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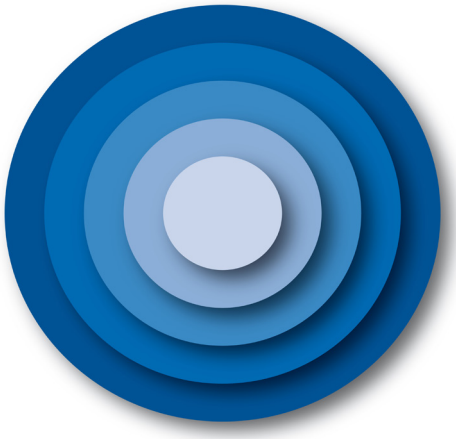

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A randomized, placebo-controlled, single and multiple dose study on animal thymus gland extracts (pTE) in healthy volunteers

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Animal thymus gland extract (pTE) may have a potential use in promoting regulation of cytokine activity in autoimmune pathologies of the cardio-vascular, neurological and osteo-articular districts. Four cohorts, with 10 healthy subjects each, were given ascending doses of placebo or animal thymus gland extract (pTE). Cohorts received ascending doses of either 1000, 2000, 3000, 4000 mg. in a 1:10 ratio. Following safety review, subjects were given the same dose regimen daily for 14 days. Safety evaluations, incidence of Treatment-Emergent Adverse Events, and pharmacokinetic parameters were evaluated. Adverse events were infrequent, and mild or moderate in intensity. There were no dose limiting toxicities or serious adverse events. Pharmacokinetic profile for single dose showed a dose proportional response, and an increasing half-life with increasing dose. Animal thymus gland extract pTE, given as a single dose or in multiple daily doses for 14 days over a dose range of 1000–4000 mg, in a 1:10 ratio, was well tolerated with no evidence of dose limiting toxicity. Further development for use in autoimmune pathologies of the cardiac, neuro- and osteo-articular districts should be considered.

Keywords: intramuscular thymus; autoimmunity; thymosin; thymus tolerability; thymus extract.

Introduction

Animal thymus gland extracts (pTE) are associated with a wide variety of physiologic functions including to resist infections due to immunosuppression induced by the diseases. Preclinical studies showed a variety of modulatory effects on the immune system(Bodey 2000, Chretien 1978, Goldstein 2009, Schulhof 1985a) and revealed that pTE this is a peptide of 28 amino acid bovine thymosin α1 and may have potential value in the promotion of regulation of cytokine activity in autoimmune pathologies of the cardio-vascular, neurological and osteo-articular districts.

Animal thymus gland extracts are processed in different steps of purification, fractionation and filtration to result in peptides mixtures. The exact composition and character of the peptides are not completely known and are subject to biological variation. Different preparations are not defined by their components but by the respective standardization of the extraction procedure.

This initial, single and multiple dose administration of animal thymus gland extract pTE to healthy volunteers will help to establish the safety, tolerance, and pharmacokinetic profile of ascending doses. The outcome of this study will facilitate future development of synthetic animal thymus gland extract pTE for use in the setting of autoimmune pathologies of the cardio-vascular, neurological and osteo-articular districts that require systemic drug administration by injection. IV doses ranged between 0.6 and 60 mg/kg, and no drug-related ad- verse events were observed.3

Methods

Trial design

This study was a randomized, double-blind, placebo-controlled, investigation to evaluate the safety, tolerability, pharmacokinetics, and the potential immunological reaction (antibody formation) of single and multiple daily doses of animal thymus gland extract pTE administered intramuscularly to healthy volunteers. In Phase 1A, four (4) cohorts of subjects (10 subjects per cohort) were administered one dose of animal thymus gland extract pTE or placebo in a 4:1 ratio. Dosing started at 1000 mg and advanced to 2000, 3000 and 4000 mg. in a 1:10 ratio. To reduce the risk to subjects, the first 3 individuals of each ascending cohort were randomized and treated at intervals of 24 h. The remaining seven subjects in each ascending cohort were randomized without any time restrictions. Following a comprehensive safety review of the single dose data, Phase 1A subjects or replacements of those Phase 1A subjects who did not continue after Phase 1A, were enrolled in the Phase 1B portion of the study. Subjects were administered animal thymus gland extract pTE or placebo daily for 14 days at the same dose level in Phase 1B as they received in Phase 1A.

