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DRAFT NON-GLP REPORT: 11-5203-N1

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AKI Therapeutic Trials

Test Article
Sentinel-001AKI

Second Draft Report Date
3/24/2016

Final Report Date
05/08/2016
(45 Days after Draft)

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STUDY SUMMARY

The purpose of the study was to study the efficacy and safety of the test article which was an extracorporeal support system containing mesenchymal stem cells (MSCs). The animals in the study underwent varying degrees of therapeutic intervention in order to test the ability of the test article to treat an acute kidney ischemic injury. A jugular catheter was implanted and the ischemic model was induced by performing a unilateral nephrectomy and the remaining kidney was clamped to obstruct the blood flow of the renal artery and vein for 90 minutes. All animals underwent the ischemic injury and the treatment groups were Group 1: baseline control of injury only, Group 2: injury with dialysis treatment with no MSCs and Group 3: injury with dialysis treatment of MSCs. Five of the animals included in Group 1 were performed under Toxikon project # 10-2996-N1. Clinical pathology including urinalysis was performed routinely during the study and histopathology of the kidney was performed. All data was reported to the Sponsor and the Sponsor is responsible for all conclusions.

STUDY DIRECTOR AND QUALITY ASSURANCE SIGNATURES AND VERIFICATION DATES

SIGNATURES

Signature Information	
Protocol Number	Not Applicable
Study Director	Christopher Parker, M.S., MBA
Study Supervisor	Allan Sleger, A.S., LAT
Company	Toxikon Corporation

VERIFICATION DATES

Verification Date(s)	
Test Article Receipt	11/2/2011
Additional Test Article Receipt	Provided each day of treatment
Project Log	11/2/2011
Study Completion	To Be Determined (TBD)

_____ _____

Quality Assurance Date

_____ _____

Christopher Parker, M.S., MBA
Study Director Date

1.0 PURPOSE

The purpose of the study was to study the efficacy and safety of the test article which was an extracorporeal support system containing mesenchymal stem cells (MSCs).

2.0 REFERENCES

The study was based upon the following references:

2.1 Sponsor specifications

2.2 ISO/IEC 17025, 2005, General Requirements for the Competence of Testing and Calibration Laboratories.

3.0 COMPLIANCE

The data and report generated from this Non-GLP protocol should not be used to support applications for research or marketing permits for products regulated by the FDA. Compliance to 21 CFR part 58 Good Laboratory Practice (GLP) is required for data/reports intended for regulatory submission to assure the quality and integrity of safety data. The Sponsor was responsible for informing the Test Facility and the Study Director if the data was for any regulatory submission purpose. It was the Sponsor's responsibility to request GLP compliance prior to study initiation. Although this study was Non-GLP, it was conducted according to the accredited Quality System in effect at Toxikon, including ISO/IEC 17025, 2005, General Requirements for the Competence of Testing and Calibration Laboratories.

4.0 IDENTIFICATION OF TEST AND CONTROL ARTICLES

The Sponsor supplied the following information on a Non-GLP Test Requisition Form or other correspondence, wherever applicable (excluding confidential or trade secret information).

4.1 Test Article:

Name	Sentinel-001AKI
CAS/Code Number	Not Supplied by Sponsor (N/S)
Lot/Batch Number	Sentinel-001AKI

4.2 Control Article (Sponsor Supplied):

Name	Blank-001AKI
Lot/Batch Number	Blank-001AKI

5.0 IDENTIFICATION OF TEST SYSTEM

5.1 Animals Used in the Study:

Number and Species: 27 Purpose-bred Mongrels

Sex: male

Weight/Age Range: 23.6–35.3 kilograms / at least 12 months old
weighed to the nearest 10 g

Health Status: healthy, previously used in other experimental procedures

Animal Purchase: Antech Incorporated, Barnhart, MO

Animal Identification: ear tattoo

Acclimation: minimum 5 days, under same conditions as for the actual test

Animal Selection: selected from larger pool and examined to ensure lack of adverse clinical signs

