0.6	9.2 +/- 0.4	6.36 - 9.42 fL
MCV	53.2 +/- 1.6	46.2 +/- 1.8	46.2 +/- 2.3	50.2 +/- 1.2	49.1 +/- 2.2	49.5 +/- 1.3	48.2 - 58.3 %
HCT	54.3 +/- 2.6	52.0 +/- 3.0	52.0 +/- 2.6	45.2 +/- 1.4	45.1 +/- 2.7	45.9 +/- 2.0	34.6 - 44.6 g/dL
HGB	17.1 +/- 0.7	16.5 +/- 1.0	16.5 +/- 0.8	14.2 +/- 0.3	14.4 +/- 0.8	14.7 +/- 0.8	11.0 - 14.3 pg
MCH	16.8 +/- 0.4	16.8 +/- 0.8	16.8 +/- 0.6	16.1 +/- 0.4	15.8 +/- 0.6	15.9 +/- 0.6	15.8 - 19.0 g/dL
MCHC	31.6 +/- 0.5	31.9 +/- 0.5	31.9 +/- 0.7	32.2 +/- 0.3	31.8 +/- 0.5	32.1 +/- 0.5	30.0 - 35.0 g/dL
RDW	14.7 +/- 1.7	15.1 +/- 0.8	15.1 +/- 1.3	13.5 +/- 2.1	14.0 +/- 1.3	13.1 +/- 1.4	13.0 - 17.0 G/L
WBC	5.5 +/- 1.8	8.4 +/- 1.9	8.4 +/- 2.3	2.9 +/- 1.5	2.8 +/- 1.2	3.0 +/- 1.0	0.8 - 6.8 G/L
LYMF	4.6 +/- 1.3	6.6 +/- 1.6	6.6 +/- 1.7	1.9 +/- 1.2	2.0 +/- 0.9	2.1 +/- 0.7	0.7 - 5.7 G/L
GRAN	1.3 +/- 0.3	1.6 +/- 0.4	1.6 +/- 0.5	0.9 +/- 0.2	0.7 +/- 0.2	0.8 +/- 0.3	0.1 - 1.8 G/L
MON	0.2 +/- 0.1	0.2 +/- 0.1	0.2 +/- 0.1	0.1 +/- 0.1	0.1 +/- 0.1	0.1 +/- 0.1	0.0 - 0.0 %
LYN	75.7 +/- 4.4	78.6 +/- 4.8	78.6 +/- 2.9	63.0 +/- 7.4	70.8 +/- 3.2 *	68.9 +/- 7.2+	55.8 - 90.6 %
GRA	21.6 +/- 2.9	19.2 +/- 4.6	19.2 +/- 2.6	31.6 +/- 6.2	25.7 +/- 2.9 *	27.0 +/- 6.2+	8.6 - 38.9 %
MON	2.8 +/- 1.5	2.1 +/- 0.4	2.1 +/- 0.5	5.4 +/- 1.4	3.5 +/- 0.4 *	4.2 +/- 1.1 +	1.8 - 6.0 %
EOS	0.0 +/- 0.0	0.0 +/- 0.0	0.0 +/- 0.0	0.0 +/- 0.0	0.0 +/- 0.0	0.0 +/- 0.0	0.0 - 0.0 %

*** - Significance before and after 5 weeks in the PolyNac Group (Kruskal Wallis Test) p < 0.05. + - Significance before and after 5 weeks in the ImmunoD Group (Kruskal-Wallis Test) p < 0.05.**

Nac and ImmunoD group. All measured CRP levels were within the normal range (0 - 5 mg/mL).

DISCUSSION

Vitamin D obviously plays an important role in cancer development and defense against various cancer entities like breast cancer [1-4,7-14], colon cancer [15], prostate cancer [16-18] or melanoma [19-21].

The most described form of vitamin D in cancer is 25-hydroxy-vitamin D also called calcidiol. It is a prehormone that is produced in the liver by hydroxylation of vitamin D_3 (cholecalciferol) by the enzyme cholecalciferol 25-hydroxylase [22,23]. Physicians worldwide measure this metabolite to determine a patient's vitamin D status [23]. At a typical daily intake of vitamin D_3, its full conversion to calcifediol takes approximately 7 days [24].

