A Dose Range Finding and Pharmacokinetics Study of RUC-4 Succinate Alone and In Combination with Aspirin in Nonhuman Primates

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Abstract

RUC-4 is a novel αIIβ3 antagonist being developed for prehospital therapy of myocardial infarction. The objectives of this study were to determine the pharmacokinetics and maximum tolerated dose (MTD) of RUC-4 alone and in combination with aspirin when administered by subcutaneous (SC) injection. RUC-4 was given SC twice (4 hours apart) at 0, 1, 10, and 100 mg/kg/dose and by intravenous injection (IV) once at 1 mg/kg/dose to cynomolgus monkeys (one/sex/group). RUC-4 caused a dose and time-point dependent inhibition of platelet aggregation. RUC-4 at doses up to 10 mg/kg/dose caused no clinical signs of toxicity; no effects on body weight, body temperature, or food consumption; and no clear test article-related effects on hematology and clinical chemistry. Potential test article-related increases in creatine kinase and lactate dehydrogenase at 100 mg/kg dose were noted, which could be skeletal muscle-related due to physical activities during dosing. The maximum tolerated dose for RUC-4 alone was considered to be 10 mg/kg/dose (20 mg/kg/day). After a 21 day wash-out period, the effect of aspirin on MTD dose of RUC 4 was determined. Animals received aspirin (81 mg/dose) by oral gavage followed 2 hours later by 2 SC injections of RUC-4 (10/mg/kg/dose) 4 hours apart. Aspirin's effect was confirmed by the complete inhibition of platelet aggregation to arachidonic acid prior to dosing with RUC-4. RUC-4 + aspirin caused no clinical signs of toxicity; no effects on body weight, body temperature, or food consumption; and no clear test article-related effects on hematology and clinical chemistry. C_{max} and AUC_{last} values increased with increasing dose levels, but the increases were not proportional to dose administered, indicating non-linear kinetics at 1, 10, or 100 mg/kg. RUC-4 was rapidly cleared from blood within 0.25 hours following IV administration. There was no clear indication that administration of aspirin had an effect on RUC-4 levels in the NHP at the dose tested. In summary, the maximum tolerated dose (MTD) for RUC-4 alone or in combination with aspirin was considered to be 10 mg/kg/dose (20 mg/kg/day).

Methods and Materials

The study was conducted in two phases. In Phase 1, RUC-4 succinate salt formulated in saline was administered to male and female cynomolgus monkeys by subcutaneous injection (SC) at nominal doses of 0 (vehicle control), 1, 10, and 100 mg/kg/dose and by intravenous injection (IV) at 1 mg/kg/dose. Animals administered RUC-4 subcutaneously were dosed twice with a 4-hour interval between doses, and animals administered RUC-4 intravenously were dosed once. In Phase 2, monkeys from Phase 1 were arbitrarily assigned to Groups 6-8, one/sex/group, after a 21 day wash out period, and treated in the following manner: Group 6 was administered vehicle (disodium succinate; 3.22 mg/kg/dose) twice by subcutaneous injection, with a 4-hour interval between doses. Group 7 received a single oral gavage dose of aspirin (81 mg/dose). Group 8 received a single oral gavage dose of aspirin (81 mg/dose) followed 2 hours later by a subcutaneous injection of RUC-4 (10 mg/kg/dose), and again 4 hours later by a second similar injection of RUC-4 (10 mg/kg/dose).

Study Design

Phase 1: Maximum Tolerated Dose (MTD) and Bioavailability

Dose Group	Treatment	Dose Level (mg/kg/dose)[1]	Total Dose (mg/kg/day)	Concentr. (mg/mL)	Dose Route	Number of Animals
1	Vehicle[2]	0	0	0	Subcutaneous	1 M / 1 F
2	RUC-4	1	2	0.5	Subcutaneous	1 M / 1 F
3	RUC-4	10	20	5	Subcutaneous	1 M / 1 F
4	RUC-4	100	200	50	Subcutaneous	1 M / 1 F
5	RUC-4	1	1	0.5	Intravenous	1 M / 1 F

[1]Concentrations/doses listed above are based upon the RUC-4 monosuccinate salt form.
[2]Saline used as the vehicle.