Eligibility

Male and female volunteers between 18 and 79 and in good health, with no underlying medical condition that would place them at risk, were evaluated for study participation. All subjects gave informed consent prior to

any study related procedures. Volunteers were excluded from participation if any of the following were present:

(1) Malignancy.
(2) Tobacco use.
(3) Clinically abnormal safety labs; or vital signs outside the normal ranges.
(4) GFR < 80 mL/min/1.7 m2.
(5) Use of any medications.
(6) Ana, anca, Il2α and β, Il10.

Volunteers were excluded if there was evidence of an increased risk of malignancy, as indicated by

(1) Family history of cancer.
(2) Skin lesions that were risk factors for melanoma.
(3) Environmental exposure such as asbestosis.
(4) Male subjects over the age of 40 with an eleva ted PSA, positive fecal occult blood, alpha-fetoprotein, or CEA outside of the normal range.
(5) Female subjects over the age of 50 with an ab normal mammogram, PAP smear, positive fe cal occult blood, alpha fetoprotein, CEA, CA 19-9, CA 15-3, or CA 125 outside of the nor mal range, in menopause since un year.

Procedures

An Ethics Review Board approved all study related documents. Qualified subjects for Phase 1A were admitted to the clinical pharmacology unit on Study Day 1 (minus 1) and were discharged from the unit on Study Day 2. Animal thymus gland extract pTE or placebo was administered as a single dose on Day 1. Safety parameters including: vital signs, CBC, Chemistries, UA, were recorded on Study Days 1, 2, 7, 14, and 28. Physical exams were recorded on Days −1, 2, 7, 14, and 28. Evaluation of treatment emergent adverse events was performed throughout the study.

Pharmacokinetic samples were obtained on Study Days 1 and 2. Blood samples were collected for serum antibody determinations to animal thymus gland extract pTE at Screening and Day 28.

During Phase 1B, subjects who participated in Phase1A were requalified. For those Phase 1A subjects who did not continue, replacement subjects matched for gender were screened and enrolled to ensure that 10 subjects were randomized into each cohort. Subjects were admitted to the pharmacology unit on Day 1 and remained confined to the unit through Day 15. Animal thymus gland extract pTE or placebo was administered as a single intramuscular dose starting from Day 1, and then daily for 14 days. Safety labs, EKGs, vital signs, evaluation of adverse events, and physical exams were performed intermittently throughout the 14 day dosing period. Pharmacokinetic samples were obtained on Study Days 1, 2, and Days 12 to 15. Subjects were discharged on Day 15 and returned for out-patient evaluations on Days 21, 28, and 42. Blood samples were collected for serum antibody determinations to animal thymus gland extract pTE at Screening and Day 28.

Subjects were evaluated regarding any de novo diagnosis of cancer via a telephone interview which occurred approximately 6 months after Day 28 for subjects who elected not to participate in Phase 1B. Subjects participating in Phase 1B had a cancer follow-up call 6 months after Study Day 42 of Phase 1B.

Investigational product was supplied as a frozen sterile, aqueous solution in vials of animal thymus gland extract pTE, 100 mg/mL, or placebo, 0 mg/mL, by Homo Novus GmbH.

Frozen vials were thawed, and the appropriate volume of solution was prepared by an unblinded pharmacist in a sterile manner and subsequently administered by the investigator. The solution was given intramuscolarly During Phase 1A, escalation to the next dosing cohort occurred after a blinded review of all cohort safety data through Day 7. During Phase 1B a safety review of all cohort data occurred after Day 21.

Stopping rules for enrollment included:

(1) Occurrence of 3 or more adverse events (AEs) assessed as severe and possibly, probably or definitely related to study drug and within the same organ class.

(2) Occurrence of 2 or more nonfatal, nonlife threatening serious AEs assessed as possibly, probably or definitely related to study drug.

Stopping rules for treatment included:

(1) Occurrence of 1 or more fatal or life- threatening serious AE as possibly, probably, or definitely related to study drug.

