Study Protocol

Study Title: Compound A: Effects on the Heart Rate, Blood Pressure, and the Electrocardiogram by Oral Administration in Conscious Monkeys

Study Number: CJUGSEND00

Study Director: Taro Sendo

Test Facility: Example Contract Lab Name

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1. STUDY TITLE

Compound A: Effects on the Heart Rate, Blood Pressure, and the Electrocardiogram by Oral Administration in Conscious Monkeys

2. STUDY PURPOSE

The purpose of this study is to examine the effects of Compound A on the heart rate, blood pressure, and the electrocardiograms by single oral administration in conscious monkeys.

3. REGULATORY COMPLIANCE

This study will not be conducted under regulatory compliance.

4. ANIMAL WELFARE

This study is approved by the Institutional Animal Care and Use Committee (Approved No. IACUC00) and is performed in accordance with the animal welfare bylaws of Example Contract Lab Name, which is accredited by AAALAC International.

5. SPONSOR

Example Sponsor Inc.

6. TEST FACILITY

Example Contract Lab Name
1000 Anywhere Street, Honolulu, HI 11111
USA
TEL: 099-999-9999 FAX: 099-999-9999

7. KEY PERSONNEL

Study Director: Taro Seno
Statistical Analysis Responsibility:
Hanako Sendo

8. STUDY SCHEDULE

The day before the first dosing day is designated as Day −1. The first dosing day is designated as Day 1.

Study Initiation: July 29, 2014 (The day which study director signs protocol)
Acclimation Initiation: July 30, 2014
Acclimation Completion: August 5, 2014

Surgical Operation:	August 6, 2014 and August 7, 2014
First Dosing (Day 1):	September 3, 2014
Second Dosing (Day 8):	September 10, 2014
Third Dosing (Day 15):	September 17, 2014
Fourth Dosing (Day 22):	September 24, 2014
End of Observation (Day 23):	September 25, 2014
Final Report Creation /Study Completion:	January 30, 2015

9. MATERIALS AND METHODS

9.1. Test Article

Name:	Compound A
Source:	Example Sponsor Inc.
Lot No.:	123-456

9.2. Control Article / Vehicle

Name:	0.5 w/v% Methylcellulose (MC) solution
Manufacturer	
MC (Metolose SM-400):	Shin-Etsu Chemical Co., Ltd.
Water for Injection:	Otsuka Pharmaceutical Factory, Inc.

9.3. Dosing Formulations

9.3.1. Control Article / Vehicle Formulations

(SOP: TSB/002 and TSB/004)

Preparation Method:	The required amount of MC is weighed, adds to hot water for injection equal to 40% of the final volume, and the mixture is stirred until thorough dispersed. Cooled water for injection is then added to dissolve. This preparation is transferred to a measuring cylinder, makes up to the final volume with water for injection, and stirrers with a magnetic stirrer.
Expiration Date:	Within 30 days after preparation
Storage Condition:	Refrigerated (acceptable range: 2-8°C)
Storage Place:	Refrigeration Room in the Test Article Depository of Example Contract Lab Name

9.3.2. Test Article Formulations

(SOP: TSB/002 and TSB/004)

Concentration:	2, 6 and 20 mg/mL
Conversion Factor:	None
Preparation Method:	The three test article formulations (2, 6 and 20 mg/mL) are prepared separately. The required amount of test article is weighed and transferred to an agate mortar. Vehicle is gradually added to the agate mortar to prepare a suspension while gently mixing. The suspension is transferred to a measuring cylinder and made up to the final volume of formulation with vehicle.
Preparation Frequency:	Once for each concentration, prepared just before use

9.4. Dosing of the Test and Control Article Formulations

(SOP: GTX/210 and GTX/211)

Dosing Route:	Oral gavage
Justification for Dosing Route:	In accordance with the intended clinical route
Dosing Method:	The dosing formulations are administered into the stomach via the nasal cavity using a disposable catheter and syringe. The remaining dosing formulation in the catheter is flushed into the stomach with approximately 5 mL of water.
Justification for Dosing Method:	Commonly used method for oral dosing to cynomolgus monkeys
Dosing Frequency and Interval:	Single dose The control article is administered once and test articles are administered three times at 7–day interval
Dosing Volume:	5 mL/kg Individual dosing volumes are calculated based on the body weight measured on the day before dosing.
Dosing Time:	Between 09:00 a.m. and 12:00 p.m.
Sham Dosing:	The water in 5 mL/kg is dosed to each animal in the same manner as the control and test article formulations once daily on Day -6, Day -5, and Day -2 in order to acclimatize the animals to the dosing procedure. Individual dosing volumes are calculated based on the body weight measured on the day before first sham dosing (Day -7).

9.5. Test System

Species:	Cynomolgus monkey (purpose-bred)
Gender:	Male
Body Wight (At Initiation of Acclimation):	3 to 7 kg

Age (At Initiation of Acclimation):
3 to 7 years

Origin: China

Date of Receipt: July 30, 2014

Number of Animals Allocated: 4 males

Number of Animals Used: 4 males

Source: Example Supplier

Rationale for Selection of the Animal Species:
Cynomolgus monkeys are a commonly used non-rodent species for safety pharmacology studies.

