

Safety and Tolerability of the Hexadecapeptide AOD9604 in Humans

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Abstract

Background: The human growth hormone (hGH) has properties making it a potential candidate to treat obesity, however safety issues limit its long-term use. AOD9604 is a peptide fragment of the C-terminus of hGH (Tyr-hGH₁₇₇₋₁₉₁), which harbors the fat reducing activity of hGH, without its negative effects. In this paper the safety data of AOD9604 obtained in clinical trials are summarized.

Methods: Six randomized, double-blind, placebo-controlled trials were performed with AOD9604. Special focus was given to undesired effects associated with hGH treatment: increases in IGF-1 levels, insulin resistance, and impaired glucose tolerance. Blood samples were analyzed for presence of anti-AOD9604 antibodies to exclude immunogenicity.

Results: AOD9604 had no effect on serum IGF-1 levels, which confirms the hypothesis that AOD9604 does not act via IGF-1. Results of oral glucose tolerance test demonstrated that, in contrast with hGH, AOD9604 has no negative effect on carbohydrate metabolism. There were no anti-AOD9604 antibodies detected in any of the patients selected for antibody assay. In none of the studies did a withdrawal or serious adverse event occur related to intake of AOD9604.

Conclusion: AOD9604 displayed a very good safety and tolerability profile indistinguishable from placebo. AOD9604 did not result in any of the adverse effects associated with full-length hGH treatment.

Keywords: AOD9604; Peptide fragment; C-terminus of Human

growth hormone; Clinical trial

Introduction

The human growth hormone (hGH) is a 191-amino acid long, single-chain polypeptide secreted by the pituitary gland under the regulation of the hypothalamus. It regulates a variety of biological processes in various tissues. When binding to the hGH-receptor it activates one of various signaling cascades, depending on the tissue. In the liver hGH induces the secretion of Insulin-Like Growth Factor-1 (IGF-1). IGF-1 can stimulate growth in almost every tissue in the body, including skeletal muscle, cartilage, bone, liver, kidney, nerves etc. [1].

The hGH plays different roles over the entire life time of a human being. For example, in children the most obvious function of hGH is promoting growth, but hGH also plays an important role in adults in metabolism [2, 3].

Amongst its metabolic effects, hGH can induce inhibition of lipoprotein lipase activity in adipose tissue, stimulating lipolysis in adipocytes, which results in the reduction of fat cell mass [4-7]. Moreover, a correlation has been found between adiposity and the reduced circulating levels of hGH [8]. When applied systemically, hGH reduces body fat mass and influences fat distribution [9]. Therefore, treatment with hGH should theoretically have a positive impact on obesity. However, long term treatment with hGH is associated with various health risks, including glucose intolerance and insulin resistance, diabetes, acromegaly, cancer, edema, and hypertension [10-13].

In plasma, different isoforms and fragments of hGH were found [10]. Research on specific domains and fractions of the protein revealed that they can be assigned to different actions of the protein: In vitro and in vivo experiments have shown that several fragments of the amino terminal region of hGH, namely 1-15, 1-42, 6-13, and 32-46, exhibit an insulin-potentiating action [14-16]. The region hGH 108-129 was found to evoke high mitogenic responses [17], while the carboxy terminus hGH₁₇₇₋₁₉₁ seemed to be a lipid mobilizing domain, inhibiting the acetyl-CoA carboxylase activity

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in adipocytes and hepatocytes [18].

AOD9604 is a peptide fragment of the C-terminus of human growth hormone (Tyr-hGH₁₇₇₋₁₉₁). It is prepared by solid phase peptide synthesis and contains an additional tyrosine residue at the N-terminal end that stabilizes the peptide. Investigation on the secondary structure of AOD9604 showed similarities to the homologous region in the naturally occurring hGH molecule [19]. Animal experiments confirmed the fat reducing potential of AOD9604, which seems to act directly on fat metabolism without influencing appetite. In genetically obese strains of rats and mice, AOD9604 was shown to affect body weight reduction, stimulation of lipolysis and inhibition of lipogenesis. Adverse effects, as seen in similar studies using intact hGH [18, 20, 21], were not observed with AOD9604 supplementation.

Recently, AOD9604 was determined by an appropriately qualified GRAS panel of experts who are qualified by scientific training and experience to be 'Generally Recognized As Safe' (GRAS) under conditions of intended uses of AOD9604 in foods.