(2) Occurrence of AEs assessed as severe and possibly, probably, or definitely related to study drug within the same organ class and in more than half of the subjects currently enrolled in the group receiving the highest dose.

During Phase 1A, pharmacokinetic parameters were determined from plasma samples collected from each subject prior to dosing and 10, 30, 60, 90 and 120, postdose, and at 3, 9, 12, and 24 h postdose. Plasma was prepared and analyzed for concentrations of animal thymus gland extract pTE. Concentration time data for each subject dosed with animal thymus gland extract pTE was subjected to noncompartmental pharmacokinetic analysis. During Phase 1B samples were obtained at similar time points on Day 1 and 14 and through plasma samples obtained on Day 12 and 13.

Results

Demographic and baseline characteristics.

The study volunteers had fairly consistent characteristics across all cohorts, with the exception of cohort 3 that showed a slightly lower mean age (Table 1). Twenty of the original 40 subjects in Phase 1A were re- placed in the multidose Phase 1B. Scheduling was the predominant reason for failure to enroll in Phase 1B. Replacement subjects were matched for gender and all cohorts had similar number of replacement subjects.

Adverse events

Phase 1A. There were a total of 18 adverse events re-

corded across all cohorts (Table 2), and all of them were treatment emergent adverse events (TEAEs). The frequency of AEs did not increase with increasing dose. Fifteen (15) adverse events were judged to be unrelated to study drug. Based on the temporal nature of the event, the remaining three adverse findings were judged to be possibly related to animal thymus gland extract pTE or placebo and included: a single subject who received placebo, (cohort 2) with a laboratory finding of leukocytosis, a second subject who received 2000 mg, (cohort 2) with dizziness which persisted postdose for 7 days, and one subject receiving placebo, (cohort 3) with headache, temporally associated with dosing that occurred approximately 3 h after dosing and persisted for approximately 18 h. Five of 18 AEs were classified as grade 2 (CTCAE), and 1 AE (elevated CPK) as grade 4. These 6 AEs were judged to be unrelated to study drug on the basis of other probable etiologies and lack of temporal association. These included: elevated CPK in 3 subjects; two subjects receiving 1000 mg and one subject receiving placebo; neutropenia in two subjects, 2000 and 3000 mg; and pharyngitis in 1 subject receiving 4000 mg. in a 1:10 ratio. There were no clinically significant findings Day 1 and 14 and through plasma samples obtained on Day 12 and 13. There were no serious adverse events or dose limiting toxicities. No subjects withdrew due to an adverse event, and there were no deaths. There were no reported cancers at the 6-month follow-up for subjects who did not participate in **Phase 1B.**

Phase 1B. There were a total of 64 adverse events across all cohorts, (Table 3); of those, 62 AEs were treatment emergent adverse events. The frequency of AEs was varied with increasing dose, including 15 AEs in five subjects for placebo, 8 AEs in five subjects

Table 1. Subject demographics

Parameter	Placebo 1 *n* = 8	1000 mg *n* = 8	2000 mg *n* = 8	3000 mg *n* = 8	4000 mg *n* = 8
Study phase			**Phase 1A**		
Age (years)					
Mean	29.9	35.0	34.1	26.0	32.3
Range (years)	18–38	27–54	18–42	20–40	21–54.0
African American	0	0	0	0	0
Caucasian/Non-Hispanic	6	7	5	5	3
Hispanic	2	1	3	3	5
Gender					
Male	6	7	7	7	6
Female	2	1	1	1	2
Height/weight					
Mean (inches)	68.7	68.0	68.7	65.8	68.2
Mean (lbs)	178.2	168.7	182.6	160.8	175.3
Study phase			**Phase 1B**		
Age (years)					
Mean	28.8	28.9	32.9	30.9	32.1
Range (years)	18–40	22–35	21–42	22–41	21–44
African American	0	0	0	0	0
Caucasian/Non-Hispanic	6	6	6	5	6
Hispanic or Asian	2	2	2	3	2
Gender					
Male	6	7	7	7	6
Female	2	1	1	1	2
Height/weight					
Mean (inches)	68.4	66.2	68.0	67.2	67.0
Mean (lbs)	187.1	164.6	183.3	174.8	165.2