Unfortunately, the bioavailability of this vitamin D form is very low and because of its hydrophobic reaction, up to now, not available for intra-venous injection. There also is increasing evidence in the literature that calcitriol possesses anticancer properties. This has been demonstrated in both human and animal subjects. Several mechanisms for the antitumor activities of calcitriol have been postulated, including cell cycle arrest, induction of apoptosis, and inhibition of angiogenesis via anti-vascular endothelial growth factor actions [25,26]. It is well known that calcitriol induces toxicity in both human and animal subjects, which is particularly evident at higher doses. Some of the calcitriol-associated toxicities in humans are hypercalcemia; hyperphosphatemia; primary renal failure; pancreatitis; and calcifica-

Table 2. Estimation of electrolytes and CRP concentrations in plasma of isotonic saline (sham, n = 4), Poly-Nac (n = 7), and ImmunoD® (n = 7) treated mice.

	Sham (n = 4)	PolyNac (n = 7)	ImmunoD (n = 7)
Na [mM]	154.3 +/- 1.0	152.4 +/- 4.5	152.4 +/- 2.4
K [mM]	5.5 +/- 1.0	6.4 +/- 1.2	4.5 +/- 0.1
Ca [mM]	2.17 +/ 0.14	2.10 +/- 0.16	1.86 +/- 0.22
Mg [mM]	0.98 +/- 0.05	0.97 +/- 0.11	1.03 +/- 0.05
Cl [mM]	117.0 +/- 0.8	118.9 +/- 3.9	118.0 +/- 0.8
CRP [mg/mL]	0.17 +/- 0.05	0.14 +/- 0.06	0.15 +/- 0.07

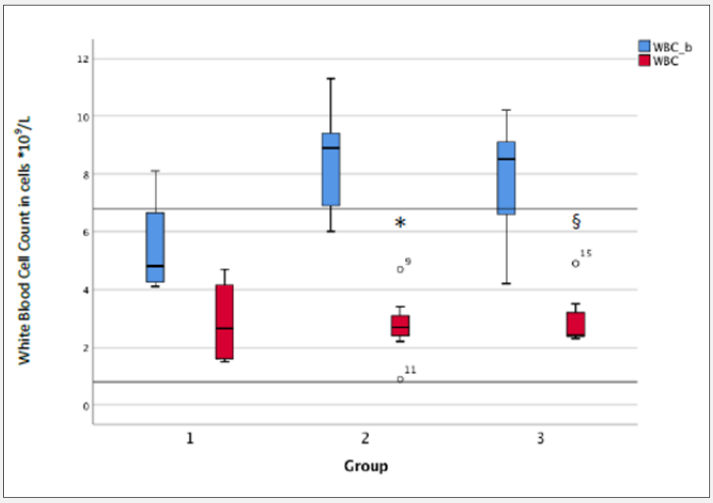

Figure 1A. Comparison of white blood cell count between sham (1), PolyNac (2), and ImmunoD before (WBC_b) and after 5 weeks (WBC). * Significance between PolyNac before and after study: p = 0.018. § Significance between ImmunoD before and after study: p = 0.018.

tion of the kidney, aorta, heart, and lung [27-29]. In mice, calcitriol and its derivatives are known to induce hypercalcemia, kidney calcifications, weight loss, lethargy, and death [30,31].

In early studies of vitamin D analogs, aiming to treat cancer in animals, a large proportion of mice died because of the toxic effects of calcitriol. To avoid or decrease such calcitriol-induced mortality in mice due to toxicity, a common practice for investigators was administering less than the target doses or even skipping doses. The same problem is likely to exist with other drugs as well (e.g., chemotherapeutic agents) [32]. It has long been assumed that vitamin D intestinal absorption is a passive process, but new data show that it is actually far more complex than previously thought [33]. Reboul et al. have described the fate of vitamin D in the human upper gastrointestinal lumen during digestion and focused on the proteins involved in the intestinal membrane and cellular transport of vitamin D across the enterocyte.

Although recent data have significantly improved our understanding of vitamin D intestinal absorption, further studies are needed to increase our knowledge of the molecular mechanisms underlying this phenomenon [33].

