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Safety, Tolerability and Pharmacokinetics of Sodium Tungstate (OXO-001) in Healthy Female Volunteers of Childbearing Age: A Randomized, Double-Blind, Dose-Finding, and Placebo-Controlled Phase I Study

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Abstract

Background: Phase I study to assess the effects of single oral doses of 100, 200, and 300 mg/day of sodium tungstate (OXO-001) in healthy women of childbearing age.

Materials and Methods: A randomized, double-blind, dose-finding, and placebo-controlled phase I study was conducted in healthy weight (body mass index [BMI] 18.5-24.9 kg/m2) and overweight (BMI 25 to ≥30 kg/m^2) volunteers who received OXO-001 or placebo during a menstrual cycle (maximum 28 days). Data recorded were adverse events (AEs), vital signs, electrocardiogram (ECG), laboratory tests, pharmacokinetics (PK) parameters, and transvaginal ultrasound.

Results: Thirty women were included in the safety analysis, and 29 completed the study. Thirty-eight treatment emergent adverse events (TEAEs) were reported by 20 participants (15 in the OXO-001 group and 5 in the placebo group). TEAEs were related to OXO-001 administration in 13.2, 10.5, and 15.8% of cases of the 100, 200, and 300 mg doses, respectively. None of the participants discontinued the study, and no serious AEs or deaths were recorded. Differences in TEAEs by BMI were not found. The PK profile showed a fast absorption rate and proportional increases of OXO-001 plasma concentration to increasing doses, suggesting linear PK, with higher concentrations in BMI <25 kg/m^2 group higher than in the ≥25 kg/m^2 group. There were no relevant changes in vital signs, ECG, ovarian follicle development, endometrial morphology, and laboratory tests before and after the administration of OXO-001 or placebo.

Conclusion: The administration of OXO-001 in volunteers of childbearing age was safe and well tolerated, with consistent PK linear profile within doses studied and without detrimental effect on endometrium or ovary-related variables, with similar effects in healthy weight and overweight participants. The maximum studied dose (300 mg/day) was safe and well tolerated. These data are sufficient to support further clinical trials (registration number: 2016-001276-30).

Keywords: Fertility, Pharmacokinetics, Phase I study, Safety, Sodium Tungstate

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Introduction

Female infertility, defined as the failure to achieve a clinical pregnancy due to women's causes, is present in around 70% of couples. According to the Global Burden Disease Study, female infertility was a prevalent disease that affected 43 to 83 million women, rising from 22.2 to 32.5% from 2007 to 2017 (1). The reasons for the increase in infertility are numerous including lifestyle and nutritional factors, delay of the first pregnancy until the third decade of life, sexually transmitted diseases, uterine organic diseases, prolonged exposure to environmental pollutants and chronic stress, and gonadotoxic oncological treatments in cancer survivors (2). Assisted reproduction techniques (ART) are the most common therapeutic approach to female infertility, with *in vitro* fertilization (IVF) accounting for more than 80% of the ART procedures. However, despite the advances in the last decades, the overall pregnancy rates and delivery rates of IVF procedures do not overcome 30% per started cycle (3). In women undergoing IVF, 65% of pregnancy losses are due to implantation failure and early pregnancy loss (4, 5) and unfortunately, there is no pharmacological intervention that enhances the implantation process.

Cytokines produced by maternal endometrium and their downstream signaling pathways appear to have a crucial role in the early and late implantation process (6-8). Leukemia inhibitory factor (LIF) or leptin, among other cytokines, and the signaling transducer and activator of transcription 3 (STAT-3), have been shown to be essential for embryo implantation. In experimental models with deficiencies in any of these molecules, implantation incompetence has been reported (9-11).

Sodium tungstate, an inorganic salt, and a phosphatase inhibitor, also named OXO-001, enhances cytokine effects in several models (12) and modulates hypothalamic gene expression increasing the activity of several kinases and proteins involved in the leptin signaling system, such as the Janus Kinase 2 (JAK-2)/STAT-3 signaling pathway (13). Our group showed for the first time that OXO-001 acts on the reproductive system to restore and improve fertility in non-diabetic infertile mammals. In an insulin receptor substrate 2 (IRS2) knock-out female mice, a model used for the study of infertility associated with polycystic ovary syndrome (PCOS) (14), OXO-001restored the estrous cycle and achieved high fertility rates with a direct effect on embryo implantation process independently of insulin-deficient states (15). These results solve the doubts of previous studies in female diabetic rats about whether the partially reversed infertility was a direct OXO-001 effect on the reproductive system or partial restoration of the diabetic state (16).