In order to demonstrate safety, several human studies were performed with AOD9604 (supplementary data): 1). METAOD001: A Phase I (double-blind, placebo-controlled, dose escalation) safety study with doses (ranging from 25 to 400 µg/kg AOD9604) administered intravenously to 15 healthy adult male volunteers presenting with a BMI between 24 and 30 kg/m². A single dose of recombinant hGH (0.12 international units/kg) was administered intravenously as positive control. 2). METAOD002: A Phase IIa (double-blind, placebo-controlled 4 × 4 Latin Square design) safety study with single doses (25, 50 and 100 µg/kg AOD9604) administered intravenously to 23 healthy clinically obese males presenting with a BMI ≥ 35 kg/m². 3). METAOD003: A Phase IIa (double-blind, placebo-controlled 4 × 4 Latin Square design) safety study with single doses (9, 27 and 54 mg AOD9604) administered orally (capsules) to 17 healthy, clinically obese males presenting with a BMI ≥ 35 kg/m². 4). METAOD004: A Phase IIa (double-blind, placebo-controlled, dose escalation) safety study with multiple daily doses (9, 27 or 54 mg AOD9604) administered orally (capsules) for seven days in 36 healthy clinically obese males presenting with a BMI ≥ 30 kg/m². 5). METAOD005: A Phase IIb (randomized, double-blind, placebo-controlled) study to assess the efficacy (reduction in body weight), safety and tolerability of 12 weeks treatment with daily doses (1, 5, 10, 20 or 30 mg AOD9604) administered orally (capsules) in 300 healthy, clinically obese males, and females of non-child bearing potential, with a BMI ≥ 35 kg/m². 6). METAOD006: A Phase IIb, randomized, double-blind, placebo-controlled study to assess the efficacy (reduction in body weight), safety and tolerability of 24 weeks treatment with different doses of AOD9604 tablets (0.25 mg, 0.5 mg, 1 mg, or placebo) in 502 obese adults.

The objective of this paper is to summarize and discuss

the safety and tolerability data obtained from these human clinical trials.

Materials and Methods

All studies were performed according to the Declaration of Helsinki (as amended in Edinburgh, Scotland, October 2000) and the ICH Guidelines for Good Clinical Practice (GCP) (E6). Further, independent ethics review committees of up to 16 Australian hospitals and medical centers have approved each of them. The two largest studies (METAOD005 and METAOD006) were registered at the Therapeutic Goods Administration's Clinical Trial Notification (CTN) Scheme in Australia.

Study design and study population

All studies were performed as double-blind placebo-controlled trials with specific design adaption depending on the question that was to be answered. All, but the first, were performed on obese, but otherwise healthy, adults. In the first 4 studies, only male subjects were included (supplementary data). Approximately 900 adult subjects participated in these 6 clinical trials.

Treatment and duration

The test substance was either administered intravenously (studies METAOD001 and METAOD002) or as a capsule/tablet. The hexadecapeptide AOD9604 was produced under cGMP conditions by PolyPeptide Laboratories (Torrance, CA, USA). For studies 1 and 2, the product was supplied in a lyophilized form and reconstituted before usage with the designated volume of sterile water for intravenous injection. The capsules/tablets were manufactured using a common excipient mix (Capsules: Mannitol, PEG3350 (in studies METAOD003, METAOD004 and METAOD005)); or Tablets: L-Arginine Free Base, Microcrystalline Cellulose, Fumed silica, Magnesium Stearate (in study METAOD006)).

Within all the clinical trials the subjects received either the active treatment AOD9604 Tyr-hGH₁₇₇₋₁₉₁ (Metabolic Pharmaceuticals Ltd.; amino acid sequence: YLRIVQCRS-VEGSCGF; CAS Registry Number: 38624-39-7; INCI Name: 27701 sh-Oligopeptide-74) or placebo (vehicle of mix of excipients). In addition, in study METAOD001 individual subjects were treated with rhHG (0.12 IU/kg; supplied by Unichem in the form of somatropin (Saizen® - Serono)) as a positive control. The administered doses of the study product ranged from 25 µg up to 400 µg per kg bodyweight for the injectable product (i.v. administration in study METAOD001 and METAOD002) and from 0.25 mg/day to 54 mg/day for the orally administered capsules/tablets (capsules METAOD003 - METAOD005; tablets: METAOD006).