Therefore, an administration of vitamin D in case of vitamin D deficiency seems to be essential, whereas the precise mechanism of vitamin D administration is not completely known.

To avoid the negative side effects of iv. administration

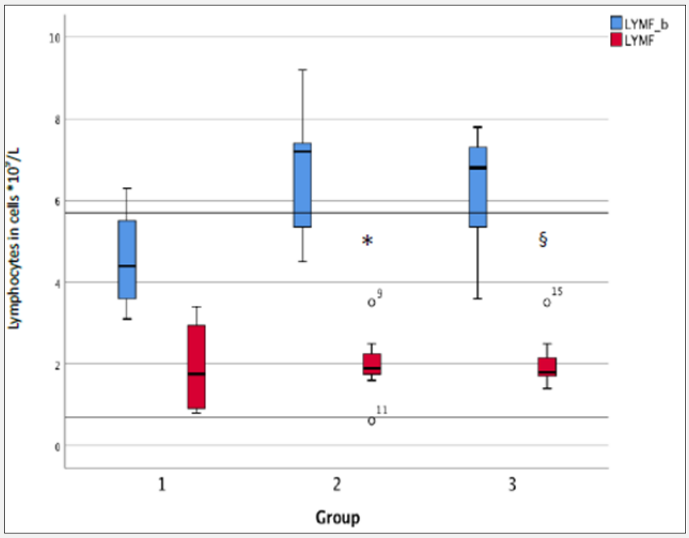

Figure 1B. Comparison of lymphocyte count between sham (1), PolyNac (2), and ImmunoD before (LYMF_b) and after 5 weeks (LYMF). * Significance between PolyNac before and after study: p = 0.018. § Significance between ImmunoD before and after study: p = 0.018.

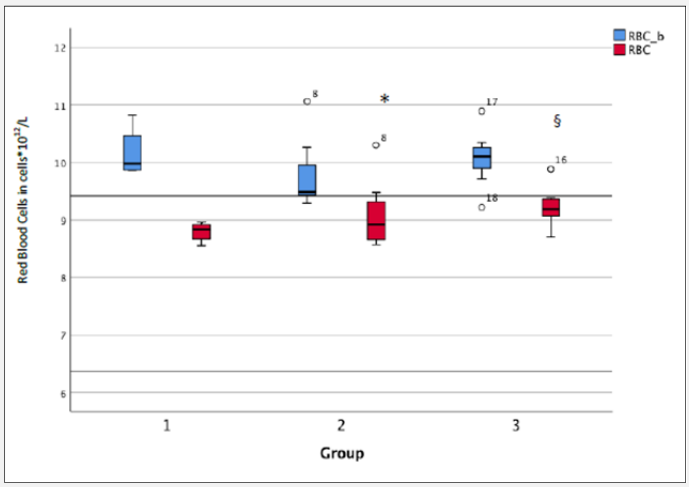

Figure 1C. Comparison of red blood cell count between sham (1), PolyNac (2), and ImmunoD before (WBC_b) and after 5 weeks (WBC). *Significance between PolyNac before and after study: p = 0.018. § Significance between ImmunoD before and after study: p = 0.018.

of calcitriol and to supplement the body with a significant amount of usable vitamin D, we have recently developed a protein derived vitamin D transport mechanism to the body [34]. We developed a vitamin D-protein complexed dimer for better administration and better availability, especially in case of vitamin D deficien-

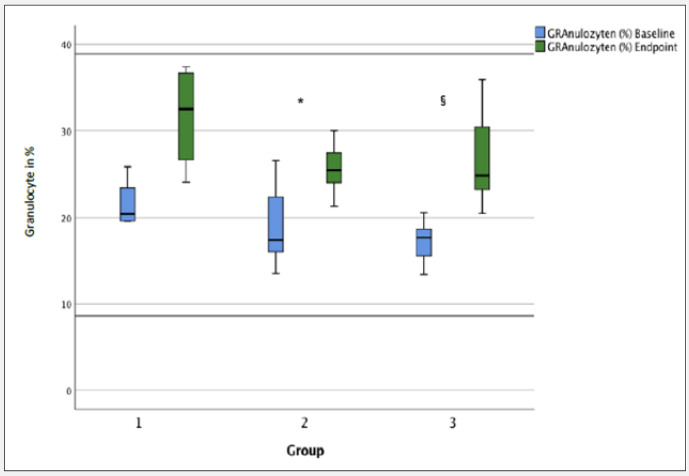

Figure 1D. Comparison of granulocyte count between sham (1), PolyNac (2), and ImmunoD before (GRAnulozyten_b) and after 5 weeks (GRAnulozyten).