Thus, the potential effect of OXO-001 on fertility seems reasonable and aims for further analysis in human fertility, advancing first to ensure safety in women of childbearing age. Previous phase I studies (maximum tolerated dose, tolerability, and pharmacokinetics [PK] single dose and tolerability and PK multiple dose) and a phase II (proof of concept in human obesity) clinical trial conducted in males and postmenopausal women have shown good safety and tolerability of OXO-001 with doses up to 300 mg/day (17, 18). However, there is no experience in women of childbearing age. Therefore, a phase I clinical trial was conducted to confirm the safety and tolerability of OXO-001 and to characterize the PK profile and pharmacodynamic effects of different doses of OXO-001 in normal weight and high weight healthy women in their fertile age.

Materials and Methods

Design and participants

Between September 2016 and August 2017, a randomized, double-blind, dose-finding, and placebo-controlled phase I study was designed, the main objective of which was to assess the safety profile and tolerability of three orally administered doses (100, 200, and 300 mg/day) of OXO-001 during one menstrual cycle in normal weight and high weight healthy female volunteers of childbearing age. Secondary objectives were to define the PK profile and pharmacodynamic effects of the three doses of OXO-001 and to determine the optimal dose based on the risk-benefit ratio of the different dose levels.

Inclusion criteria were as follows: age ≥18 and ≤45 years; body mass index (BMI) ≥18 and ≤25 kg/m^2 (normal weight) and ≥25 and ≤50 kg/m^2 (high weight) with weight stability in the previous 3 months; regular menstrual cycles (21-35 days, bleeding duration <8 days); normal gynecological findings on transvaginal ultrasound (TVUS) and ovarian reserve (antral follicle count >5); unrevealing medical history and physical examination; normal results of standard laboratory tests; unintended pregnancy during the study and 1 month later; use of a barrier method of contraception; and signed informed consent. Exclusion criteria were pregnant, lactating or postmenopausal women; ovulatory dysfunction; recurrent miscarriage or abortion within the previous year; BMI outside the range of the inclusion criteria; active smoking; alcohol use > 24 g/day; any medical diseases or abnormalities of laboratory tests; hormonal treatment for fertility or use of metformin, or history of gynecological surgery in the previous 3 months; and any other condition deemed ineligible by the investigator.

The study protocol was approved by the Clinical Research Ethics Committee (CEIC) of Hospital de la Santa Creu i Sant Pau (Barcelona, Spain) (HSCSP code 16/128, KPl-001-CL-004, approval date July 26, 2016). All participants provided written informed consent. The study was registered in the European Clinical Trials Database (EudraCT) (EudraCT trial number 2016-001276-30).

Study procedures

The study was performed in two stages: stage 1 included the administration of OXO-001 100 mg/day (dose level I) and 200 mg/day (dose level II) or placebo, which was followed by stage 2 which included the administration of 300 mg/day (dose level III) or placebo. Normal weight and overweight participants were randomized to receive the active drug or placebo in a 2:2:1 ratio for stage 1 and in a 2:1 ratio for stage 2. Each subject could participate only in one dose level. The randomization list was generated by an independent contract research organization using the Statistical analysis system (SAS, SAS Institute, Cary, NC, USA, version 9.4) for Windows. After all subjects in stage 1 finished the treatment cycle, a blinded interim analysis of safety data was performed by a safety monitoring committee (principal investigator, medical expert and the sponsor based on safety considerations and plasma concentration data) to continue to stage 2 with the next dose.

The study included pre-treatment (after screening visit), treatment, and post-treatment phases, consisting of one menstrual cycle each. The screening visit was scheduled within 3 months before starting treatment and included an assessment of eligibility criteria; a complete medical history and a physical examination [weight, height, heart and respiratory rate, axillary temperature, systolic (SBP) and diastolic blood pressure (DPB)]; 12-lead electrocardiogram (ECG); laboratory tests [hematology, biochemical profile, serology for hepatitis B and C, and human immunodeficiency virus (HIV) infection, human chorionic gonadotropin (hCG), luteinizing hormone (LH), antimullerian hormone (AMH), serum leptin, ghrelin, and adiponectin]; urine drug screening for commonly abused drugs; gynecological examination with TVUS; and written consent to take part in the study. Additionally, during the pretreatment phase, vaginal cytology and endometrial biopsy were performed 7 days after ovulation (assessed by serial LH determinations and TVUS). The endometrial biopsy was included in the protocol given that transvaginal sonography and clinical symptoms are not sufficient to infer changes in cellularity.