5.2 Animal Care and Maintenance:

Animal Room Temperature: 68 ± 5 °F

Animal Room Relative Humidity: 30–70%

Air Exchanges per Hour: a minimum of 10 changes per hour

Lights: 12–hour light/dark cycle, full spectrum fluorescent lights

Housing: individually housed

Cages: concrete steel pens

Bedding: pine shavings, P.W.I. Industries, St–Hyacinthe, Quebec, Canada (contact)

Animal Rations: Teklad 8755 Dog Food, Harlan Laboratories, Madison, WI, *ad libitum*

Water: tap water, *ad libitum*

There were no known contaminants present in the feed, water, or bedding expected to interfere with the test data.

The laboratory and animal rooms were maintained as limited–access facilities.

6.0 JUSTIFICATION OF TEST SYSTEM AND ROUTE OF ADMINISTRATION

6.1 Justification of Test System:

Canines are an established model for vascular dialysis and the size and/or anatomy of the specie is best or uniquely suited to the procedure. The vascular system of the mongrel hound canine provides a sufficient flow rate to enable dialysis to be performed using a clinical scale device prototype.

6.2 Route of Administration:

The test article was used as a part of an extracorporeal dialysis infusion system connected to the animals via an implanted jugular catheter.

7.0 EXPERIMENTAL DESIGN AND DOSAGE

7.1 Preparation of Test and Control Articles:

The test and control articles were supplied by the Sponsor on each day of dialysis. The Sponsor was responsible for all test and control article preparation.

7.2 Pre–Dose Procedure:

Prior to surgery, each animal was weighed, observed for routine clinical observations and then bled for hematology, clinical chemistry and coagulation analysis. Additionally, urine was collected from each animal for urinalysis.

On the first day of the study (Day 0) all animals underwent surgery in order to induce the ischemic injury model. Groups 2 and 3 were additionally implanted with a jugular catheter in order to enable dialysis on Day 1 of the study. The animals were pre-medicated with appropriate analgesics and acepromazine before being anesthetized with ketamine and diazepam. The animals subsequently received an endotracheal tube and were placed on 1-4% of isofluorane. Once prepared for surgery, one jugular vein exposed and an intravenous dialysis catheter was implanted and anchored to the animal using suture (Groups 2 and 3). The catheter was then flushed with heparinized saline. For all groups (1, 2 and 3), the left kidney was then removed by a nephrectomy and then the renal artery and vein of the remaining kidney were clamped for 90 minutes. Following surgery, the animals were appropriately closed and allowed to recover. As appropriate, animals were bled and urine collected for clinical pathology analysis following surgery.

7.3 Dose Administration:

Animal assignments into treatment groups are as detailed in Table 1:

TABLE 1
Animal Assignment

Group #	Animal #	Study Date	Treatment Group	Cell Dose (in Million Cells)
Group 1	7X214*	5/3/2011	No Dialysis	N/A
	6X214*	6/8/2011		
	2X95*	6/8/2011		
	3X224*	7/12/2011		
	A1221*	7/12/2011		
	21342	9/11/2012		
Group 2	1171	11/1/2011	Dialysis + Blank Device	
	9X245	11/29/2011		
	1X323	12/13/2011		
	91117	12/13/2011		
	51177	2/28/2012		
	31252	3/20/2012		
	41267	4/24/2012		
	51150	5/15/2012		
	51174	6/5/2012		
	31290	6/19/2012		
Group 3	6179	11/1/2011	Dialysis + Cells	192
	2177	11/29/2011		139
	21136	2/28/2012		560
	31136	3/20/2012		560
	51267	4/24/2012		250
	11150	5/15/2012		560
	11154	6/5/2012		560
	61319	6/19/2012		250
	51322	9/11/2012		522
	4292	11/28/2012		540
	9292	11/28/2012		530

*Animal was tested as a part of Project # 10-2996-N1 and is included in the current study (11-5203-N1) per Sponsor request.
N/A = Not Applicable
Note: Test article cell load data was as provided by the Sponsor

The animals in Group 1 were used as a baseline control and were not dosed with the test or control article.

