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Applicant: Kamada, Ltd

Product: Alpha 1 Proteinase Inhibitor (Human) Intravenous, 2% Solution for Injection

Subject: Final Nonclinical Pharm-Tox Review

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Background Summary

The active ingredient in Kamada-API is purified human Alpha-1 Proteinase Inhibitor (A1-PI) formulated at a final concentration of 2% active A1-PI in a phosphate-buffered saline solution for injection. A1-PI is an inhibitor of serine proteases, most importantly elastase activity in the lungs. Human A1-PI, especially its reactive site is conserved among mammalian species suggesting a conserved inhibitory activity. The final specifications are tabulated in Table 1.

Indication and dosage: Kamada-API is indicated for chronic augmentation and maintenance therapy in adults with emphysema due to congenital deficiency of alpha-1-proteinase inhibitor at a dosage of 60 mg/kg body weight administered once weekly by intravenous infusion at a rate not to exceed 0.08 mL/kg body weight/minute.

Table 1: Release specifications

Test	Specification
Appearance	The solution is clear and colorless to yellow-green. May contain a few particles.
Identification	
---(b)(4)---	---(b)(4)-----
-(b)(4)-	---(b)(4)-----
Potency	
Total Active API Content	---(b)(4)-----
Active API Content	---(b)(4)-----
Specific Activity	---(b)(4)-----
Excipients	
Sodium	---(b)(4)-----
Chloride (as NaCl)	---(b)(4)-
Phosphate	---(b)(4)-----
Purity and Impurities	
------(b)(4)----- -----	---(b)(4)-----
Residual TnBP	---(b)(4)-----
Residual Tween 80	NMT 20 ppm
------(b)(4)-----	---(b)(4)-----
Safety	
Bacterial Endotoxin	---(b)(4)-----
Pyrogenicity	Pass

Test	Specification
Sterility	Pass
General Safety Test	Meets Requirements
General tests	
pH	-(b)(4)--
Extractable Volume	---(b)(4)---

Nonclinical Studies, Summary

Table 2 summarizes the toxicology studies performed with A1-PI. In two GLP-compliant single dose general toxicology studies two formulations of 2% A1-PI were evaluated, one containing sodium phosphate-buffered saline solution and the other containing sodium phosphate-buffered --- (b)(4)--- solution. The current formulation being evaluated (i.e. 2% A1-PI in --(b)(4)- sodium phosphate buffer and -(b)(4)- NaCl) was evaluated in repeated-dose toxicity study in rabbits.

Table 2: Toxicology Summary

Study type	Route of administration, regimen and dose levels	Species	GLP compliance	Study Number
Acute toxicology study in rats	Single Intravenous bolus injection 0, 60 and 640-650 mg/kg	Sprague-Dawley rats	Yes	KAM/029/AIT
Acute toxicology study in rabbits	Single Intravenous bolus injection 0, 60 and 600 mg/kg	New Zealand White rabbits	Yes	KAM/030/AIT
Repeated dose toxicology study in rabbits	Intravenous bolus injection once daily for 5 consecutive days 0 and 300 mg/kg	New Zealand White rabbits	Yes	KAM/031/RIT
Neoantigenicity study in rabbits	Intradermal, intramuscular, subcutaneous 20 mg	New Zealand White rabbits	Yes	-(b)(4)-071902

Other studies performed to support this BLA are two *in-vitro* cytotoxicity studies as well as a Pharmacokinetic (PK) study comparing single injections of Kamada-API and Prolastin™ in rabbits.

Main Findings

- There were no test article-related effects on clinical observations, body weights or hematology indices after single dose IV administration of Kamada-API in rats.
 - A statistically significant decrease in group mean Alanine aminotransferase (ALT) levels was seen on Day 15 in animals treated with low- (one times human dose) and high-dose (more than ten times human dose) Kamada-API in saline when compared to vehicle control. This change was minor in magnitude, dose-independent and had no microscopic correlates.
- There were no test article-related changes in clinical observations, body weights, body weight gains or gross pathology after single dose IV administration of Kamada-API in rabbits.
 - There were no test article-related effects on serum chemistry indices after the 14-day recovery period.