at 1000 mg, 8 AEs in four subjects at 2000 mg, 14 AEs in five subjects at 3000 mg, and 17 AEs in seven subjects at 3000 mg. There was no single AE that showed increased frequency with increased dose. The most common adverse event was headache and this was noted in all dose groups including placebo. Fifty-five of 62 TEAEs were judged to be unrelated to study drug. Based on the temporal nature of the event, the remaining seven adverse findings were judged to be possibly related to Animal thymus gland extract pTE or placebo and included: one subject with head pressure (coded as headache) and one subject with feverish feeling (coded as pyrexia) at the 2000 mg dose; one subject with an elevated GGT level at the 4000 mg dose; in a 1:10 ratio, one subject with nausea in the placebo group; and one subject in the placebo group with three AEs including headache, elevated PTT, and elevated INR. Ten (10) of 62 TEAEs were classified as grade 2 (CTCAE) and nine of theses were judged to be unrelated to study drug. The only adverse event that was graded at level 2 and possibly related to animal thymus gland extract pTE, was head pressure (coded as headache) at the 2000 mg dose. All treatment emergent adverse events resolved at the time of final follow-up except for one subject with a residual traumatic neuropathy that was improving. This was not treatment related.

Table 2. Summary of single dose MedDRA coded treatment emergent adverse events by dose for Phase 1A

Preferred term	Placebo	1000 mg	2000 mg	3000 mg	4000 mg	All
Headache	1					1
Nasal congestion		1				1
Diarrhea			1			1
Neutropenia			1	1		2
Leukocytosis	1					1
Eye discharge			1			1
Pharyngitis				1		1
Edema peripheral					1	1
Blood potassium increased		1				1
Aspartate aminotransferase	1					1
Blood creatine phosphokinase increased	1	2				3
Blood lactate dehydrogenase increased	1					1
Gamma-glutamyltransferase increased			1			1
Proteinuria		1				1
Dizziness			1			1
Total adverse events	5	5	5	2	1	18
Number (%) of subjects reporting	3 (38%)	5 (63%)	3 (38%)	1 (13%)	1 (13%)	13 (33%)

Table 3. Summary of multiple dose MedDRA coded treatment emergent adverse events by dose for Phase 1B

Preferred term	Placebo	1000 mg	2000 mg	3000 mg	4000 mg	All
Headache	1	1	3	3	1	9
Upper respiratory tract infection	1		1	1	1	4
Constipation	1				2	3
Anemia	2			2		4
Pyuria		1			1	2
Nausea	1			1		2
Back pain					2	2
Abdominal pain	1				1	2
Blood potassium decreased		1			1	2
Gamma-glutamyltransferase increased			1		1	2
Other adverse events	8	5	3	7	7	30
Total adverse events	15	8	8	14	17	62
Number (%) of subjects reporting	5 (63%)	5 (63%)	4 (50%)	5 (63%)	7 (88%)	26 (65%)

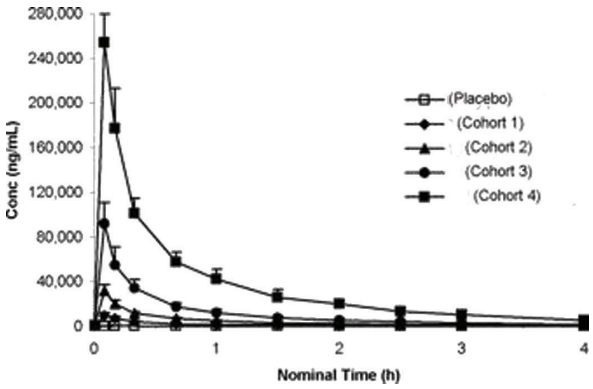

Figure 1. Mean (±SD) plasma concentrations (ng/mL) of Animal thymus gland extract (pTE) following single intramuscular administration to healthy human subjects (0–4 h expanded scale, linear–linear plot).

Day 1 (baseline), with the exception of one subject with a papular skin lesion on the forearm that was not related to study drug.

There were no Grade 3 or higher adverse events and there were no serious adverse events or dose limiting toxicities. There were no deaths. Thirtynine of 40 subjects received all 14 doses. A single subject received study drug through Day 11 and had to leave the study for a personal issue.