The duration (treatment and follow up) of the individual studies depended on the type of study (supplementary data). The first three clinical trials were single dose treatments (METAOD001 - METAOD003); the longest was a phase IIb clinical trial (METAOD006) with a four weeks run-in phase, followed by a six months treatment phase and a 30 day follow-up phase.

Accessed safety parameters

Within all clinical trials the subjects underwent physical examination. The vital signs were observed, laboratory parameters were analyzed (hematology; biochemistry, urinalysis, lipid analysis), and ECG were measured before and after treatment (or in between and follow-up depending on the duration of the study). All subjects were interviewed at each visit with regard to any adverse events (AEs) they had experienced since the previous visit. The causality of AEs (namely their relationship to trial treatment) was assessed by the Principal Investigator. Special attention was made to on the evaluation of Serious Adverse Events (SAE).

Due to the similarity of AOD9604 to the C-terminus component of hGH some parameters that are associated with hGH activity have been carefully monitored in the long-term studies: 1). Oral glucose tolerance tests (OGTT) were conducted to assess the effect of treatment on glucose handling. 2). Testing for any anti-AOD9604 antibodies in blood samples of participants was conducted to investigate if oral administration of AOD9604 resulted in the generation of neutralizing antibodies. 3). Serum levels of IGF-1 were measured to confirm the hypothesis that unlike hGH, AOD9604 does not act via IGF-1 or raise IGF-1 levels.

Statistical analysis

All analyses of the safety and tolerability parameters were performed in the data safety set. It consists of all subjects who received at least one dose of study treatment and was the analysis set for the safety endpoints. Subjects were analyzed according to the actual treatment received.

Results

Between 2001 and 2006 six human clinical trials with the hexadecapeptide AOD9604 have been performed, 893 healthy, in all but one study, clinically obese adults participated in these studies and are the basis of this safety evaluation. The details of the individual studies are listed in supplementary data. The first 3 studies were dose-escalating studies investigating the acute effects of various dosages and two application routes (i.v. and oral) in healthy or obese male subjects. These single dose studies were followed by a 7-day multiple dose study (METAOD004) as well as two

long-term clinical trials (METAOD005 and METAOD006) where the safety and tolerability of chronic oral treatment with AOD9604 was investigated.

In the first two clinical trials (METAOD001 and METAOD002) AOD9604 was delivered by i.v. injection. Therefore, the safety results of these two trials will be evaluated separately from the other 4 trials (METAOD003 to METAOD006), in which AOD9604 was administered orally.

Administration route: IV infusion

Single dose, dose-escalating study

In the first dose-escalating study (METAOD001) 15 healthy male subjects received 3 single dosages of AOD9604 and placebo as single dosages each separated by a 7-day washout period (range 25 to 400 µg/kg bodyweight; single IV infusion doses over 20 minutes). One subject terminated the study due to personal reasons, 14 subjects completed the study. In total twenty-nine AEs were reported by twelve subjects during the study. No SAEs occurred during this study. The most common AEs reported during the study were headache (6 times). The remainder were related to fatigue (4), hypoglycemia unspecified (3), dizziness (3), nasopharyngitis (2), cough (2) and lethargy, tonsillitis, abdominal pain unspecified, application site reaction unspecified, sore throat unspecified, injection site bruising, rhinitis seasonal, anorexia, injection site pain, all with an incidence of 1. None of the AEs were of severe intensity. The majority of AEs were mild in intensity with possible relationship to study treatment, equally distributed between the various concentrations of AOD9604 and placebo treatment. The adverse event profile was similar following administration of all treatments.

There were no clinically significant changes in vital signs, physical examinations findings, clinical laboratory parameters or ECG during the study. No significant glycerol, glucose or IGF-1 trends were observed for AOD9604, rhGH and placebo administrations.

A similar clinical study was conducted in obese subjects (METAOD002). In that double-blind placebo-controlled, 4 sequence, 4 period Williams' Latin Square design study 23 subjects participated. The subjects were 19 to 50 years old and had a BMI ≥ 35 kg/m² (range 36 to 67 kg/m²). Each subject received 4 single doses (25, 50 and 100 µg/kg AOD9604 or placebo; single IV infusion doses over 20 minutes), separated by a 7-day washout period.

