Synopsis for Clinical Study Report Final Version 1.0, from February 13th, 2019

Synopsis Version Final 1.1

Effects of Mg-orotate on cardiocirculatory performance and adaptations at the muscular level: A double-blind, randomized, explorative, placebo-controlled, cross-over pilot study

Originaltitel: *Einfluss einer Mg-Orotat-Gabe auf die kardiozirkulatorische Leistungsfähigkeit, die muskuläre Konzentration von Phosphokreatin und die Adaptation auf muskelzellulärer Ebene: Eine doppelblinde, randomisierte, explorative placebo-kontrollierte, cross-over Pilotstudie Verum versus Placebo über 3 Monate*

EudraCT Nr.: 2013-003418-42
Study-ID: WOE-2013-TUE

Phase II

Sponsor:
Wörwag Pharma GmbH & Co. KG

Study Site:
Universitätsklinikum Tübingen, Abteilung Sportmedizin

CONFIDENTIAL

Change history:

Version 1.1. 23.06.2020	Completion of site address, remove of title page, inclusion of lot numbers for IMP and information about amendments. Information was part of trial report V1.0 13.02.2019, therefore report didn't change.

SYNOPSIS

NAME OF SPONSOR Wörwag Pharma GmbH & Co. KG **NAME OF FINISHED PRODUCT** *magnerot*® Classic N **NAME OF ACTIVE INGREDIENT(S)** Magnesium orotate dihydrate	**INDIVIDUAL STUDY TABLE REFERRING TO MODULE 5 OF THE CTD** Volume: Not applicable Page: Not applicable	**(FOR NATIONAL AUTHORITY USE ONLY)**

Title of Study	Effects of Mg-orotate on cardiocirculatory performance and adaptations at the muscular level: A double-blind, randomized, explorative, placebo-controlled, cross-over pilot study Originaltitel: *Einfluss einer Mg-Orotat-Gabe auf die kardiozirkulatorische Leistungsfähigkeit, die muskuläre Konzentration von Phosphokreatin und die Adaptation auf muskelzellulärer Ebene: Eine doppelblinde, randomisierte, explorative placebokontrollierte, cross-over Pilotstudie; Verum versus Placebo über 3 Monate*		
Investigator(s)	[redacted]		
Study centre(s)	University Hospital Tübingen, Department of Sports Medicine [redacted] 72076 Tübingen Germany		
Publication	N/A		
Study period	From: September 2016 To: February 2018	Phase of development	Phase II – pilot study
Objectives	Primary Objective: Change of maximum oxygen uptake after 4 weeks of treatment in comparison to placebo Secondary Objective: • Change of oxygen uptake at the anaerobic threshold after 4 weeks of treatment in comparison to placebo • Change of lactate threshold after 4 weeks of treatment in comparison to placebo • Change of maximum power after 4 weeks of treatment in comparison to placebo • Change of muscular mRNA parameters after 4 weeks of treatment in comparison to placebo		
Methodology	Spiroergometry, mRNA analysis of muscle biopsy		

Number of subjects	Planned: 12 Analysed: 12

NAME OF SPONSOR Wörwag Pharma GmbH & Co. KG **NAME OF FINISHED PRODUCT** *magnerot*® Classic N **NAME OF ACTIVE INGREDIENT(S)** Magnesium orotate dihydrate	**INDIVIDUAL STUDY TABLE REFERRING TO MODULE 5 OF THE CTD** Volume: Not applicable Page: Not applicable	**(FOR NATIONAL AUTHORITY USE ONLY)**

Diagnosis and main criteria for inclusion	Healthy male volunteers between 18 and 35 years of age. No intake of concomitant medication and without clinically relevant laboratory parameters.	
Test product, dose and mode of administration	3x 1500 mg per day oral intake of Magnerot Classic N (Magnesium orotate dihydrate; LotNo: 14D057)) or placebo (LotNo: 00710301) in randomised order.	
Duration of treatment	4 weeks	
Criteria for evaluation	Primary:	
	$\Delta VO2max_{0-1}$	Change of maximum oxygen uptake (VO2max) from start to end of period (period 1: V4-V2; period 2: V8-V6)
	Secondary:	
	ΔAT_{0-1}	Change of oxygen uptake (VO2) at anaerobic threshold (AT) from start to end of period (period 1: V4-V2; period 2: V8-V6)
	ΔLT_{0-1}	Change of lactate threshold (LT) from start to end of period (period 1: V4-V2; period 2: V8-V6)
	$\Delta Pmax_{0-1}$	Change of maximal power (Pmax) from start to end of period (period 1: V4-V2; period 2: V8-V6)
	FC $MSTN_{0-1}$	Change of muscular myostatin mRNA from start to end of period (period 1: V4-V2; period 2: V8-V6)
	FC $LDHB_{0-1}$	Change of muscular Lactate Dehydrogenase mRNA from start to end of period (period 1: V4-V2; period 2: V8-V6)
	FC $TRIM63_{0-1}$	Change of muscular Tripartite Motif Containing 63 / muscle-specific RING Finger Protein 1 mRNA from start to end of period (period 1: V4-V2; period 2: V8-V6)
	FC $FBXO32_{0-1}$	Change of muscular F-Box Protein 32 / Atrogin-1 mRNA from start to end of period (period 1: V4-V2; period 2: V8-V6)

