A method development study to assess the effectiveness of measures to prevent cross contamination during topical application of a test article to the Göttingen minipig

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ABSTRACT

Although the rabbit is commonly used for the assessment of primary dermal irritation, pigs have generally been considered to be a better model for the more sophisticated study of dermal permeability and toxicity. Many studies have shown the close resemblance of human and minipig skin in terms of architectural structure/morphology, histology and physiological characteristics.

As a model in toxicology, the minipig has been used extensively in dermal/topical application studies. The Gottingen Minipig has sparse hair coverage and uniform coloration of the skin and are the smallest available minipig. Males reach sexual maturity at 3 to 4 months and weigh 7 to 9 kg and females at 4 to 5 months and weigh 9 to 12 kg. This makes them an ideal animal model for dermal toxicology assessment where test article may be limited.

One of the most significant issues for dermal studies is the extensive measures required to prevent cross-contamination of the animal, blood or tissue samples. Obviously blood samples collected to allow assessment of systemic exposure to the test article can give a damming picture of any contamination. To allow an assessment of the optimal measures to control cross contamination ITR has completed a method development topical application study utilizing the Göttingen minipig.

The results on this study confirmed that the measures we employed were able to ensure that there was no cross contamination of control animals or samples.

INTRODUCTION

The objective of the study was to determine whether the measures detailed in the standard handling procedures in this protocol prevent test article contamination of the Control animals following non-occluded topical application of Lidocaine 2.5% cream (EMLA©) for 4 hours/day over 7 consecutive days to the minipig.

The study did not meet the requirements of GLP but followed ITR's Standard Operating Procedures (SOP's).

The measures identified as being pivotal to the success of the study were defined by an in-house taskforce which had a remit to identify all the areas where there was a possibility of cross contamination.

The test material Lidocaine 2.5% cream (EMLA©) was selected because it has good permeability and a well characterized analytical procedure.

EXPERIMENTAL DESIGN

The test and control/vehicle articles were administered by topical application (non-occluded) for 4 hours/day, for 7 consecutive days to Göttingen Minipigs as described in the table below:

Group Number	Group Designation	Dose Level (mg/kg)	Dose Concentration (mg/g of cream)	Number of Animals
				Male
1	**Control***	**0**	**0**	**2**
2	**TA-treated**	**37.5**	**25**	**2**

* The Control animals will receive the vehicle (containing no test article) alone.

All animals including Controls received 1.5 g of cream/kg.

Dose levels were selected based on the available published data including the concentration of active in the commercially available cream based formulation and the profile of adverse effects following topical anesthesia application. The vehicle used for the study was Glycerin USP.

Batch numbers are retained on File at ITR and all materials were used within the expiry date presented by the manufacturer.

TEST SYSTEM

The Göttingen Minipigs were supplied to ITR by Marshall Bioresources, Inc. North Rose, New York 14516 USA. A Total of 4 males and 1 spare male were selected for the study. At onset of treatment the selected animals were in the body weight range of 20.0-23.9 kg and were aged between 16 to 22 months old.

Each minipig was housed in a stainless steel cage equipped with an automatic watering system supplemented by water bottles as appropriate and were fed a standard commercial minipig chow (400g of LabDiet Certified Lab Mini-pig Diet #5081) which was made available to each minipig twice daily (200g per occasion) during feeding periods of approximately 2 hours, but up to a maximum of 4 hours, for each occasion.

Due to the nature of the test system and the procedures to be undertaken, the animals were habituated to human contact from arrival. Where possible the same technicians were used to accustom the animals to handling and some dosing procedures on a daily basis. This was intended to reduce stress in the animals associated with the performance of study procedures. Treats (certified) were offered to the animals after any acclimation or study related procedure was performed (as appropriate).

During the pretreatment period, an area on the upper dorsum (across the midline) of each minipig was clipped free of hair over a 400 cm2 area of approximately 25 cm x 16 cm. The dermal test site was cleared of hair as often as necessary during the treatment period and care was taken to not damage the skin. The dermal test site was defined by dots in each of the four corners using indelible ink.

ANIMAL TREATMENT

The test and control/vehicle articles were administered once daily for 4 hours by topical application (non-occluded) to the dermal test site on the upper dorsum of the minipigs for 7 consecutive days. The appropriate volume of test material was weighed in the pharmacy department onto a piece of aluminum foil up to 3 days prior to the day of dosing. The control article was dispensed in the animal room using a syringe. The actual amount administered to each minipig was adjusted based on the most recent body weight of each animal.

The test and control/vehicle article formulations were applied directly to the dermal test site from the aluminum foil or using a syringe. The administered dose was then spread over the dermal test site using a gloved fingertip or fingertips to gently spread the cream/control material over the animal's skin. Attention was paid to ensure that the full dose was applied to the skin of the animal, inside the defined area, and that as little cream/control material as possible was left of the glove.

The 4 hour exposure period was curtailed by gentle washing of the dermal test site using warm municipal tap water.