In total 118 AEs were reported. No SAEs were reported. The most common adverse event reported by 16/23 subjects (69.6%) was mild or moderate headache. From all the reported AEs three events were reported of severe intensity (one in the 50 µg/kg AOD9604 group, 2 in the placebo group), with one of those events (a feeling of chest tightness) deemed possibly related to the AOD9604 treatment. Mild or moderate euphoria deemed possibly related to treatment, was reported

Table 1. Changes in IGF-1 Levels (Mean \pm SEM) After 12 Weeks of Treatment With AOD9604

Treatment group	IGF-1 changes from baseline	P-value (mmol/L) compared to placebo
Placebo	0.12 (0.56)	
1 mg AOD9604	-0.12 (0.58)	1.00
5 mg AOD9604	-0.18 (0.55)	0.99
10 mg AOD9604	0.41 (0.52)	0.99
20 mg AOD9604	1.28 (0.53)	0.38
30 mg AOD9604	0.63 (0.59)	0.95

by 5/23 subjects, during the periods when the AOD9604 was administered. There were no reports of euphoria during placebo administration. In total, there was no observable trend between the different treatment groups with respect to the incidence of certain AEs.

As in the previous study there were no clinically relevant changes observed in safety laboratory parameters 24 hours following administration of AOD9604 or placebo. Similarly, there were no clinically relevant changes in vital signs (blood pressure, radial pulse rate and temperature) or ECGs recorded at any of the scheduled time points up to 24 hours post dose. There were no significant changes in glucose or IGF-1 levels following AOD9604 treatment compared with placebo.

In total it could be concluded, that the administration of AOD9604 as single IV doses to healthy and healthy obese male volunteers was well tolerated in the concentration range between 25 μ g - 400 μ g/kg bodyweight, and that the safety profile of AOD9604 was comparable with the reference treatment, rhGH and placebo.

Administration route: Oral

Single dose or short-term studies

The objective of the 3rd study (METAOD003) was to assess the safety, tolerability and pharmacodynamics of single oral doses of AOD9604 in healthy, clinically obese males. 17 subjects (n = 15 completed the study), age 35 to 54 years, with a BMI \geq 35 kg/m² (range 35 to 56 kg/m²) subsequently received 3 increasing doses of AOD9604 (9, 27 and 54 mg) or placebo. Each dose administration was separated by a 2-week wash-out period.

A total of 97 AEs were reported by 17/17 subjects during this study. Most of them were of mild or moderate in intensity, with the exception of two SAEs, one of which (diar-

rhoea) was deemed “possibly related” to study treatment (54 mg AOD9604) and one (bronchial pneumonia) deemed to be “unrelated” to the study treatment (54 mg AOD9604). The most common adverse event reported was mild or moderate headache followed by events related to the digestive system, specifically diarrhea, flatulence, increased appetite and nausea. There was no observable trend between the AOD9604 groups or the placebo with respect to the incidence. The only event deemed definitely related to the treatment was taste perversion occurring 10 minutes following dose administration of the placebo.

There were no significant changes in IGF-1 values observed during 24 hours following any AOD9604 dose compared with placebo. No significant differences in glucose levels were observed following AOD9604 administration compared with placebo with the exception of one isolated time point (8% increase 12 hour post treatment in one subject receiving 54 mg AOD9604). There were no clinically significant observable trends in vital signs, physical examinations, abnormalities noted in the ECG measurements, or findings in the safety-related laboratory tests throughout the study.

Based on the findings of this study the investigator concluded that there were no safety related concerns during the conduct of the study, and that AOD9604 was well tolerated over the oral doses of 9 mg, 27mg and 54 mg.

The first multiple dose study was performed as a randomized, double-blind, placebo-controlled Phase IIa study (METAOD004), 36 healthy, clinically obese male volunteers, age 18 to 54 years, with a BMI \geq 30 kg/m² (range 30 to 47 kg/m²) were treated with doses of either 9, 27 or 54mg of AOD9604 or placebo (n = 9 each) for 7 days.