[*] Significance between PolyNac before and after study: p = 0.028. [§] Significance between ImmunoD before and after study: p = 0.043.

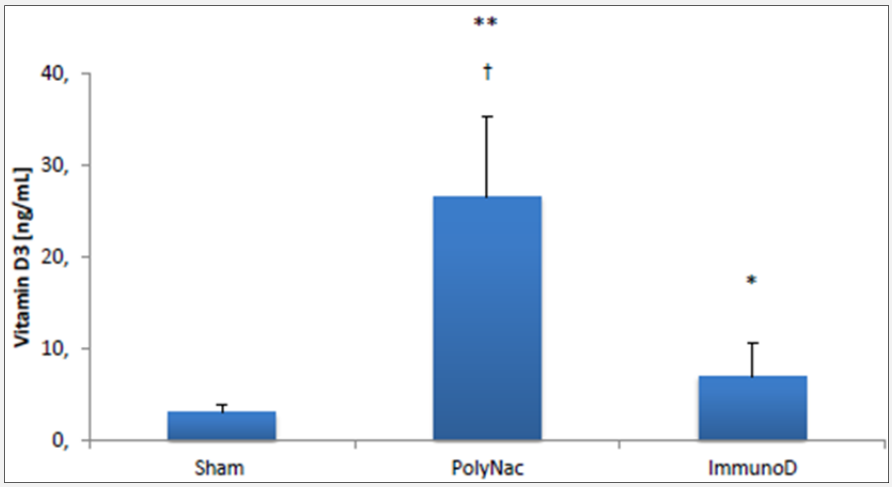

Figure 2. Comparison of vitamin D level (ng/mL) after iv. injection of 5 weeks in the sham, ImmunoD, and PolyNac treated mice.

[*] - Significance between ImmunoD and the sham group: p = 0.03, [**] - Significance between PolyNac and sham group: p = 0.001, Significance between PolyNac and ImmunoD: [†] - p = 0.007.

cies and higher vitamin D consumption [35].

To achieve a sufficient Vitamin D supplementation without the known side effects, we developed the dimer ImmunoD, which has been proven to be effective, among others, e.g. in autistic children under oral supplementation [35].

To ensure higher bioavailability, we performed this study to prove the effectiveness and safety of this newly developed dimer under intra-venous injection.
ImmunoD-Vitamin D dimer is a combination of cholecalciferol with a recombinantly produced protein deriving from GC-protein. Additionally, PolyNac is an equivalent dimer, enhanced with additional poly-N-acetyl-galactosamine to avoid early inactivation via proteins like nagalase.
Previous pre-clinical toxicology studies of human Gc globulin in mice, rats, guinea pigs, rabbits, and Shetland ponies showed no toxic effects after injection. Therefore, Phil et al. concluded that the safety profile of Gc globulin appears to be consistent to that required for use in humans [36].
To prove the safety of Cholecalciferol supplementation via ImmunoD and PolyNac injection as a newly developed dimer including a protein carrier, we performed a placebo-controlled murine study with either substance. After five weeks of bi-weekly injection, no side effects could be observed in either group, especially any signs of hypervitaminosis. At the end of the study, all blood results were within the normal range. Animals in the treatment groups showed a significant elevation of vitamin D. In the ImmunoD group the vitamin D level was double the value of the control group, the PolyNac group showed a value of eight times higher than control.

CONCLUSION

Therefore, we conclude that this newly developed complexed cholecalciferol is a safe and highly effective method of oral and iv. substitution of vitamin D. Further investigations have to be performed to verify the value in treatment of vitamin D deficiency related diseases.

Declaration of Interest:

R.H. is CEO of HG Pharma, which supported this study, and J.F. G. and M.G. are partial owners of HG Pharma.

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