On Day 1 of the study, the animals in Groups 2 and 3 underwent dialysis. Prior to and following dialysis, as appropriate, animals were bled and urine collected for clinical pathology analysis. On the day of dialysis, the Sponsor primed the dialysis system and randomly chose which animal was to be treated with either the test or control article as available. Each animal received a bolus of 4000 units of heparinized saline prior to dialysis and then were maintained on a continuous infusion of 51 mL/hour of 18.6 U/mL heparinized saline in order to prevent clotting within the dialysis system during treatment. The animals were maintained on dialysis for approximately 12 hours, unless otherwise affected by clinical or technical complications, after which they were returned to their cage.

7.4 Post–Dose Procedure:

Each animal was observed daily for clinical signs of toxicity. All observations were recorded. Observations included the following systems: skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous system, somatomotor activity, and behavior pattern. Particular attention was directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma. Clinical observations made on an animal were not open ended nor had gaps during the course of study.

Animals were observed once daily for morbidity/mortality. Animals whose condition made it unlikely that they would survive until the next observation, based upon the criteria established by the Study Director in concert with the veterinary staff, were sacrificed immediately and necropsied. Animals which were found dead were necropsied as soon as possible if found to be appropriate by the study director and pathologist. If possible clinical pathology samples were collected prior to sacrifice.

The animals were maintained for a period of up to 7 days. Animals were bled and urine collected for clinical pathology analysis on each day of the study. If urine could not be collected due to insufficient volume or other reason, it was noted in the raw data.

Other than the therapeutics involved in surgery and dialysis, no other medications were provided to the animals unless they were required based upon veterinary treatment requirements.At the end of the observation period, the animals were weighed and sacrificed by an overdose of an injectable barbiturate.

At necropsy, the kidney in each animal was collected and photographed. One half of each kidney, cut longitudinally, was immediately placed in 10% neutral buffered formalin for histopathological analysis by staining with routine hematoxylin and eosin (H&E) and periodic acid–Schiff (PAS). The half of each kidney submitted for histopathology was serially sectioned (8 or 20 sections depending on the individual animal and discussions between the Sponsor and the pathologist) equally along the entire tissue. The serially sectioned kidney tissues were microscopically assessed for signs of inflammation and renal health. The other half of each kidney was sectioned, flash frozen and stored at -80 °C for Sponsor analysis.

8.0 EVALUATION CRITERIA

Control of Bias Statement:

This study and its design employed methodology to minimize uncertainty of measurement and control of bias for data collection and analysis, which included but was not limited to: control

group, animals selected from a larger pool, baseline values for the studied parameters, quality control samples for performance monitoring, system suitability assessment, randomization, and method controls such as blanks and replicates.

9.0 RESULTS

A total of 5 animals from Toxikon project # 10-2996-N1 performed for the Sponsor are being reported as a part of the current study in Group 1 per Sponsor request (Animal #s 7X214, 6X214, 2X95, 3X224 and A1121).

9.1 Clinical Observations (Attachment A):

A total of 7 animals were humanely sacrificed or found dead during the study, three in Group 1, three in Group 2 and one in Group 3. In Group 1, Animal # A1221 was humanely sacrificed on Day 3 and intussusception of the small intestine was observed. Animal # 6X214 was found dead on Day 6 and Animal # 3X224 was humanely sacrificed on Day 6 due to significant signs of lethargy and renal failure. In Group 2, Animal # 31290 was humanely sacrificed after it was observed to have potentially had a seizure during dialysis on Day 1. Animal # 41267 was humanely sacrificed on Day 4 due to significant vomiting and Animal # 51177 was humanely sacrificed on Day 6 due to lethargy and vomiting and was noted to have a swollen abdomen potentially related to the nephrectomy surgical procedure. In Group 3, Animal # 51267 was humanely sacrificed on Day 6 due to significant vomiting and lethargy. A significant loss of weight was also a contributor to the decision to humanely sacrifice a number of the above animals. The most common clinical observations across all groups included decreased food consumption, lethargy, diarrhea and vomiting. All of these clinical observations were not unforeseen considering the model that was induced.