A total number of 207 AEs were reported by 36/36 subjects. All but one was of mild to moderate intensity (placebo-treated subject, soft tissue injury to left shoulder, unrelated to study treatment). No SAE occurred during the 7-day treat-

Table 2. Summary of Serious Adverse Events (SEA) Reported

	Placebo (n = 125)	AOD9604 0.25 mg (n = 127)	AOD9604 0.5 mg (n = 125)	AOD9604 1 mg (n = 125)	Total (n = 502)
Any serious adverse event	8	3	1	6	18 (3.6%)

ment and the 7-day follow-up time. The rate as well as the AE profile was comparable in the 9 mg, 27 mg AOD9604 and the placebo group. There was no observable trend between treatment groups with respect to the incidence of certain AEs, however subjects who received 54 mg AOD9604 experienced a greater number of headaches, diarrhea and flatulence.

Serum IGF-1 levels remained relatively constant over the dosing period with no apparent differences between treatment groups. Fasting plasma glucose and serum insulin levels remained unchanged throughout the treatment period. Furthermore, no changes in any of the OGTT parameters were observed from day 1 to day 7 of treatment. There were no study related clinically significant findings in the safety related laboratory tests, vital signs, or ECG measurements.

Long-term studies

Two long-term studies have been performed with AOD9604 (METAOD005 and METAOD006).

The first was a double-blind, placebo-controlled, parallel group, multi-center study (5 Australian hospitals) (METAOD005). In this study 300 healthy obese males and females (BMI ≥ 35 kg/m²; Median BMI: 40 kg/m², range: 35 to 67 kg/m²; 30 to 65 years old; 54% males and 46% females) were randomized to a 14-week period of daily oral dosing. The treatment period comprised a 2-week single-blind placebo run-in period followed by 12 weeks administration of either placebo or AOD9604 (1, 5, 10, 20 or 30 mg AOD9604 or placebo once daily; n = 50 per group).

The analysis of the AE was divided into the run-in phase and the treatment phase. During the run-in placebo treatment phase 70% of subjects experienced at least one AE, the incidence of which was similar across treatment groups (range 64.6% to 79.6%); the body system organ classes with the highest incidences (> 10%) of events were the nervous system (32.2%; mainly headache, 30.4%), gastrointestinal system (19.4%) (mainly diarrhea unspecified, 5.5%) and infections and infestations (16.6%, mainly viral infections, 3.5%).

After the commencement of the active treatment 88.9% of subjects experienced at least one AE, whereby the distribution was similar in the 5 AOD9604 groups and the placebo group. There was a higher incidence (48.4%) of nervous system disorders (mainly headache, 42.6%), gastrointestinal

disorders (30.4%, mainly diarrhea unspecified, 9.0%) and infections and infestations (45.3%), than seen before the commencement of active treatment. The distribution of the intensity of AEs was similar across all treatment groups. The percentage of AEs that deemed to be possibly or probably related to the study medication was similar across all treatment groups, including placebo.

Five patients in the study reported a serious AE; three in the AOD9604 20 mg group (basal cell carcinoma, moderate lipoma and squamous cell carcinoma), one in the 5 mg group (breast cancer) and one in the 10 mg group (malignant melanoma). According to the investigator, none of the SAEs reported were considered to be possibly, probably or definitely related to study medication (see discussion).

There were no significant changes or notable trends in standard laboratory parameters (hematology, biochemistry and urinalysis), vital signs, or ECGs throughout the study in any AOD9604 or placebo group.

The effect of AOD9604 treatment on IGF-1 levels was of particular interest, since hGH administration is associated with increases in IGF-1 levels. Prolonged increases in IGF-1 may have a variety of undesirable effects, including an increase in cancer risk [22, 23]. In this study the levels of circulating IGF-1 were measured at the baseline visit and at the end of the 12-week treatment period. There was no significant change in the levels of IGF-1 in any of the treatment groups (Table 1).

There were no significant changes or obvious trends in any mean values of the parameter from the OGTT in any treatment group at any time point. In the AOD9604 treatment groups, there was a trend for improved glucose tolerance; however, there were no statistically significant changes in any parameters measured.

Anti-AOD9604 antibody analysis was performed at baseline, after 4, 8, and 12 weeks of treatment. In none of the subjects at none of the timepoints were antibodies against the peptide detected in serum collected during the study.