No terminal procedures were performed on the animals after the last blood sampling occasion.

SPECIFIC PROCEDURES TO PRECLUDE CROSS CONTAMINATION

a) Animal Housing
The following strict guidelines were followed regarding housing and husbandry:
Control animals were housed in cages situated as far away from treated animals as possible.
Animal exercising was not performed during the 6-hour exposure period.
The control animals were exercised first when required, not with treated animals and in a clean area.

b) Animal Handling and Dosing
During the handling of the animals the following strict guidelines were followed:
Control animals were always handled and treated in advance of the test article-treated animals.
Where appropriate, the test article-treated animals were treated in ascending dose level order.
Any clothes that have been used while handling the treated animals were not used for the controls and they was stored separately (eg on a separate rack) to avoid cross contamination.
Disposable gloves were changed whenever procedures are performed on different animals. This included dosing, handling and bleeding or manipulating blood samples.

c) Toxicokinetic Blood Samples
During the collection of blood from the animals the details presented above were followed regarding handling with specific attention paid to the following:
Individuals removing and/or restraining the animals used one lab coat for controls and another for treated animals.
Gloves were changed between consecutive blood draws.
For each of the two different groups of animals (treatment and control) two separate and clearly marked V-boards were used, for bleeding procedures.

d) During the processing and analysis of TK blood samples
Blood collection tubes were clearly marked with the group color (color coded).
Blood samples obtained from control animals were centrifuged separately.
Blood samples for Control animals were processed separately from Treated animals.
Gloves and all disposable equipment were discarded between animals as in normal practice.
A separate pipette was used to harvest/inject biological samples obtained from control animals.
Pipette tips were changed following each single use as per normal practice.
The work area was cleaned between each animal.

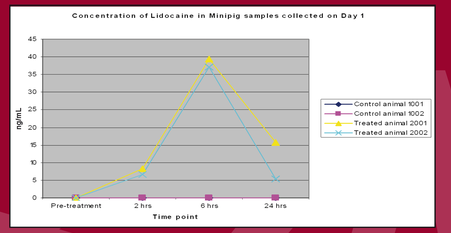
SAMPLE ANALYSIS

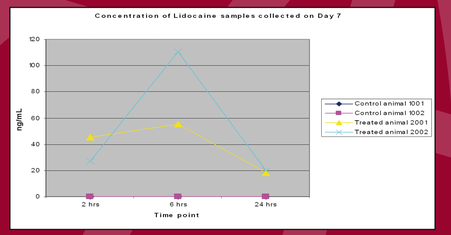
The plasma samples collected from the animal at pre-treatment and on Days 1 and 7 at 2, 6 and 24 hours after application of Lidocaine were analyzed at ITR by LC/MS/MS using a validated method.

RESULTS

There was no mortality and there were no clinical signs or dermal observations noted during the study. Body weight and food consumption were also unaffected. Minor changes at the dermal test site only were apparent during the study.

No measurable drug levels were noted in the control animals at any time point during the study. All treated animals did show appropriate drug levels that were seen to increase up to the 6 hour time point and then decrease to the 24 hour time point.





Concentrations of Lidocaine in minipig plasma samples determined by LC/MS/MS

Animal Number	Pre-treatment	Time Point/Lidocaine Concentration (ng/mL)					
		Day 1			Day 7		
		2 hours	6 hours	24 hours	2 hours	6 hours	24 hours
Control animal 1001	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Control animal 1002	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ-
Treated animal 2001	BLQ	8.38	39.33	15.67	45.25	55.54	18.11
Treated animal 2002	BLQ	6.67	37.07	5.42	27.20	110.49	20.03

BLQ = Below Limit of Quantification of <1 ng/mL

DISCUSSION

The results obtained on the study suggested that the procedures employed did preclude cross contamination in a small population of pigs. It is also important to analyze these procedures and the appropriateness for use on larger and longer duration studies.

Training of technicians must be a top priority to ensure that the process is followed.
The ideal situation would be to maintain Control animals in a separate area at least during exposure.
To allow separation of laboratory coats used for control animals, it is best to have hangers placed in another area of the room so that they are not in close proximity.
It is important to ensure that all departments dealing with animals or biological samples follow the same rules.

CONCLUSION

In conclusion, the objective of the study was to determine whether the measures detailed in the standard handling procedures in this report prevent test article contamination of the Control animals following non-occluded topical application of Lidocaine 2.5% cream (EMLA©) for 4 hours/day over 7 consecutive days to the minipig.

Results from the analysis of blood samples collected from control animals revealed that there were no measurable levels of drug in the control animals at any time point. Treated animals, however, showed appropriate levels of drug at all time points with the exception of the pre-treatment time point. The drug level noted for animal 2002 at the 6 hour time point was within the expected range following the exposure. It is assumed that even when the exposure is completed, levels of drug in the blood can still remain high and increase due to release from the tissue into the systemic circulation.

It can be concluded therefore, that the measures employed on the study were effective in preventing contamination of control animals and samples with drug.