9.2 Body Weight Changes (Table 2 and Attachment A):

All animals in the study decreased in weight. The weight loss corresponds to the observed clinical observations which were generally pervasive across all groups.

9.3 Clinical Pathology (Tables 3, 4, and 5, and Attachment A):

All clinical pathology data was collected and the Sponsor is responsible for the interpretation of the results.

9.4 Extent of Renal Injury (Table 6 and Attachment A):

The extent of renal injury at the time of dialysis was determined for Groups 2 and 3. This was considered as the increase in BUN and Creatinine values from each animal's pre-surgery baseline values to the pre-dialysis blood collection time point values.

9.5 Gross Pathology (Attachment A):

Primary gross findings were related to the condition of the kidney and were not unexpected due to the ischemic injury model. Two animals in Group 1 and one in Group 2 were noted to have inflamed/discolored small intestines. The level of visual discoloration and other damage to the kidney appeared to be relatively comparable across the groups.

9.6 Histopathology (Table 7 and Attachment B):

Each kidney was serially sectioned and each section was evaluated for signs of inflammation and general renal health.

10.0 CONCLUSION

All data was collected and reported to the Sponsor and the Sponsor is responsible for drawing all conclusions.

11.0 RECORDS

11.1 Original raw data will be archived by Toxikon Corporation.

11.2 A copy of the final report and any report amendments will be archived by Toxikon Corporation.

11.3 The original final report will be forwarded to the Sponsor.

11.4 The test article was returned by Toxikon.

11.5 Test article retention upon study completion is the responsibility of the Sponsor.

12.0 CONFIDENTIALITY AGREEMENT

Per corporate policy, confidentiality shall be maintained in general, and in specific accordance with any relevant agreement specifically executed between Toxikon and the Sponsor.

13.0 ANIMAL WELFARE STATEMENT

The Sponsor assured that, to the best of their knowledge, this study did not unnecessarily duplicate previous testing and that there were no non-animal alternatives acceptable for the evaluation of the test article as defined by the protocol.

All evidence of pain and distress was reported to the Veterinarian and/or Study Director. All clinical observations correlated with the veterinary findings.

Toxikon strictly adhered to the following standards, where applicable, in maintaining the animal care and use program:

United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service, 9 CFR Ch. 1 (1/1/95 edition), Subchapter A-Animal Welfare.

“Guide for the Care and Use of Laboratory Animals,” National Research Council, 2011. (NIH).

Office for Laboratory Animal Welfare (OLAW), “Public Health Service Policy on Humane Care and Use of Laboratory Animals,” Health Research Extension Act of 1985 (Public Law 99-158 November 20, 1985), Reprinted 1996.

ISO 10993–2, 2006, Biological Evaluation of Medical Devices – Part 2: Animal Welfare Requirements.

Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

14.0 UNFORESEEN CIRCUMSTANCES

Any unforeseen circumstances were documented in the raw data. However, no unforeseen circumstances that affected the integrity of the study were noted.

APPENDIX I
Software Systems

Software	Use	Publisher/Vendor	Location
Adobe Acrobat 8, 9, and 10 Professional	Document preparation	Adobe Systems, Inc.	San José, CA
Lotus Domino Rel. 5.0	Client–server application for Sponsor, sample, test codes, and quotation management application databases	IBM Corporation	Armonk, NY
MS Office 2007 and/or 2010 Small Business Suite and MS Office 2013 Professional Suite	Business software (suite includes Word, Excel, PowerPoint, Outlook, Publisher, Office tools)	Microsoft Corporation	Redmond, WA
Rees CentronSQL System 2.0	Environmental monitoring and metrology system	Rees Scientific	Trenton, NJ
TMS Web 7	Document management for SOPs and training records management software system	Quality Systems Integrators	Eagle, PA

ATTACHMENT A
Individual Animal Data

ATTACHMENT B
Pathology Reports and Microscopic Observations