3. There were small hematology and serum chemistry changes seen in the repeated-dose toxicity study in rabbits receiving 300 mg/kg, or five times human dose Kamada-API, such as small reductions in group mean lymphocytes, small reductions in group mean Creatinine Phosphokinase (CPK), and minor increase in group mean neutrophils. Recovery was observed at the end of the 14 day recovery period.
4. There was no cytotoxicity potential of Kamada-API on lung tissue using *in vitro* Safety Pharmacology studies.
5. There was no increase in antigenicity potential of Kamada-API after manufacturing change.
 - a. Viral inactivation step added in the manufacturing process of A1-PI did not cause neoantigenic changes to Kamada-API as measured using -(b)(4)-.

Excipients and Impurities

TnBP

Kamada-API contains -----(b)(4)----- of TnBP in the final formulation. Thus, the greatest amount of TnBP that could be administered with the highest weekly dose of 60 mg/kg Kamada-API is -(b)(4)-.

1. Similar exposure to TNBP is obtained after intravenous use of other approved products, such as different immune globulin preparations.
2. The NOAEL of TNBP in rabbits receiving 13 weeks of daily intravenous administrations was shown to be -(b)(4)- or more than 13 times the human once weekly dose. At this dose spleen and thymus weight increases were observed but this finding was not considered adverse.

In conclusion, the exposure of TnBP resulting from the clinical use of Kamada-API according to the PI is considered safe.

PS80

Kamada-API contains no more than 20 ppm (0.002%) of polysorbate 80 in the final formulation. The greatest amount of polysorbate 80 that could be administered per a 60 mg/kg dose of Kamada-API is 0.06 mg/kg. This amount is less than other plasma derived IV preparations for example immune globulin preparations that have a long and safe use in the clinic.

In conclusion, the exposure of PS80 resulting from the clinical use of Kamada-API according to the PI is considered safe.

Conclusions

There are no preclinical issues that would prevent this product from approval.

Complete Review of Preclinical Studies

Study Number KAM/029/AIT

Title: -(b)(4)-API Acute Intravenous (IV) Toxicity in Rats

Model: Sprague-Dawley rats

Design: N=48 rats weighing within 20% of the mean value were randomized in one of the following 12 groups which received IV injections (tail vein) of Kamada-API in the 2 formulations at dosages 60 and 640-650 mg/kg or the vehicle controls. Animals in the low dose group received a bolus injection whereas high-dose level and vehicle-treated groups received slow IV injection at a rate of about 1 ml/minute.

Group No.	N (M/F)	Treatment	Day of Sacrifice Post-Dosing
1	2/2	-(b)(4)-	Day 3
2	2/2		Day 14
3	2/2		Day 3
4	2/2		Day 14
5	2/2		Day 3
6	2/2		Day 14
7	2/2		Day 3
8	2/2		Day 14
9	2/2		Day 3
10	2/2		Day 14
11	2/2		Day 3
12	2/2		Day 14

Two animals per sex in each dose level were sacrificed 3 days following dose administration, and another 2 animals/sex for all dose groups were maintained for 14-days after treatment to assess reversibility, persistence or delayed occurrence of any toxic effects.

Outcome measurements: Clinical signs, body weight, clinical pathology including hematology, gross pathology. Histopathology slices of lung, liver, kidney and heart were collected but there was no histopathology performed.

Results

There were no test article-related effects on clinical observations, body weights or hematology indices for either formulation of Kamada-API. There was a statistically significant decrease in group mean ALT levels in animals treated with 60 mg/kg and 640-650 mg/kg Kamada-API in -(b)(4)- NaCl, when compared to vehicle control, on Day 15. This change was minor in magnitude, dose-independent and had no microscopic correlates. A statistically significant increase in group mean urea was observed on Day 4 in the 60 mg/kg Kamada-API/---(b)(4)-group. There were no findings on necropsy and the values were normal on day 15.