In the treatment phase, single daily doses of film-coated tablets of the active drug (50 mg×2 in dose level I, 100 mg×2 in dose level II, and 150 mg×2 in dose level III) or identically appearing placebo tablets were administered from day 1 (D1) (first day of the menstrual cycle) to D28 (or until the last day of the menstrual cycle in case of shorter cycles) at the study center, in fasting conditions and the presence of the investigator. Food and water intake was not allowed until 4 and 2 hours post-medication, respectively. At the beginning of the treatment phase, participants were admitted to the study center for two subsequent overnight stays for the daily administration of the assigned medication. On D1 and D2, collection of blood samples to measure plasma levels of the investigational medicinal product (IMP) (D1: pre-dose and serial sampling post-dose; D2: pre-dose) and safety laboratory tests (hematology, biochemical profile, urinalysis) were performed. Tolerability variables (vital signs, ECG, laboratory tests, adverse events [AEs] and concomitant medication monitoring) were assessed on D1 and D2. On the following days (D3 to D28), volunteers came back to the study center for administration of the assigned medication, blood sampling for measuring PK parameters (predose: D3, D4, D7, D14, D21, D28, and 12, 24, 48, and 72 hours after the last dose administration), safety laboratory tests (D3, D7, D14, D21, and D28), tolerability assessments (D4, D7, D14, D21, and D28), and monitoring of AEs and concomitant medication (D3 to D28). Also, a TVUS and an endometrial biopsy were performed 7 days after ovulation.

During a post-treatment cycle within 4 weeks after the last drug administration, a complete physical examination and assessment of safety and tolerability variables were performed on days 3 and 28 post-treatment. Gynecological examination and serum leptin, ghrelin, and adiponectin levels were performed on day 3.

Evaluation criteria

Safety and tolerability variables included AEs, findings on physical and gynecological examinations, vital signs (heart rate, SBP, DBP, axillary temperature), 12-lead ECG, and laboratory tests (hematology, biochemistry, hormone analyses, and urinalysis). AEs spontaneously referred by participants and using a complementary check-list were rated on a 3-point scale of intensity (mild, moderate, severe), in relation to the study medication (unassessable, unlikely, possibly, probable/likely, definitive/certain), and coded according to the system organ class (SOC) and preferred term (PT) using the medical dictionary for regulatory activities (MedDRA) (18). Treatment emergent adverse events (TEAEs) were AEs that occurred after drug administration for the first time or, if present before, worsened during drug exposure up to 1 day after the last administration of the given drug.

Laboratory tests included hematology (hemoglobin, glycosylated hemoglobin, hematocrit, blood cell counts); biochemical (electrolytes, liver and renal function tests); hormonal determinations [hCG, LH, follicle-stimulating hormone (FSH), estradiol, prolactin, testosterone, insulin, sex hormone binding globulin (SHBG), thyroid stimulating hormone (TSH), T3, T4, 17-hydroxyprogesterone (17-OHP), delta-4-androstendione, and dehydro-epiandrosterone sulfate (DHEA-S)]; and urinalysis (pH, leukocytes, nitrites, proteins, glucose, ketone bodies, bilirubin, urobilinogen, and erythrocytes).

The optimal dose of OXO-001 was defined as the highest tested dose in the absence of safety concerns or the dose level below the dose limiting toxicity (DLT). Pharmacokinetic parameters of included peak (Cmax) and minimum (Cmin) plasma concentration (expressed as tungsten ng/ml), time to reach Cmax (Tmax), appar-

ent volume of distribution (Vd), clearance (Cl), elimination half-life (t½), elimination rate constant (kel), area under the concentration-time curve (AUC) from zero to infinite (AUC0-∞) and from zero to time "t" (AUC0-t). Pharmacodynamic variables included serum levels of leptin, ghrelin and adiponectin (ELISA test) evaluated on days 3 of both the pre-treatment and post-treatment cycles; total number of follicles >15 mm and dominant follicle size, endometrial morphology (hypoechoic, isoechoic, hyperechoic), thickness and line pattern (not visible, only unclearly, visible) evaluated by TVUS on menstrual cycle days 14 of both the pre-treatment and treatment cycles, and histological studies of biopsies.