The second long-term study (METAOD006) was a randomized, double-blind, placebo-controlled, multi-center, parallel group study conducted at 16 Australian hospitals and medical centres. In that study 534 were enrolled but of those 502 clinically obese subjects (BMI ≥ 30 kg/m² and ≤ 45 kg/m²; Median BMI: 36.3 kg/m², range: 30 to 45.2 kg/m²; 44% males and 56% females) were randomized to receive a

daily dose of 0.25, 0.5 or 1mg AOD9604 or placebo for 24 weeks. Prior to this treatment period all subjects underwent a 4-week single-blind placebo run-in period. After cessation of the treatment a 4-week follow-up phase was performed.

Prior to commencement of active treatment, 48.4% of subjects experienced at least one AE. The body system organ classes with the highest incidences of events (> 10%) were the nervous system (17.5%; mainly headache, 14.5%), infections and infestations (15.9%, mainly nasopharyngitis and upper respiratory tract infection, 4.0%), gastrointestinal system (12.4%, mainly diarrhea 3.2%) and musco-skeletal and connective tissue disorders (12.0%, mainly back pain, 4.0%), 32.9% of subjects experienced mild AEs, 38.6% experienced moderate AEs and 36 (7.2%) patients experienced severe AEs. The intensity of AEs was similar across all treatment groups. None of the AEs were deemed to be definitely related to the study treatment.

After the commencement of active treatment, 78.7% of subjects experienced at least one AE, with the incidence ranging from 75.6% to 83.2% across all treatment groups (83.2% placebo group; 75.6% 0.25 mg AOD9604 group). There was a high incidence of infections and infestations (46.8%, mainly nasopharyngitis, 17.1%), nervous system disorders (30.1%, mainly headache, 25.9%), musculo-skeletal and connective tissue disorders (25.5%, mainly back pain, 8.2%), and gastrointestinal disorders (22.9%, mainly diarrhea, 7.8%). Although the percentage of subjects experiencing AEs in these body systems is higher than in the run-in period there was no obvious pattern with respect to treatment received, suggesting that the increase was likely due to the longer period of assessment in the active treatment phase.

Overall, there were no AEs that were deemed to be “definitely related” to the study treatment. The percentage of AEs that were deemed to be “probably” or “possibly related” to study treatment was similar among all treatment groups including placebo. The most common classes of AE deemed to be “probably” or “possibly” related to study treatment were gastrointestinal disorders (5.2% overall) and nervous system disorders (4.9% overall).

A total of 18 patients (3.6%) reported at least one SAE. The distribution of SAEs was similar among all treatment groups (Table 2). The most common SAEs reported were in the injury, poisoning and procedural complications body system class (6 patients, 1.2%). The others were general disorders and administration site conditions (2 patients; 0.4%), infections and infestations (2 patients; 0.4%), musculo-skeletal and connective tissue disorders (2 patients; 0.4%), and vascular disorders (2 patients; 0.4%).

There were no changes in laboratory parameters or vital signs in any treatment group. There were no clinically significant abnormalities in vital signs, safety tests, or ECGs during the studies. At no time were statistically significant differences in IGF-1 levels among the treatment groups and placebo detected. The overall mean changes in IGF-1 were

1.76 nmol/L and 1.24 nmol/L after 12 and 24 weeks of treatment, respectively. There were no statistically significant differences between the treatment groups or placebo ($P = 0.50844$ after 12 weeks and $P = 0.75754$ after 24 weeks).

An OGTT was performed at screening and after 12 and 24 weeks of treatment. At these visits blood samples for assessment of glucose and insulin were collected immediately prior to and 2 hours after an oral glucose load. After 12 weeks the overall change in pre-load glucose was -0.02 units and there were no significant differences between the randomized treatment groups ($P = 0.73488$). The changes in pre-load glucose in the placebo group differed by -0.08, -0.06, and -0.07 units from those obtained in the AOD9604 0.25 mg, 0.5 mg, and 1 mg treatment groups, respectively; none of these differences were statistically significant. Similar results have been obtained after 24 weeks of treatment. The overall change in pre-load glucose after 24 weeks treatment was 0.04 units, and there were no significant differences among the treatment groups ($P = 0.62787$). Estimated differences from placebo in change in pre-load glucose were -0.03, 0.02, and 0.06 units for the AOD9604 0.25 mg, 0.5 mg, and 1 mg treatment groups, respectively; none of these differences were statistically significant.

There were no anti-AOD9604 antibodies detected in the subset of patients selected for antibody assay.