Phase 2: Combination Therapy

Dose Group	Treatment	Dose Levels	Dose Route	Number of Animals
6	Disodium Succinate	3.22 mg/kg/dose (BID)	Subcutaneous	1 M / 1 F
7	Aspirin	81 mg	Oral	1 M / 1 F
8	Aspirin + RUC-4	81 mg 10 mg/kg/dose (BID)	Oral Subcutaneous	1 M / 1 F

In Phase 1, blood drug levels were analyzed prior to and at three timepoints after dosing. Clinical pathology determinations were performed on samples from animals in Groups 1-5 on Days -1, 2, and 7. Platelet aggregation assays were performed on whole blood samples from animals in Groups 1-5 prior to dosing and at four timepoints after dosing. An ophthalmologic examination was performed on Day 2. In Phase 2, blood drug levels were analyzed prior to and at three timepoints after dosing. Clinical pathology determinations were performed on samples from animals in Groups 6-8 on Days -2, 1, 2, 4, and 7. Platelet aggregation assays were performed on blood samples from animals in Groups 6-8 prior to dosing and at two timepoints after dosing. In Phases 1 and 2, clinical observations, body weights, body temperature, and food consumption data were collected.

Results

Phase 1

Mortality: At nominal doses of 1, 10, 100, and 1 (IV) mg/kg/dose RUC-4 (2, 20, 200, and 1 mg/kg/day on Day 1, respectively), there were no test-article related deaths.

Body Weights, Body Temperatures and Food Consumption: At nominal doses of 1, 10 and 100 mg/kg/dose RUC-4 there were no test article-related effects on body weight and body temperature. At nominal doses of 1 and 10 mg/kg/dose there were no test article-related effects on food consumption. At the nominal dose 100 mg/kg/dose, there appeared to be a reduction in food consumption on Days 3 and 4.

Clinical Observations: At nominal doses of 1, 10 and 100 mg/kg/dose RUC-4, no clinical signs of toxicity were observed. At nominal doses of 1 (IV), 10, and 100 mg/kg/dose, swelling (hematoma) in the groin, the site of venipuncture, was observed, approximately 15-30 minutes after the first dose of RUC-4.

Hematology: Decreases in erythrocyte counts, hemoglobin levels, and/or hematocrit levels were noted on Days 2 and/or 7 amongst the various groups, including the vehicle group.

Clinical Chemistry: Elevations in enzyme levels [aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LD) and creatine kinase (CK), were noted in male and female NHP from the various groups, including the vehicle group, on Days 2 and/or 7. Highest elevations in CK and LD were present in the 100 mg/kg/dose group.

Coagulation: Changes in fibrinogen did not appear to be associated with RUC-4 administration.

Ophthalmology: No ophthalmoscopic changes observed that were considered to be due to RUC-4 administration.

Platelet Aggregation Inhibition: Inhibition of platelet aggregation was noted at all RUC-4 dose levels (1, 10 and 100 mg/kg/dose) evaluated. Generally, with the adenosine diphosphate (ADP, 20 µM) test, at nominal doses of 1 and 10 mg/kg/dose platelet aggregation was inhibited at 0.25 hours after the first dose and 4 hours later platelet aggregation had returned to baseline levels. In contrast, at a nominal dose of 100 mg/kg/dose platelet aggregation in both sexes was inhibited from 0.25 (~98 inhibition) to 4 hours (~50% inhibition) after the first dose.

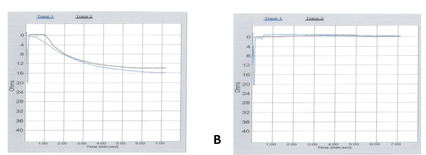

Figure 1. Representative original tracings demonstrating the effect of RUC-4 on ADP (20 µM)-induced aggregation on NHP platelets 4 hours after the SC administration of RUC-4. At 0 mg/kg/dose (A), there was no effect on ADP-induced platelet aggregation. At 100 mg/kg/dose RUC-4 (B), ADP-induced platelet aggregation was inhibited.