Study endpoints

The primary endpoint was the safety and tolerability of single repeated oral doses of OXO-001 during the treatment cycle and at follow-up as compared with placebo. Secondary endpoints included the optimal dose of OXO-001, PK and pharmacodynamic profiles.

Statistical analysis

A total of 32 participants were planned to be included (n=10 for 100 mg/day dose regimen, n=10 for 200 mg/day, and n=12 for 300 mg/day) to have at least 24 completers (3 of the 4 subjects for cohort and group), which were considered sufficient to fulfil the study aim. Demographics, safety and tolerability analyses were performed in the safety population (all randomized participants who took at least one dose of the study medication), and PK and pharmacodynamic analyses in the per-protocol (PP) population (all participants who completed the study), in whom at least one PK and one pharmacodynamic parameter were available, respectively. Calculations of PK parameters were based on a non-compartmental approach using the linear trapezoidal rule. Values < lower limit of quantification (LLOQ) were set to 0. Categorical variables are expressed as frequencies and percentages, and continuous variables as mean and standard deviation (SD), median, minimum and maximum. Data were analyzed with the statistical analysis system (SAS) (SAS Institute, Cary, NC, USA) version 9.4 for Windows. Descriptive statistics are reported.

Results

Sixty-six volunteers were recruited but 36 were ineligible because the inclusion criteria were not met, so 30 participants were randomized and included in the study. At dose level I, however, one woman assigned to the active treatment was excluded as her menstrual cycle was >35 days. Therefore, 29 participants completed the study. The flow chart distribution of participants is shown in Figure 1. The mean age and BMI of participants is shown in Table 1. Data of anamnesis and results of physical examination, laboratory tests, gynecological examination and endometrial biopsies were unrevealing.

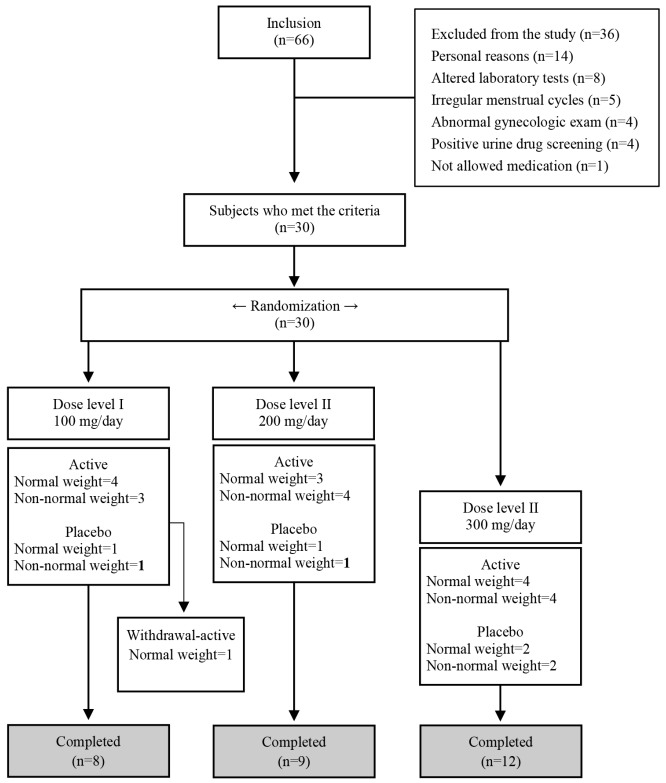

Fig.1: Flow chart of the study population (safety and tolerability analysis were performed in 30 patients and pharmacokinetics (PK) and pharmacodynamic analyses in 29).

Table 1: Demographic characteristics of participants

Variables	All participants		Healthy weight	Overweight
	OXO-001	Placebo		
Age (Y)	33.2 ± 5.9	30.0 ± 4.9	30.1 ± 6.7	33.9 ± 6.0
BMI (kg/m^2)	24.7 ± 3.7	25.2 ± 3.4	22.0 ± 1.5	27.7 ± 2.6

Data are presented as mean ± SD. BMI; Body mass index.

Safety and tolerability

No serious AEs were recorded during the study. A total of 38 TEAEs were reported by 20 participants (15 in the OXO-001 group and 5 in the placebo group). Headache was the most common complaint (21.1%) (8 cases, 3 with placebo) followed by nausea (15.8%) (6 cases, 1 with placebo), and somnolence (10.5%) (4 cases, 2 with placebo). None of the participants discontinued the study because of TEAEs, and no serious AEs or deaths were recorded during the study.