In total the treatment of obese subjects for 24 weeks with daily dosages of 0.25 mg to 1 mg AOD9604 displayed a good safety and tolerability profile that was indistinguishable from placebo.

In all of the presented studies no treatment-related withdrawals or SAEs, clinically significant AEs, changes of clinical significance in vital signs, safety laboratory tests or ECGs were observed. Therefore the daily intake of AOD9604 in dosages of 1 mg/day over 24-weeks can be stated as being safe and well tolerated.

Discussion

Obesity is no longer a health problem of just the industrialized countries but is now recognized as being present all over the world. It has global implications for health and disease associated with significant morbidity and mortality, particularly diabetes and cardiovascular disease.

Human Growth Hormone (hGH) is not only important for growth processes during childhood, but plays a pivotal role in lipid metabolism throughout life. It is well known that hGH is involved in the regulation of lipolysis and lipogenesis. Therefore, hGH was implicated as a good potential candidate for the treatment of obesity. However undesired side effects, such as induction of glucose intolerance and insulin resistance, diabetes, acromegaly, cancer, edema, and hypertension [10-13] rendered therapeutic doses of hGH unsuitable for long-term treatments in humans.

In vitro and in vivo investigations revealed a specific region within the hormone molecule that is responsible for the molecular events associated with lipid metabolism [18, 24, 25]. AOD9604 is a peptide fragment of the C-terminus or lipolytic domain of hGH (hGH₁₇₇₋₁₉₁), with an additional tyrosine residue at the N-terminal end for stabilization. In vitro and in vivo experiments have shown similar effects of AOD9604 and hGH on lipid metabolism when chronically applied to mice [20, 21]. Interestingly, AOD9604 mimics the effect of hGH on lipid metabolism, without having growth promoting or pro-diabetic effects. The safety and tolerability of AOD9604 has been studied in the human clinical trials described in this paper.

Up to the date of this paper, six clinical trials (three single dose studies and three multiple dose studies from which 2 were long-term studies) have been conducted with AOD9604. In none of the three acute dose-escalating studies (METAOD001 - METAOD003) were there reported any AEs related to the intake of AOD9604, withdrawals from the study caused by AOD9604 or SAEs reported. No treatment related differences were identified upon administration of AOD9604 by IV injection (25 µg - 400 µg/kg bodyweight) or orally (9 mg - 54 mg). Treatment with AOD9604 as a single dosage had no effect on physical examination, vital signs, laboratory parameters, ECG, blood glucose and IGF-1 levels, with results indistinguishable from placebo.

In the multiple dose and long term studies, AOD9604 was well tolerated over the entire dose range. In none of the studies did any drug-related withdrawals or drug-related serious AEs occur. No drug related clinically significant AEs, or changes of clinical significance in vital signs, safety laboratory tests or ECGs were detected during the studies. There were no observable trends in the incidence of AEs between the 0.25 mg, 0.5 mg, 1 mg, 9 mg and 27 mg AOD9604 and placebo treatment groups. The highest dose administration (54 mg), however, was associated with an increased incidence of GI-related AEs.

It is well known that hGH is associated with increased IGF-1 levels. IGF-1 may have a variety of undesirable effects, including an increase in cancer risk [22, 23]. In the studies discussed herein, IGF-1 levels were monitored in all long-term studies but did not reveal clinically significant differences between dose groups or placebo. Therefore the 5 SAEs that occurred in the 12 week treatment study (three in the AOD9604 20 mg group (basal cell carcinoma, moderate lipoma and squamous cell carcinoma), one in the 10 mg group (malignant melanoma) and one in the 5 mg group (breast cancer)) could not be attributed to increased IGF-1 levels. The Principal Investigator considered none of the reported SAEs to be “possibly”, “probably” or “definitely related” to the study medication. The rationale behind this judgment was that none of the cancer forms occurred in the highest dosage group (30mg AOD9604/day), therefore a dose effect can be excluded. Further examination of the

SAE cases indicated that these subjects had neglected their personal medical care for a longer period of time, so that the higher incidence of cancer may well have occurred due to the natural incidence rate of cancer events in the population.

It has to be noted that three of the SAEs were skin cancer forms. Since the study was performed in Australia, a country with the highest incidence rate of skin cancer (<http://globocan.iarc.fr/>), this cumulative incidence is not improbable. Furthermore the study was performed on clinically obese subjects with a BMI ≥ 35 kg/m² (BMI ≥ 35 kg/m²; Median BMI: 40 kg/m², range: 35 to 67 kg/m²). It is known that the incidence of several types of cancers is associated with increased BMI [26].