There were no reported cancers at the 6-month follow-up call.

Pharmacokinetics and antibody response Phase 1A: Mean concentration-time profiles for plasma animal thymus gland extract pTE following single intramuscular administration at doses ranging from 1000 to 4000 mg/subject were qualitatively similar at all doses (Fig. 1). Concentrations attained Cmax at the first postdose sampling time (5 min; t max) for all but one subject (t max of 10 min) and then declined with time in an apparent multiphasic manner.

Concentrations were measurable through median times of 1.8 h at 1000 mg/subject, 3.6 h at 2000 mg/subject, 6.0 h at 3000 mg/subject, and 9.0 h at 4000 mg/subject.

Mean terminal half-life estimates increased with dose, from 0.95 h at 1000 mg/subject, to 1.2 h at 2000 mg/subject, to 1.9 h at 3000 mg/subject, and to 2.1 h at 4000 mg/subject. Mean Cmax and mean AUClast each increased approximately proportionally with dose over the range of doses studied. The mean volume of distribution increased with dose from 8740 mL (8.74 L) at 1000 mg/subject to 21,700 mL (21.7 L) at 4000 mg/subject, but mean clearance appeared to be independent of dose, ranging from 6870 to 7330 mL/h (Table 4). Pharmacokinetics results for Phase 1B multidose and antibody response await further evaluation.

Discussion

Pharmacokinetics for a single dose would suggest that over the IV doses studied:

(1) Cmax and AUClast are approximately dose proportional.

(2) Mean terminal half-life appears to increase with dose.

(3) Mean clearance appears to be independent of dose.

Single IV administration of synthetic animal thymus gland extract pTE given over a dose range of 1000 to 4000 mg in a 1:10 ratio appears tobe

Table 4. Summary of Animal thymus gland extract (pTE) meana plasma pharmacokinetic parameters following single intramuscular administration to healthy human subjects

Dosage mg	C_{max} ng/mL	t_{max} h	t_{max} h	AUC_{last} ng/h/mL	$AUC_{0\text{-}inf}$ ng/h/mL	$t_{½}$ h	V_z mL	mL/h
1000	9,610	0	1,8	5,630[d]	6,500[d]	0.95[d]	8,740[d]	6870[d]
2000	31,800	0	3,6	18,700	20,500	1.2	11,800	6940
3000	91,900	0	6,0	53,500[e]	50,100[e]	1.9[e]	19,400[e]	7300[e]
4000	254,00	0	9,00	172,00	175,000	2.1	21,700	7330

a Median for t max and t last; n = 8.
b A time of "zero" indicates t max for observed concentrations was at the first postdose sampling time (a nominal time of 5 min postdose) due to IV dosing which took only 1–3 min, because, for the pharmacokinetic analysis, the concentration at time zero was assigned the same value as that observed at the first postdose sampling time.
d n = 6.
e n = 7.

safe and well tolerated with no dose limiting toxicity or serious adverse events. The most frequent adverse event observed was an elevated CPK level, but these elevations were noted in both placebo and active subjects. These elevations occurred during the out patient visits and likely reflected the increased physical activity common in this population.

Multiple daily dosing for 14 days also appears to be safe and well tolerated with no reported or observed dose limiting toxicity or serious adverse events. The most frequent adverse event observed was headache, but this was seen in all doses including placebo, and there was no increase frequency or severity with increasing doses. The adverse event profile for multiple doses does not show any trend in type, severity, or frequency across increasing doses from 1000 to 4000 mg. in a 1:10 ratio. The reported potential of animal thymus gland extract pTE to influence the metastatic potential of certain malignancies through its ability to promote angiogenesis and stimulate cell migration warranted close follow-up for any potential cancers.4,5 No cancers were identified during a 6-month follow-up period.

This study is the first reported investigation of intramuscular animal thymus gland extract pTE in healthy subjects.

These initial results should allow further development of a planned efficacy trial in autoimmune pathologies of the cardiac, neuro- and osteo-articular districts.

Acknowledgments

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Conflicts of interest

Cerifos, head of this research project, being a non-profit organization, has no conflict of interest. The only goal is the verification of scientific data already existing in literature.

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