Table 2 shows the details of TEAEs according to intensity and by SOC and PT of MedDRA classification. In relation to the SOC, 13 (34.2%) TEAEs were included in the category of "nervous system disorder", 9 (23.7%) in "gastrointestinal disorders", 6 (15.8%) in "infections and infestations", 5 (13.2%) in "reproductive system and breast disorders", 4 (10.5%) in "general disorders and administration site conditions", and 2 (5.3%) in "neoplasms benign, malignant and unspecified". The distribution of TEAEs in the study groups is shown in supplement Figure 1 (See supplementary online information at www.ijfs.ir). In the placebo group, TEAEs were related and not related to drug use in 21 and 10.5% of cases, respectively. TE-

AEs were related to OXO-001 administration in 13.2% of cases of the 100 mg/day group, 10.5% of the 200 mg/day group, and 15.8% of the 300 mg/day group. TEAEs not related to the study drug occurred in 5.3% of cases of the 100 mg/day group, 10.5% of the 200 mg/day group, and 13.2% of the 300 mg/day group. Differences in the occurrence of TEAEs among normal weight and high weight participants were not found.

Table 2: Summary of 38 treatment emergent adverse events (TEAEs) in the placebo and OXO-001 study groups

Study group	Mild		Moderate		Severe		Total
	Related	Not related	Related	Not related	Related	Not related	
Placebo (n=8)							
Nervous system disorders							
Headache			3 (7.9)				5 (13.2)
Somnolence	2 (5.3)						
Reproductive system and beast disorders							
Dysmenorrhea				1 (2.6)			2 (5.3)
Ovarian pain	1 (2.6)						
Gastrointestinal disorders							
Nausea	1 (2.6)						2 (5.3)
Toothache				1 (2.6)			
General disorders/administration site							
Pyrexia		1 (2.6)					1 (2.6)
Neoplasms benign, malignant, unspecified							
Uterine polyp	1 (2.6)						1 (2.6)
Infections and infestations							
Nasopharyngitis			1 (2.6)				1 (2.6)
Total TEAEs							12 (31.6)
OXO-001, 100 mg/day (n=7)							
Nervous system disorders							
Headache			2 (5.3)				3 (7.9)
Dizziness			1 (2.6)				
Infections and infestations							
Nasopharyngitis			1 (2.6)				2 (5.3)
Tonsillitis				1 (2.6)			
Neoplasms benign, malignant, unspecified							
Fibroadenoma		1 (2.6)					1 (2.6)
Gastrointestinal disorders							
Nausea	1 (2.6)						1 (2.6)
Total TEAEs							7 (18.4)
OXO-001, 200 mg/day (n=7)							
Infections and infestations							
Pharyngitis			1 (2.6)				3 (7.9)
Nasopharyngitis			1 (2.6)				
Tonsillitis			1 (2.6)				
Reproductive system and beast disorders							
Metrorrhagia	1 (2.6)						1 (2.6)
General disorders/administration site							
Discomfort	1 (2.6)						2 (5.3)
Pyrexia	1 (2.6)						
Nervous system disorders							
Headache			1 (2.6)				1 (2.6)
Gastrointestinal disorders							

Table 2: Continued

Study group	Mild		Moderate		Severe		Total
	Related	Not related	Related	Not related	Related	Not related	
Total TEAEs							8 (21.0)
OXO-001, 300 mg/day (n=8)							
Nervous system disorders							
Headache	1 (2.6)		1 (2.6)				4 (10.5)
Somnolence	1 (2.6)		1 (2.6)				
Gastrointestinal disorders							
Nausea	2 (5.3)		1 (2.6)				4 (10.5)
Vomiting			1 (2.6)				
Reproductive system and beast disorders							2 (5.3)
Dysmenorrhea				1 (2.6)			
Ovarian pain	1 (2.6)						
General disorders/administration site							
Asthenia	1 (2.6)						1 (2.6)
Total TEAEs							11 (28.9)

Data are presented as a number of adverse events (frequency within the group, %).

Regarding vital signs, ECG, and laboratory data, no relevant consistent pattern of changes was observed and all values of laboratory tests were within the normal range at the post-treatment assessment on D3 and 28.