Blood Drug Levels and Toxicokinetics: For NHP administered a SC dose of RUC-4, C_{max} and AUC_{last} values increased with increasing dose level. However, the increases in mean C_{max} and AUC_{last} values were not linear with increasing dose level; this was particularly evident for animals given the highest dose level.

Toxicokinetic Parameters Calculated from Concentrations of RUC-4 in Blood following SC Administration to Nonhuman Primates

Group	Dose Level[a] (mg/kg/dose)	Sex	Animal	T_{max}[b] (hr)	C_{max}[c] (ng/mL)	AUC_{last}[d] (hr·ng/mL)
2	1	F	2F14662	0.25	48	6
2	1	M	2M14656	0.25	178	22
3	10	F	3F14663	0.25	900	701
3	10	M	3M14658	0.25	1145	1137
4	100	F	4F14664	0.25	35400	50006
4	100	M	4M14657	0.25	16600	22332

[a]Animals were given two SC doses of RUC-4 (1, 10, or 100 mg/kg/dose) approximately 4 hours apart.
[b]Time maximum concentration of RUC-4 was observed in blood
[c]Maximum concentration of RUC-4 observed in blood
[d]Area under the blood concentration versus time curve calculated from 0 to the last time point the concentration of RUC-4 was above the level of quantitation

For administered an IV dose (1 mg/kg), RUC-4 was quantifiable in whole blood at only one time point and at a low concentration (0.25 hours; 46 ng/mL) after dose administration to the male animal and at no time points after dose administration to the female animal. Due to the absence of quantifiable levels of RUC-4 in plasma over time following IV administration, the absolute bioavailability of RUC-4 could not be assessed following SC administration.

Results

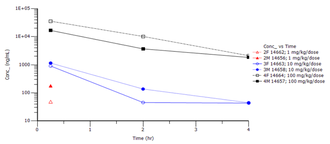

Figure 2. Concentrations of RUC-4 in blood following SC administration of 1, 10, or 100 mg/kg/dose to male and female nonhuman primates (Phase 1).

Phase 2

Mortality: At a dose of 10 mg/kg/dose RUC-4 (20 mg/kg/day on Day 1), there were no test article-related deaths.

Body Weights, Body Temperatures and Food Consumption: At a dose of 10 mg/kg/dose RUC-4, there were no test article-related effects on body weights, body temperatures and food consumption.

Clinical Observations: Swelling was observed in the groin of animals of both sexes in the aspirin group and the tail of the female in the aspirin + RUC-4 group. Discoloration was observed in multiple sites in the aspirin and aspirin + RUC-4 groups, particularly in the hindlimbs. As in Phase 1, the discoloration in Phase 2 was likely attributed to physical restraint and venipuncture procedures.

Hematology: Hematologic changes (decreases in erythrocyte counts, hemoglobin levels, and/or hematocrit levels) were considered equivocal.

Clinical Chemistry: Elevations in enzyme levels AST, ALT, LD and CK, noted in male and female NHP form the various groups, including the vehicle group, on Days 1, 2, 4, and/or 7.

Coagulation: Changes in fibrinogen did not appear to be associated with RUC-4 administration.

Platelet Aggregation Inhibition: Platelet aggregation was inhibited in blood samples taken 2 hours after aspirin administration in the arachidonic acid (AA, 0.5 mM)) test in the groups treated with aspirin and aspirin + RUC-4. Platelet aggregation was inhibited in blood samples taken 4 hours after the first RUC-4 administration in the ADP test in the male and female animals treated with aspirin + RUC-4.