Conclusions

A single administration of Kamada-API in both formulations was well-tolerated by the rats.

Study Number KAM/030/AIT

Title: -(b)(4)--API Acute Intravenous (IV) Toxicity in Rabbits

Model: New Zealand White Rabbits weighing 2.6-3.2 kg at study initiation

Design: The design was identical with the rat study reviewed above (study # KAM/029/AIT) with the location of the dosing being one of the marginal ear veins.

Results

There were no test article-related changes for either formulation in clinical observations, body weights, body weight gains or gross pathology. Hematology changes were minor increases in group mean RBC, hemoglobin and hematocrit parameters in the high-dose (600 mg/kg) animals receiving Kamada-API in ----(b)(4)-- at terminal sacrifice on Day 4. Changes in clinical chemistry parameters consisted of decreases in alkaline phosphatase in the low- and high-dose Kamada-API/-(b)(4)- NaCl groups and glutamyl transpeptidase levels in the high-dose Kamada-API/-(b)(4)- NaCl group on Day 4. There were no test article-related effects on serum chemistry indices after the 14-day recovery period. There was an increase in mean potassium concentration on Day 15 in animals receiving 60 mg/kg Kamada-API /-(b)(4)- NaCl, but this was considered incidental since it was minor in magnitude and dose-independent.

Conclusions

A single administration of Kamada-API in both formulations was well-tolerated by the rabbits.

Study Number KAM/031/RIT

Title: Repeated Intravenous (IV) Toxicity in Rabbits

A GLP-compliant, 5-day repeated dose, general toxicology study

Test Article: -(b)(4)--API 2% batch numbers: -(b)(4)- (23 mg/ml in Na-Phosphate buffer containing -(b)(4)- NaCl), -(b)(4)- (22 mg/ml in Na-Phosphate buffer containing ----(b)(4)---). Stability confirmed for 24 months.

Design: New Zealand White rabbits weighing 2.8-3.2 kg were randomly assigned into 8 groups, N=2M/2F group receiving 300 mg/kg Kamada-API either formulated in -(b)(4)- sodium phosphate buffer containing -(b)(4)- sodium chloride or -(b)(4)- once daily for 5 successive days. Groups 1-4 were sacrificed one day after the last administration. Groups 4-8 were recovery groups and were maintained for 14-days following the end of treatment to assess reversibility, persistence or delayed occurrence of any effects.

Group No.	Treatment	Day of Sacrifice
1	-(b)(4)-	1
2		
3		
4		
5	-(b)(4)-	14
6		

Group No.	Treatment	Day of Sacrifice
7	-(b)(4)-	
8		

Outcome Measures: Clinical signs, body weight, clinical pathology (hematology and chemistry), necropsy and macroscopic examination, histopathology

Results

Small reductions in group mean lymphocytes, Creatinine Phosphokinase (CPK) and a minor increase in group mean neutrophils in animals receiving 300 mg/kg Kamada-API/-(b)(4)- NaCl was observed on Day 6. In addition, a small, but statistically significant, elevation in group mean urea was observed with 300 mg/kg Kamada-API/-(b)(4)- on Day 6.

A slight but statistically significant increase in group mean absolute spleen weight and spleen weight to body weight ratio in the Kamada-API/-(b)(4)- treated group was observed on Day 6 without microscopic correlates. There were no differences with the controls on Day 15.

There were minimal to mild perivascular hemorrhage and inflammation in the local injection site, minimal blood vessel thrombus and minimal epidermis encrustation in both test article and formulation controls. These changes could be related to needle injury.

The histopathology is limited to liver, kidney, lungs and injection sites.

Conclusions

Repeat administration of Kamada-API over 5 consecutive days was well-tolerated in the rabbits with no apparent adverse toxicities.