Pharmacokinetic data

The PKs of OXO-001 were characterized by a fast absorption and elimination phase for most of the volunteers showing a proportional increase of OXO-001 plasma concentration proportionally to increasing doses (Fig.2A). As may be expected, plasma drug concentrations of all doses in the normal weight group were higher than in the high weight group (Fig.2B, C). The mean values of all PK variables are shown in Table 3. The mean Tmax ranged from 4.9 hours (100 mg dose) to 4.5 hours (300 mg dose). In the normal weight group, Tmax was 4.5 hours as compared with 5.2 hours in the high weight group. In relation to t½, values ranged from 4.2 hours (100 mg dose) to 4.4 hours (300 mg dose), with a slight difference as doses increased. The mean of peak plasma concentrations (Cmax) for OXO-001 was 1370 ng/ml, 2047, 1 ng/ml 2885, and 2 ng/ml in 100 mg, 200 mg and 300 mg doses respectively. Showing a good correlation (r^2=0.854). Similar findings were observed in the normal and high weight groups. Also, Kel (t-1) remained constant (0.2) in all doses and normal and high weight groups.

The systemic exposure to OXO-001 was characterized by a proportional increase of AUC0-t with increasing doses of 100, 200, and 300 mg (8626.5 ng·hour/ml, 14460.0 ng·hour/ml, and 20774.7 ng·hour/ml, respectively) with a good correlation coefficient (r^2=0.872) and proportionality for each dose increase. Similar findings were observed for the extent of systemic exposure, with AUC0-∞ values from 8766.4 ng·hour/ml for the 100 mg dose to 21227.2 ng·hour/ml for the 300 mg dose (r^2=0.664).

Table 3: Summary of pharmacokinetic (PK) parameters in the study groups receiving different doses of OXO-001

PK parameters	OXO-001 doses		
	100 mg	200 mg	300 mg
Cmax (ng/ml)	1370.0 ± 1.4	2047.1 ± 1.2	2885.2 ± 1.3
Tmax (hour)	4.9 ± 1.0	4.7 ± 0.5	4.5 ± 1.1
T1/2 (hour)	4.2 ± 0.4	4.4 ± 0.5	4.4 ± 0.7
AUC0-∞ (ng·hour/ml)	8766.4 ± 1.3	14737.5 ± 1.2	21227.2 ± 1.3
AUC0-t (ng·hour/ml)	8626.5 ± 1.3	14460.0 ± 1.2	20774.4 ± 1.3
Kel (h^{-1})	0.17 ± 0.02	0.16 ± 0.02	0.16 ± 0.03
Vd (L)	78.0 ± 1.2	99.2 ± 1.4	92.2 ± 1.4
Cl/F (L/h)	13.2 ± 2.6	16.2 ± 4.3	15.3 ± 4.9

Data are presented as mean ± SD. Cmax; Peak plasma concentration, Tmax; Time to reach Cmax, t½; Elimination half-life, AUC0-∞; Area under the concentration-time curve from zero to infinite, AUC0-t; Area under the concentration-time curve from zero to time "t" (t=24 hours), Kel; Elimination rate constant, Vd; Apparent volume of distribution, and Cl/F; Apparent clearance. PK parameters were calculated on study day 1 after subjects had received the first dose. Results are expressed as tungsten.

Pharmacodynamic data

There were no relevant changes in endometrial variables, including endometrial thickness, visualization of the three endometrial layers, endometrial morphology, total number of follicles >15 mm, and dominant follicle size, as well as serum levels of leptin, ghrelin and adiponectin before and after the administration of OXO-001 or placebo (Table 4). Differences in pharmacodynamic data between the groups of normal weight and high weight participants were not found. Histological studies of endometrial biopsies did not show any abnormality.

Finally, based on safety and tolerability data, and PK and pharmacodynamic profiles, the maximum tolerated dose of OXO-001 to be considered was the higher dose administered of 300 mg/day.