	FC $ABRA_{0-1}$	Change of muscular Actin Binding Rho Activating Protein mRNA / STARS from start to end of period (period 1: V4-V2; period 2: V8-V6)
	FC LPL0-1	Change of muscular Lipoprotein Lipase mRNA from start to end of period (period 1: V4-V2; period 2: V8-V6)

NAME OF SPONSOR Wörwag Pharma GmbH & Co. KG **NAME OF FINISHED PRODUCT** *magnerot*® Classic N **NAME OF ACTIVE INGREDIENT(S)** Magnesium orotate dihydrate	**INDIVIDUAL STUDY TABLE REFERRING TO MODULE 5 OF THE CTD** Volume: Not applicable Page: Not applicable	**(FOR NATIONAL AUTHORITY USE ONLY)**

	FC $COX4I1_{0-1}$	Change of muscular Cytochrome C Oxidase Subunit 4I1 mRNA from start to end of period (period 1: V4-V2; period 2: V8-V6)
	FC $MYL3_{0-1}$	Change of muscular Myosin Light Chain 3 mRNA from start to end of period (period 1: V4-V2; period 2: V8-V6)
	FC $NDUFB8_{0-1}$	Change of muscular NADH:Ubiquinone Oxidoreductase Subunit B8 mRNA from start to end of period (period 1: V4-V2; period 2: V8-V6)
	FC $GPT2_{0-1}$	Change of muscular Glutamic-Pyruvic Transaminase 2 from start to end of period (period 1: V4-V2; period 2: V8-V6)
	FC $SLC41A1_{0-1}$	Change of muscular mRNA Solute Carrier Family 41 (Magnesium Transporter), Member 1 mRNA from start to end of period (period 1: V4-V2; period 2: V8-V6)
	FC $PRKAG3_{0-1}$	Change of muscular Protein Kinase AMP-Activated Non-Catalytic Subunit Gamma 3 mRNA from start to end of period (period 1: V4-V2; period 2: V8-V6)
	FC $VEGFA_{0-1}$	Change of muscular Vascular Endothelial Growth Factor A mRNA from start to end of period (period 1: V4-V2; period 2: V8-V6)
	FC $UCP3_{0-1}$	Change of muscular Uncoupling Protein 3 mRNA from start to end of period (period 1: V4-V2; period 2: V8-V6)
	FC $PHKA1_{0-1}$	Change of muscular Phosphorylase Kinase Regulatory Subunit Alpha 1 mRNA from start to end of period (period 1: V4-V2; period 2: V8-V6)
	FC $PPP1R3A_{0-1}$	Change of muscular Protein Phosphatase 1 Regulatory Subunit 3A mRNA from start to end of period (period 1: V4-V2; period 2: V8-V6)

NAME OF SPONSOR	**INDIVIDUAL STUDY TABLE REFERRING TO MODULE 5 OF THE CTD**	**(FOR NATIONAL AUTHORITY USE ONLY)**
Wörwag Pharma GmbH & Co. KG **NAME OF FINISHED PRODUCT** *magnerot*® Classic N **NAME OF ACTIVE INGREDIENT(S)** Magnesium orotate dihydrate	Volume: Not applicable Page: Not applicable	

Statistical methods	3-way variance analysis with fixed effects for medication and training and random effects for subjects. Subject and medication effects are combined. Cross-over design is considered by using intra-individual differences. Period effect and cross-over effects are checked. All parameters are described with descriptive statistics (sample size, absolute frequency, relative frequency, maximum, minimum, median, mean, standard deviation, 95% confidence interval).

SUMMARY CONCLUSIONS

EFFICACY RESULTS

Primary endpoint: Maximal oxygen uptake (VO2max) was increased in the training group by a mean of 2.25 (95% CI 4.82 - -0.32) mL/min/kg body weight (range -0.5 to 6.5), whereas VO2max was slightly reduced in the sedentary group (mean change -0.50 (95% CI 3.03 - -4.03) mL/min/kg body weight, range -4.5 to 5.0). The impact of training on the changes of VO2max was statistically significant (p=0.0394; one sided). The administration of Mg-orotate had no significant impact on the changes of VO2max (p=0.337). No interaction between medication and training could be proven (p=0.203).

Secondary endpoints: Oxygen uptake (VO2) at the anaerobic threshold (AT) was increased in the training group by a mean of 1.0 (95% CI 4.74 - -2.74) mL/min/kg body weight (range -2.0 to 8.0), whereas VO2 at the AT was slightly reduced in the sedentary group (mean change -0.33 (95% CI 1.14 - -1.81) mL/min/kg body weight, range -2.0 to 1.0). However, the impact of training on the changes of VO2 at the AT was statistically not significant (p=0.4588).