The full activation of the hGH-receptor requires dimerization of two receptor molecules by one intact growth hormone molecule. The hGH has two different binding regions, site 1 and site 2, which bind in a sequential manner to two different regions of the receptor. Only if this trimer of one hGH molecule and two receptors is formed, does the subsequent signal transduction pathway become initiated [27, 28]. The hexadecapeptide AOD9604 consists only of amino acids 177-191 of hGH with an additional tyrosine residue at the N-terminus. The binding site 1 of the hGH, which is located in the fourth helix [27], is partially overlapping with the sequence of AOD9604. However, binding site 2 of hGH is completely missing in AOD9604. Therefore, it was hypothesized that AOD9604 is unable to induce dimerization and thereby activation of the receptor. This has been confirmed in previous in vitro experiments. Competition binding assays in cells transfected with the 125I-hGH receptor have shown that AOD9604 is incapable of competing with hGH for binding [20]. In a highly sensitive BaF3 cell proliferation test Heffernan et al (2001) also showed that AOD9604 did not induce cell-proliferation even in very high dosages [20].

Nevertheless, the hypothesis that AOD9604 does not activate the hGH/IGF-1 axis had to be tested in humans. The studies presented here confirm the in-vitro results. In these studies no clinically relevant changes of IGF-1 levels were observed and no differences to the placebo treatment were found. Together with the lack of any other symptoms associated with known IGF-1 mediated effects, such as sodium retention, tissue oedema, hypertension, or impaired glucose tolerance, the results demonstrate that AOD9604 does not activate the hGH/IGF-1 pathway and therefore has no growth promoting effect.

Prolonged use of intact hGH may result in negative effects on glucose metabolism, such as glucose intolerance and insulin resistance. Given that AOD9604 is a peptide fragment of the C-terminus of hGH, its effect on glucose control was monitored. OGTT testing was carried out on all patients during the screening phase as well as at various time points during the treatment period. No significant changes or obvious trends in the OGTT in any treatment group were observed, suggesting that AOD9604 supplementation does not

deteriorate glucose control or induce insulin resistance. In contrast, treatment with AOD9604 seemed to have a positive effect in subjects with impaired glucose tolerance. In the 12 weeks treatment study, patients with impaired glucose tolerance supplemented with AOD9604 were less likely to develop diabetes during the study than subjects taking placebo. This hypothesis, however, has to be confirmed in a separate clinical trial. In contrast, in a recent clinical trial of hGH effects in obese women, hGH caused an increase in IGF-1 levels over a 6 month dosing period and decreased glucose tolerance in a subset of trial participants [29].

These results also demonstrate that, unlike hGH, AOD9604 has no negative effect on carbohydrate metabolism. This was previously demonstrated in mice. Chronic administration of hGH to ob/ob mice depressed glucose oxidation and increase plasma glucose levels. AOD9604 had no such effects, no changes in circulating plasma glucose in either lean nor obese ob/ob mice have been observed [20]. AOD9604 did not cause hyperglycemia or affect insulin sensitivity in rats and mice [18, 20].

Finally, the hexadecapeptide AOD9604 did not induce allergic reactions when consumed over 24 weeks. Blood of patients was analyzed for the presence of anti-AOD9604 antibody formation at various times and at the end of the studies (latest time point after 24 weeks). In none of the performed studies, at no time, were anti-AOD9604 antibodies detected in serum collected from any subjects in any treatment group.

In conclusion, the evaluation of these clinical trials demonstrates that AOD9604 displayed an excellent safety profile indistinguishable from placebo. AOD9604 was also very well tolerated in all studies described.

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Declaration of Interests

David Kenley - holds a 6.9% interest in Calzada Ltd, which fully owns Metabolic Pharmaceuticals Pty Ltd. Evert Vos - is a Consultant to Metabolic Pharmaceuticals Pty Ltd. He was previously the Medical Director of the company responsible for all of the human clinical trials. Heike Stier is an employee of analyze and realize ag and has written this manuscript. Analyze and realize ag acts as a consultant to Metabolic Pharmaceuticals Pty Ltd in relation to possible novel food

applications in the European region.

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