Study Number -(b)(4)-01 and Report -(b)(4)-071902

Title: Alpha 1 Proteinase Inhibitor (human) Neoantigenicity Study in Rabbits; Immunoassay Sample Testing

Aim: A neoantigenicity study to assess whether the inclusion of viral removal/inactivation steps in the manufacturing of Kamada-API resulted in protein structural changes and novel epitope presentation.

Animal Model: New Zealand White female rabbits

Design: N=6 F rabbits/ treatment group and 3 F rabbits for the negative control group were immunized IM or SC for 25 weeks with Kamada-API not subjected to the viral inactivation step (N-API) or Kamada-API chemically modified with trinitrophenol (TNP-API, positive control), or viral inactivated Kamada-API (VI-API) or 'sterile water for injection' (negative control).

First immunization was 1 ml -----(b)(4)----- + 1 ml test article and the subsequent immunizations were 1 ml -----(b)(4)----- + 1 ml test article. The animals were immunized weekly for the first three doses and then every two weeks or monthly for the remainder of the study.

Outcome measures: Food consumption, clinical signs and body weights.

Immunology: Sera collected prior to immunization (background levels) and at the time of highest antibody titer from each group were passed through an affinity column containing N-API, which removed all antibodies directed towards native API protein. Antibodies directed towards the TNP portion of the TNP-API molecule and towards any new epitopes that may be present upon viral

inactivation remained in the flow-through. Antibodies directed specifically against N-API, TNP-API or VI-API were detected and quantitated by -(b)(4)-.

Results

TNP-API immunization resulted in antibodies that were not removed by the N-API column, but specific for TNP-API. In contrast, no difference in antibody amount between N-API and VI-API groups (on respective -(b)(4)-) was observed, indicating that the viral inactivation step in the manufacturing process does not cause neoantigenic changes to Kamada-API.

Pharmacology

Two *in vitro* Safety Pharmacology studies were performed to assess the cytotoxicity potential of Kamada-API on lung tissue.

Study Number C-13003-118-0905

Title: In Vitro Cytotoxicity of ----(b)(4)--- with Kamada-API

Aim: To assess the potential of Kamada-API to adversely affect human lung sub-bronchial gland adenocarcinoma -(b)(4)- cell line monolayer integrity by measuring the TEER.

Rationale: Monolayers of -(b)(4)- cell line have high transepithelial electrical resistances (TEER) of approximately 1000-1300 Ω cm². Loss of monolayer integrity is accompanied by a decrease in TEER to values below 600 Ω and an increase in permeability.

------(b)(4)-----

Results and Conclusions

TEER measurements for Kamada-API at all concentrations were comparable to the negative control at all time points. The positive control, 0.1% SDS, dramatically decreased the TEER values by greater than 50% at all time points and is considered toxic to the cells.

Study Number KAM/117/CTX

Title: In Vitro Cytotoxicity of Kamada-API Using -----(b)(4)-----

Aim: To investigate the potential of Kamada-API's for cytotoxicity using the ---(b)(4)-- system.

Rationale: ---(b)(4)--- system consists of normal, human-derived tracheal/bronchial epithelial cells cultured to form a multilayered model resembling the epithelial tissue of the respiratory tract. TEER in this model is similar to *in vivo* tissues, averaging $550 \pm 125 \Omega$ /cm².

------(b)(4)-----

Results and Conclusions

Cell viability for Kamada-API treated tissues was calculated relative to negative-treatment control and was 99-111%. The buffer negative control when compared to the no-treatment control had an average of 86% cell viability.

Treatment with 0.3% triton X-100 caused an average of 96% cytotoxicity.

Report -(b)(4)- 405

Title: Pharmacokinetics for Intravenously Administered human Alpha-1 Proteinase Inhibitor in Rabbits

Aim: To investigate the pharmacokinetics of Kamada-API in rabbits after a single intravenous bolus injection and compared it to Prolastin™.