9.6. Husbandry

(SOP: GTX/207)

Animal Room: Room No. 777

Temperature: Acceptable range: 23 to 29°C

Relative Humidity: Acceptable range: 30 to 70%

Ventilation Rate: 15 times/hour

Illumination: 12 hours/day of artificial light (07:00 a.m. to 7:00 p.m.)
(Except for observation and feeding time)

Animal Cages

- Material: Stainless steel
- Cage Size: 680 mm (D) × 620 mm (W) × 770 mm (H)
- Number of Animals per Cage: 1 animal/cage

Food and Feeding: Solid food (HF Primate 5K91 12G 5K9J, Purina Mills, LLC) is provided. Approximately 108 g (approximately 12 g × 9 pieces) of solid food is provided to each animal once daily between 2 to 4 p.m. . The remaining food check and removal procedure are performed between 08:00 and 11:00 a.m. on the following day. On the days of surgery, approximately half the amount of solid food (5 pieces) is provided after surgery to each animal that undergoes surgery. On dosing days (except for the sham dosing days), food is provided after 4 hours after dosing, and the remaining food check procedure is performed after 24 hours after dosing (after the telemetry analysis point). On the days before surgery and the days before sham dosing, the remaining food check and removal procedure are performed at approximately 5 p.m.

Water: Water conforming to the water quality standards is available *ad*

libitum from an automatic water supply system.

Environmental Enrichment: Enrichment toys are provided 24 hours each day. Treats (pieces of apple or sweet potato) are supplied 2 times weekly, but not on the days of surgery or dosing.

Cleaning: The cages and the animal room are washed daily with water. The cages are not exchanged from the initiation of acclimation to the end of observation.

9.7. Identification of the Animals and Cages

(SOP: GTX/502)

Animals: During the study period, each animal is identified by Animal ID tattooed on the leg. For convenience, a correspondence table between an individual Animal ID and an animal number is prepared and recorded as animal number in raw data.

Cage: During the study period, each cage is identified by a cage card listing the study number, Animal ID, sex and animal number.

9.8. Acclimation

(SOP: GTX/151, GTX/203, GTX/208 and GTX/211)

Four male cynomolgus monkeys, which have been quarantined, are received, and are then acclimated for 7 days. All animals are weighed using an electronic balance (HP-40K, A&D Co., Ltd.) once on the first and final days of acclimation. During this period, all animals are observed for clinical signs at least once daily.

9.9. Surgical Implantation of the Telemetry Transmitter

(SOP: PHA/061, PHA/504, PHA/505 and PHA/232)

9.9.1. Surgical Procedure

Surgery to implant the telemetry transmitter is performed on August 6, 2014 (Nos. M001 and M002) and August 7, 2014 (Nos. M003 and M004). The animals are weighed once before surgery. Atropine sulfate hydrate (Atropine sulfate injection 0.5 mg, Mitsubishi Tanabe Pharma Corporation, 0.02 mL/kg) is intramuscularly administered to the animals as a pre-anesthetic medication. The animals are sedated by an intramuscular injection of ketamine hydrochloride[a)] (Supriya Lifescience Ltd, 50 mg/mL, 0.2 mL/kg), after which they undergoes surgery under isoflurane inhalation anesthesia (Escain, Mylan N.V., 0.5 to 2.0%) and artificial ventilation. Immediately before surgery, buprenorphine hydrochloride (Zalban Injection 0.2 mg, Nissin Pharmaceutical Co., Ltd. 0.05 mL/kg) and antibiotic (Mycillin Sol Meiji[b)], Meiji Seika Pharma Co., Ltd., 0.05 mL/kg) are intramuscularly administered to the animals to alleviate pain and to prevent infection, respectively.

A telemetry transmitter, TL11M2-D70-PCT[b)] (Data Sciences International Inc.) is implanted intraperitoneally and fixed inside the abdominal wall. A catheter for measurement of blood pressure is inserted via the right femoral artery and positioned in the abdominal aorta, and electrodes for recording the electrocardiogram (ECG) are inserted into the thoracic cavity via the bilateral intercostals and fixed in the pericardium near the right atrium and the apex.

a） Mycillinsol Meiji [aqueous suspended injection of dihydrostreptomycin sulfate and benzyl penicillin procaine: dihydrostreptomycin sulfate (250 mg potency/mL), benzyl penicillin procaine (200000 units/mL)]

b） The transmitter contains a pressure catheter filled with a special gel to prevent the entry of blood, two electrodes and a built-in temperature sensor.

9.9.2. Surgical Animal Care

As shown in below table, an antibiotic (Mycillinsol KS, 0.05 mL/kg) and an analgesic (Zalban Injection 0.2 mg, 0.05 mL/kg and Capisten IM 50 mg, 0.1 mL/kg) are intramuscularly administered to the animals for 3 days after the surgery including the day of implantation to prevent infection and to palliate pain, respectively. The animals are allowed approximately 4 weeks to recover from surgery, and the telemetry signals (intra-abdominal body temperature, blood pressure patterns and ECG waveforms) are recorded at least once (at least 26 hours from around 08:00 a.m. on Day -6) using the telemetry system. The animals are subjected to the experiment after they are confirmed to have no abnormality in clinical signs and telemetry signals. In case the recovery of surgical wound is not sufficient enough, the day of administration are postponed by the judgment of study director.