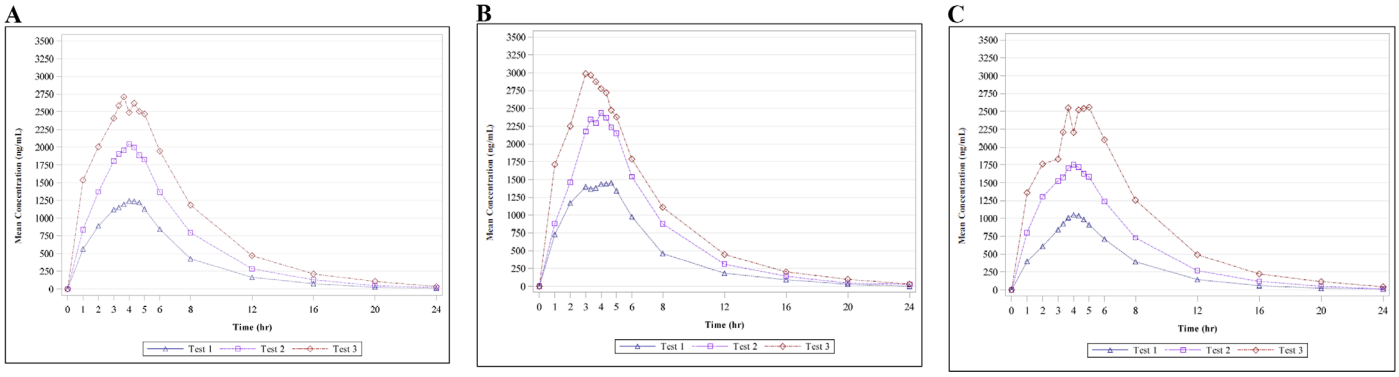

Fig.2: Mean OXO-001 plasma concentration curves after the administration of the first dose (100 mg for test 1, 200 mg for test 2, and 300 mg for test 3) **A.** In the whole set of patients, **B.** In the normal weight group and **C.** High weight group. Results are expressed as tungsten.

Table 4: Summary of pharmacodynamic parameters on TVUS and hormonal parameters in the placebo and study groups receiving different doses of OXO-001

TVUS findings	Placebo (n=8)		OXO-001, 100 mg (n=6)		OXO-001, 200 mg (n=7)		OXO-001, 300 mg (n=8)	
	Pre-treatment	Treatment	Pre-treatment	Treatment	Pre-treatment	Treatment	Pre-treatment	Treatment
Endometria variables								
Thickness (mm)[1]	9.7 ± 2.6	7.6 ± 2.1	10.6 ± 4.4	11.6 ± 2.5	9.6 ± 1.8	9.3 ± 1.7	10.8 ± 4.2	11.4 ± 2.5
Triple line visualization (%)[1]								
Not visible	0	0	16.7	33.3	28.6	28.6	25.0	37.5
Only unclearly	25.0	50.0	50.0	33.3	28.6	14.3	50.0	0
Visible	75.0	50.0	33.3	33.3	42.9	57.1	25.0	62.5
Morphology (%)[1]								
Hypoechoic	62.5	50.0	33.3	16.7	42.9	42.9	37.5	62.5
Isoechoic	25.0	37.5	50.0	33.3	14.3	0	12.5	0
Hyperechoic	12.5	12.5	16.7	50.0	42.9	57.1	50.0	37.5
Follicles >15 mm[1]	1.0 ± 0.0	0.8 ± 0.5	0.7 ± 0.5	1.2 ± 1.0	0.9 ± 0.4	0.9 ± 0.7	1.0 ± 0.0	1.0 ± 0.0
Dominant follicle size (mm)[1]	19.9 ± 5.0	21.8 ± 1.8	22.7 ± 4.2	18.1 ± 2.6	21.1 ± 3.1	21.5 ± 5.6	20.6 ± 1.9	21.4 ± 1.3
Hormones[2]								
Serum leptin (ng/ml)	18.7 ± 12.8	17.1 ± 10.3	10.5 ± 3.4	12.4 ± 5.4	16.2 ± 12.0	15.0 ± 10.3	17.5 ± 5.1	16.1 ± 4.8
Serum ghrelin (pg/ml)	73.2 ± 35.4	78.7 ± 46.3	39.4 ± 15.3	38.2 ± 19.6	48.3 ± 37.0	34.2 ± 25.7	49.6 ± 24.5	55.0 ± 31.6
Serum adiponectin (µg/ml)	10.1 ± 2.4	9.4 ± 2.9	10.5 ± 4.8	9.9 ± 2.8	9.3 ± 4.0	8.8 ± 2.7	8.8 ± 3.9	7.5 ± 2.9

Data are presented as mean ± SD. LH; Luteinizing hormone, [1]; Assessment on day 14 of the cycle, a surge of LH+24 hours (pre-treatment and treatment cycle) and [2]; Assessment on day 3 ± 1 of the cycle (pre-treatment and post-treatment cycle).