Model: New Zealand White rabbits

Design: N=2M and 2F rabbits/group were injected with 1 mg/kg Kamada-API or Prolastin using an ear vein catheter. The blood for PK measurement was withdrawn using an ear artery catheter at 10 and 30 minutes and 1, 2, 4, 6, 12, 24, 48, 72, 96, 120, and 144 hours.

Results

Kamada-API and Prolastin demonstrated similar biexponential pharmacokinetic profiles, a distribution phase with short rapid elimination, followed by a longer terminal elimination phase. The half-life of Kamada-API during the Phase 1 elimination was statistically longer than that for Prolastin (16.3 vs. 10.0 hours, respectively). The mean maximum concentration (C_{max}) 42,264 ± 3,443 ng/ml terminal t_{1/2} 68.13 ± 13.53 hours and terminal elimination rate constants -0.0046 ± 0.0014 hr⁻¹, respectively. Similar values were observed with Prolastin (46,079 ± 7,550 ng/ml, 68.30 ± 17.40 hours and -0.0047 ± 0.0009 hr⁻¹, respectively). No apparent differences between male and females were observed.

Excipient and Impurities

TnBP

Kamada-API contains ----- (b)(4) ----- of TnBP in the final formulation. The greatest amount of TnBP that could be administered per a 60 mg/kg intravenous dose of Kamada-API is ----- (b)(4) -----.

NOAEL in GLP chronic studies reported from other submissions were --(b)(4)-- mg/kg/day for mice and rats respectively or more than 800 and 300 times the dose in Kamada-API respectively.

In the single-dose toxicity studies performed by Kamada, 600 mg/kg and 650 mg/kg of Kamada-API were administered IV to rabbits and rats, respectively; the amount of TnBP administered to these animals was ----- (b)(4) -----, respectively, approximately 10-fold greater on a mg/kg basis than that expected for humans. In the repeat-dose study, rabbits were administered --- (b)(4) --- Kamada-API once daily for 5 consecutive days; this is equivalent to ----- (b)(4) ----- TnBP per daily dose (~5-fold greater/day and ~25-fold greater for a five day exposure on a mg/kg basis than expected for humans). No major toxicities were observed in any of the toxicology studies.

The NOAEL in rabbits receiving 13 weeks of daily intravenous administrations is at least -(b)(4)- ----- (the highest dose tested), which is a safety margin of >13.3 fold when compared on a mg/kg basis. At this dose spleen and thymus weight increases were observed but this finding was not considered adverse.

In conclusion, the exposure of TnBP resulting from the clinical use of Kamada-API according to the PI is considered safe.

PS80

Kamada-API contains no more than 20 ppm (0.002%) of polysorbate 80 in the final formulation. The greatest amount of polysorbate 80 that could be administered per a 60 mg/kg dose of Kamada-API is 0.06 mg/kg. This amount is much less than other plasma derived IV preparations for example -----(b)(4)----- . The FAO/WHO Expert Committee on Food Additives has established a maximum acceptable daily oral intake of Polysorbates of 25 mg/kg, 416 times the dose in Kamada-API.

In the single-dose toxicity studies with Kamada-API, 600 mg/kg and 650 mg/kg of Kamada-API were administered IV to rabbits and rats, respectively; the amount of polysorbate 80 administered to these animals was 0.65 mg/kg and 0.60 mg/kg, respectively, approximately 10-11 fold greater on a mg/kg basis than that expected for humans. In the repeat-dose study, rabbits were administered 300 mg/kg of Kamada-API once daily for 5 consecutive days; this is equivalent to 0.3 mg/kg of polysorbate 80 per daily dose (~5-fold greater/day and ~25-fold greater/week on a mg/kg basis than expected for humans). No major toxicities were observed in any of the toxicology studies.

In conclusion, the exposure of PS80 resulting from the clinical use of Kamada-API according to the PI, is considered safe.