Drugs	Analgesics	Antibiotic
Frequency	Twice a day	Once a day
On the Surgical Day	Immediately before and after surgery	Immediately before surgery
From the day after the surgical day	Morning and afternoon[a)]	Morning

a） The second dosing is conducted approximately 8 hours after dosing in the morning.

9.10. Study Design

Dosing Schedule (Dosing Day)[a)]	Test and Control Articles	Dose Level (mg/kg)	Dosing Volume (mL/kg)	Concentration (mg/mL)	Number of Animals (Animal No.)[b)] Male
First (Day 1)	Vehicle	-	5	-	4 (M001 to M004)
Second (Day 8)	Compound A	10	5	2	
Third (Day 15)	Compound A	30	5	6	
Fourth (Day 22)	Compound A	100	5	20	

a） The first day of dosing is designated as Day 1.

b） The same animals are dosed repeatedly.

9.11. Rationale for Selection of the Dose Levels

The toxicity information of Compound A is … (hereinafter abbreviated because it is not related to data set preparation).

9.12. Observations, Measurements and Examinations

9.12.1. Blood Pressure, Heart Rate, Electrocardiogram, Respiratory Rate and Intra-abdominal Body Temperature

(SOP: PHA/504, PHA/505 and PHA/506)

Telemetry data from each animal are acquired via a receiver, RMC-1 (Data Sciences International Inc.) that is placed on the cage, and the data is analyzed using a telemetry system (Dataquest Open A.R.T. version 4.34 / Ponemah Physiology Platform version 5.20-SP2, Data Sciences International Inc.). The telemetry data are recorded from at least 2 hours before dosing until at least 24 hours after dosing on each dosing day.

Number of Animals:	All animals
Analysis Points:	Twice before dosing (2 and 1 hours before dosing) and at 9 points after dosing (0.5, 1, 2, 4, 6, 8, 12, 16 and 24 hours after dosing) The mean of the values at the 2 points before dosing is regarded as the pre-dosing value (baseline).
Analysis Methods:	Systolic, diastolic and mean blood pressure (mmHg), heart rate (beats/min) derived from the blood pressure waveform, respiratory rate (breaths/min) derived from the blood pressure waveform and

intra-abdominal body temperature (°C) are averaged over a 60-second period. ECG parameters (PR interval (ms), QRS duration (ms), RR interval (ms), QT interval (ms) and QTc (corrected QT interval by Bazett formula, ms)) are averaged for 10 consecutive waveforms.
The electrocardiogram waveform to be analyzed is set within the range of 60-second in which the blood pressure is analyzed.

9.12.2. Clinical Signs

(SOP: GTX/151 and GTX/208)

Number of Animals: All animals

Observation Point and Methods

During the Telemetry Recording Period:

Each animal is observed via cage-side observation at each observation point; immediately before and after dosing, 4 and 24 hours after dosing, on each dosing day.

Other Period: Each animal is observed directly more than once daily.

9.13. Treatment of Animals after the End of Experiments

The animals are excluded from the study after observation on the final observation day (Day 23).

9.14. Statistical Analyses

(SOP: CPU/124)

Data on blood pressure, heart rate, ECG parameters, respiratory rate and intra-abdominal body temperature at each analysis point are expressed as the mean value ± standard deviation (SD).

Analysis of covariance (ANCOVA) is performed using a model including PRE, DOSE and ANIMAL. Multiple comparisons between the control article and the test article at each analysis point (sampling point) are performed by ANCOVA with Dunnett's multiplicity adjustment. These statistical analyses are performed at a two-sided significance level of 5% using SAS System for Windows, Release 9.2 (SAS Institute Inc.). Statistical analysis is not performed for clinical signs.

10. REPORT

A final report written in English is created in this test results.

11. ARCHIVE OF RECORDS, DATA AND SAMPLE

The records, data and samples generated at the Test Facility will be archived in the Archives of Example Contract Lab Name for a period of 5 years after preparation of the Final Report.

Example Contract Lab Name Data Archive Room

Protocol and amendment
Records and data on test and control articles
Records and data on test system
Records and data on husbandry
Acclimation records
Surgery records
Dosing records
Records and data on telemetry (including a DVD-R)
Records on clinical sign observations
Statistical analysis records
Final report
All other data related to the study

12. PROTOCOL AMENDMENT

Protocol amendments are created when it occurs to need to modify the protocol and it clarifies the point of change and its reason.

13. APPROVAL AND CREATION OF PROTOCOL

Creation Protocol

Study Director; Example Contract Lab Name

29 July 2014

(*Sign*)

Taro Sendo

Protocol Approval

Facility Manager; XXX Co., Ltd. Drug Safety Research Laboratories

XX YYY 2014

Manager's Name