The same effect could be shown for the administration of Mg-orotate, which increased VO2 at the AT by a mean of 1.0 (95% CI 3.48 - -1.48) mL/min/kg body weight (range -4.0 to 9.0), whereas VO2 at the AT was slightly reduced in the placebo group (mean change -0.33 (95% CI 2.41 - -3.07) mL/min/kg body weight, range -7.0 to 7.0), but also without reaching significance (p=0.4588). No interaction between medication and training could be proven (p=0.7081).

Lactate threshold (LT) was increased in the training group by a mean of 0.1 (95% CI 0.20 – 0.00) W/kg body weight (range -0.03 to 0.25), whereas LT was slightly reduced in the sedentary group (mean change -0.02 (95% CI 0.03 - -0.07) W/kg body weight, range -0.09 to 0.06). Even if changes were very small, training showed impact on LT by trend (p=0.0867).

Subjects under Mg-orotate showed a slight reduction of LT -0.03 (95% CI 0.04 - -0.10) W/kg body weight. Under placebo, LT was improved by 0.12 (95% CI 0.24 - -0.01) W/kg body weight, primarily caused by 2 outliers with extremely high, unexplainable increases during placebo intake. Due to that, the product effect

reached significance, with superiority of placebo (p=0.0408). No interaction between medication and training could be proven (p=0.4771).

Maximum power (work load) (Pmax) was increased in the training group by a mean of 26.17 (95% CI 39.25 - 13.08) W (range 10.5 to 42.0), whereas Pmax was relatively stable in the sedentary group (mean change 1.75 (95% CI 11.33 - -7.83) W, range -12.5 to -12.5). The impact of training on the changes of Pmax was statistically significant (p=0.0011).

The change under placebo and under Mg-orotate were comparable with +16.75 (95% CI 27.98 – 5.52) W and +11.17 (95% CI 23.12 - -0.79) W, respectively. The product effect was not significant (p=0.3215). No interaction between medication and training could be proven (p=0.2504).

With regard to muscular mRNA parameters, upon training, there was a significant upregulation of the *LDHB* gene, encoding the H subunit of lactate dehydrogenase, which is associated with an increased oxidative metabolism. This effect was observed both with and without Mg-orotate. There was no additive effect of Mg-orotate (p=0.6062) or an interaction between the effects of training and medication (p=0.3235). Furthermore, a significant downregulation of the mRNA encoded by the *MSTN* (myostatin) gene could be observed upon training with and without medication and there was a close-to-significant tendency towards a further, additive decrease with medication (p=0.107). Similar tendencies were observed for the *TRIM63* and the *FBXO32* genes, encoding so-called “atrogenes” involved in muscle decay and cachexia. There were no significant effects on expression of the *ABRA* (actin binding rho activating protein), *LPL* (lipoprotein lipase), *COX4I1* (cytochrome C oxidase subunit 4I1), *MYL3* (myosin light chain 3), *NDUFB8* (NADH ubiquinone oxidoreductase subunit B8), GPT2 (glutamic pyruvate transaminase 2), *SLC41A1* (solute carrier family member 1), *PRKAG3* (protein kinase AMP-activated and non-catalytic subunit gamma 3), *VEGFA* (vascular endothelial growth factor A), *UCP3* (uncoupling protein 3), *PHKA1* (phosphorylase kinase regulatory subunit alpha 1), or *PPP1R3A* (protein phosphatase 1 regulatory subunit 3A) genes.

SAFETY RESULTS

During study conduct, of 14 randomized subjects, 6 randomized persons experienced at least one adverse event (AE). In total, 10 adverse events occurred during this study. All of them were temporary, not severe, and not related to IMP intake (relatedness). There were no haematological or other laboratory abnormalities or any adverse events that led to an intervention, including withdrawal of drug treatment, dose reduction or significant additional concomitant therapy. No serious adverse events were reported. In line with these results, no negative clinically relevant effects on vital signs or any safety relevant laboratory parameter were identified for the treatment with magnesium orotate dihydrate. As a result of this observations, the intake of magnesium orotate dihydrate could not be associated with any unknown health risks.

CONCLUSION

Although in this pilot study, no significant impact of Mg-orotate was observed in terms of VO2max, VO2 at the AT or LT, these data suggest potential beneficial effects of Mg-orotate on skeletal muscle training adaptation. However, since adaptation reactions at the cellular level usually precede physiological adaptation, further studies with more extended time frames are required. In addition, in the future, it will be interesting to determine the effects of Mg-orotate in connection with training in pathological situations, specifically in patients with chronic and lifestyle-related diseases.

According to the safety relevant data collected in this clinical trial, *Magnerot*® CLASSIC N 500, containing magnesium orotate dihydrate as active pharmaceutical ingredient, continues to be considered a safe medicinal product, even in higher doses as used in this study.

Amendments to the protocol:
Amendment 1; 08.05.2015: Investigator Change

DATE OF THE REPORT: February 13th, 2019
Date of Synopsis Version 1.1: 23.06.2020