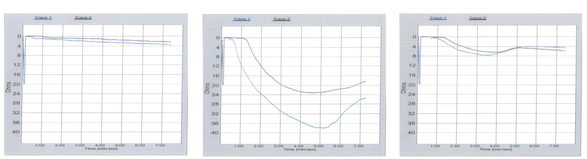

Figure 3. Representative original tracings demonstrating the effects of aspirin (81 mg) on ADP (20 µM) and AA (0.5 µM)-induced aggregation on NHP platelets prior to RUC-4 administration and the effects of 10 mg/kg/dose RUC-4 on ADP (20 µM) -induced aggregation on NHP platelets 4 hours after the SC administration of RUC-4. Subsequent to aspirin administration, AA-induced platelet aggregation was inhibited (A)), but ADP-induced platelet aggregation was not effected (B). Utilizing the ADP test, 4 hours after the first administration of RUC-4 platelet aggregation was inhibited in the animal treated with aspirin + RUC-4 (C).

Results

Blood Drug Levels and Toxicokinetics: For male and female NHP administered SC doses of 10 mg/kg/dose of RUC-4 in combination with aspirin, peak concentrations of RUC-4 were observed in blood at 15 minutes after administration of the first dose of RUC-4. C_{max} values for the male (825 ng/mL) and female (975 ng/mL) animal in Group 8 (aspirin + RUC-4) were similar to the plasma concentrations observed at 15 minutes after administration of the same dose level during Phase 1. The AUC_{last} values were higher than observed after administration of the same dose during Phase 1 but included a 24-hour sampling time which was not collected during Phase 1 (last sampling time during Phase 1: 4 hours).

Toxicokinetic Parameters Calculated from Concentrations of RUC-4 in Blood following SC Administration to Nonhuman Primates

Group	Treatment	Dose Level RUC-4 (mg/kg/dose)[a]	Sex	Animal	T_{max}[b] (hr)	C_{max}[c] (ng/mL)	AUC_{last}[d] (hr·ng/mL)
8	Aspirin	10	F	8F14662	0.25	975	1789
8	Aspirin	10	M	8M14657	0.25	825	851

[a]Animals were given two SC doses of RUC-4 (10 mg/kg/dose) approximately 4 hours apart
[b]Time maximum concentration of RUC-4 was observed in blood
[c]Maximum concentration of RUC-4 observed in blood
[d]Area under the blood concentration versus time curve calculated from 0 to the last time point the concentration of RUC-4 was above the level of quantitation

Conclusions

Phase 1

- RUC-4 was well tolerated at nominal doses of 10 mg/kg/dose BID in that it produced no clinical signs of toxicity; no effects on body weight body temperature, or food consumption; no clear cut drug-related effects on hematology
- Elevations in creatine kinase and lactate dehydrogenase were highest in the nominal 100 mg/kg/dose group, suggesting that the enzyme elevations may be test article-related.
- Maximum tolerated dose was 10 mg/kg/dose (20 mg/kg/day)
- RUC-4 exhibited a dose and time-point dependent inhibition of platelet aggregation.
- RUC-4 exhibited non-linear kinetics following SC administration of nominal 1, 10 or 100 mg/kg to male and female NHP, possibly due to saturated metabolism/elimination of RUC-4
- Due to the absence of quantifiable levels of RUC-4 in plasma over time following IV administration, the absolute bioavailability of RUC-4 could not be assessed following SC administration.

Phase 2

- In Phase 2, RUC-4 was tolerated at a dose of 10 mg/kg/dose (20 mg/kg/day on Day 1) in combination with aspirin with no clinical signs of toxicity; no effects on body weight, body temperature, or food consumption; and no clear drug-related effects on hematology and clinical chemistry parameters.
- Platelet aggregation was inhibited in samples taken 2 hours after aspirin administration in the RUC-4 in the AA test for animals treated with aspirin or aspirin + RUC-4.
- ADP tests on Day 1 on samples collected 4 hours after the first (but prior to the second) RUC-4 administration indicated inhibition of platelet aggregation for aspirin + RUC-4 in male and female NHP
- There was no clear indication that the administration of aspirin in combination with RUC-4 impacted the level of exposure of NHP administered subcutaneous doses of RUC-4

Acknowledgements

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