Discussion

This study of three different doses of OXO-001 administered to healthy female volunteers of childbearing age allows us to establish that the administration of 100, 200, and 300 mg/day of the product during 28 days or after the last day of the menstrual cycle was safe and well tolerated in both normal weight and high weight women. The maximum tolerated dose of 300 mg/day found in the present study could be adequate for testing in further phase II and phase III clinical trials.

In relation to TEAEs, headache and nausea of mild intensity were the most frequent complaints associated with the study medication, but none AEs were severe. The total number of TEAEs of mild and moderate intensity related to the drug was somewhat higher in the OXO-001 dose of 300 mg/day, but a clear dose-relationship cannot be established, particularly due to a limited number of participants. The favorable safety profile of OXO-001 consisted of the good tolerability of the product as shown by unrevealing findings on physical examination, vital signs, ECG recording, and a battery of laboratory tests. These results are consistent with a study performed by our group in a similar population and conditions with a 500 mg/day dose of OXO-001 (19). The higher dose is also safe, well-tolerated, and with similar TEAES in frequency and severity.

In the PK profile, OXO-001 showed a fast absorption, with well correlated increasing Cmax values proportionally to the dose administered, suggesting linear PK properties. Because of differences in the BMI, Cmax values were higher in the normal weight group than in the high

weight group. On the other hand, the time to reach Cmax was similar for three OXO-001 doses, which indicates a similar absorption of the parent compound. On treatment Day 1, the t½ for OXO-001 was calculated using a non-compartmental model and values range between 4.2 hours (100 mg dose) to 4.4 (300 mg dose), showing a slight difference at increasing doses of the active drug. Mean values according to normal or high weight were very similar. Additionally, on the last day of OXO-001 administration, the t½ was 13.7 with the 100 mg dose and 17.8 hours with the 300 mg dose. The difference observed in t½ between D1 and 28 could be explained by the reduced number of time points available for the assessment of t½ prior to the next dosing 24 hours later on D2. Accordingly, it would be advisable to increase the number of time points for PK assessment on the first day of drug administration in future studies. The administration of a higher dose (500 mg/day) repeated the fast peak absorption, with a mean Tmax between 4 and 5 hours, and no accumulation after 28 days of treatment. However, the linearity observed within 100 to 300 mg/day disappeared, suggesting a change in the absorption/elimination rate at some point above 300 mg/day.

Interestingly, results of all pharmacodynamic parameters were consistent with OXO-001 as a safe and well tolerated drug, with no detrimental effect on the endometrium and follicles as shown by transvaginal ultrasound, and normal findings on histopathological examination of endometrial biopsies. Moreover, serum levels of leptin, ghrelin and adiponectin did not show clinically relevant changes.

It is important to make some comments regarding the randomized, double-blind, and placebo-controlled features of the study. This design was selected to ensure balanced and homogeneous baseline characteristics of participants assigned to the different study groups, particularly regarding the distribution of women in the categories of normal weight and overweight. This allowed assessing whether there was a differential trend in the pharmacokinetic and safety profiles in the overweight group. Double-blinding is also an important methodological requirement in phase I studies for the clinical relevance of the subjective component in the perception of AEs. Since this was the first study in humans of repeated doses of OXO-001, it was necessary to include a placebo group to assess and characterize the safety profile of the investigational agent. In line with the design of phase I studies, including a placebo group is appropriate for the descriptive comparison of AEs and to be able to identify the potential occurrence of an AE related to the investigational product. It should be emphasised that the objective of the randomized, double-blind and placebo-controlled design was not to compare efficacy, given that only descriptive statistics are reported.

Conclusion

The present results of this phase I study showed that the administration of OXO-001 (sodium tungstate) in healthy female volunteers of childbearing age was safe and well tolerated, with a consistent PK linear profile within the 100 to 300 mg/day dose range and without detrimental effects on endometrium and ovary-related variables. The effects of the product were similar in normal and high weight participants. The maximum studied dose (300 mg/day) was safe and well tolerated and seems adequate for use in women of childbearing age in future clinical trials.

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Authors' Contributions

A.A., I.C., J.C., P.M.-P., J.P., R.M.A., J.C.; Participated in the study design. J.C., P.M.-P., J.P., R.M.A., J.C.; Contributed to all experimental work, Data collection and Evaluation, Drafting, and Statistical analysis. A.A., I.C., J.C., P.M.-P., M.L., R.T, M.M.-B., J.C.; Participated in the interpretation of data. I.C., A.A.; Drafted the manuscript, which was revised by J.C. All authors read and approved the final manuscript.

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