Vascular catheter locking solutions in rats: Sodium citrate as an alternative to heparin

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Abstract

Pharmacokinetic studies in rats are conducted using a chronically implanted catheter that allows for repeated blood sampling; however, maintaining continuous patency sets practical limits on its uses. Catheter patency is affected by factors including flushing regimen, catheter material, and choice of locking solutions. In this study, a recently introduced non-heparin based locking solution containing 4% sodium citrate was compared with traditional heparinized locking solutions with respect to their ability to maintain patency of indwelling polyurethane vascular catheters in rats. Locking solutions of heparinized (500 IU/ml) 50% dextrose (LOCK 1) and heparinized (500 IU/ml) glycerol (LOCK 2) were obtained from SAI infusion technologies. Sodium citrate (4%) with 30% glycerol (LOCK 3) pH adjusted to 6.2 (range 6.0 to 6.5) with citric acid was provided by Cary Pharmaceuticals, Inc. Sixty adult male 225-250 grams CD rats (Crl:CD® (SD)IGSBR) were randomly allocated into 3 groups of 20 each for LOCK1, LOCK2 and LOCK3. Standard feed, bedding and water were provided ad libitum. The study was approved by the CRL IACUC. Rats were anesthetized and a polyurethane catheter was inserted into the femoral vein as previously described (Luo et al 2000). LOCK1, LOCK2, or LOCK3 was applied, the catheter was sealed with metal plug and the extravascular portion was tunneled subcutaneously, exiting at the inter-scapula region. Patency of the catheter was checked for five animals within each lock solution group at 7, 14, 21 and 28 days post-implantation. The plug was removed, and the catheter was aspirated to determine the ability to remove the lock solution and withdraw blood. If this aspiration failed, we attempted to flush with saline. If flush solution was infused, a second aspiration was made to check patency. Catheter was considered fully patent if withdraw of blood was successful with first or second attempt. LOCK1 and LOCK2 groups (heparinized) retained 100% patency to Day21. Patency rates decreased to 40% and 25% per group (respectively) at Day28, confirming earlier findings (Luo, et. al 2000). 80% of LOCK3 group retained patency to Day7, decreasing to 40% - 60% at Day14, 21 and 28. These findings support heparinized catheter locking solutions to maintain patency; however, sodium citrate locking solution may be used as an alternative, at a lower patency rate, where heparin is contraindicated or unavailable.

Introduction

Pharmacokinetic studies in rats are most effectively and humanely preformed using a chronically implanted catheter that allows repeated blood sampling. Once a catheter is successfully implanted, the amount of time that it reliably remains patent, with or without flushing, will set practical limits on its uses. The patency life can be affected by many factors including flushing protocol, catheter material, and lock solutions used to fill the lumen of the catheter. A number of catheter materials have been studied and their advantages and disadvantages described.

Charles River has been using heparinized dextrose and heparinzed glycerol as standard vascular catheter lock solutions for years. Recently a vendor, Cary Pharmaceuticals Inc, has developed a unique catheter lock solution as an alternative to heparin based locking solutions. This unique lock solution contains 4% sodium citrate and 30% glycerol with pH adjusted to 6.2 (range 6.0 to 6.5) with citric acid. Sodium Citrate acts locally as an anticoagulant by chelating ionized calcium in the blood resulting in the blockage of calcium-dependent clotting pathways. Sodium citrate 4% has been used successfully in clinical practice as an alternative catheter locking anticoagulant with minimal to no risk of bleeding.

This study was designed to compare the heparin and non-heparin based lock solutions with respect to their ability to maintain patency of unmanipulated, indwelling polyurethane femoral vein vascular catheters in rats. In answering questions about the suitability of lock solutions in extending patency, the use of a vascular flushing regimen would impose an unwanted variable that could confound interpretation. For this reason, regular flushing was not considered in the design of this study.

Materials and Methods

Lock Solutions

Heparinized dextrose (LOCK 1): Sodium heparin (10000 IU/ml) was added to 50% dextrose solution to make a final concentration of 500 IU/ml. Lock solution was purchased from SAI infusion technologies.
Heparinized glycerol (LOCK 2): Sodium heparin (10000 IU/ml) was added to glycerol solution to make a final concentration of 500 IU/ml. Lock solution was purchased from SAI infusion technologies.
Sodium Citrate glycerol (LOCK 3): This is a 4% sodium citrate and 30% glycerol with pH adjusted to 6.2 (range 6.0 to 6.5) with citric acid. This was provided by the supplier, Cary Pharmaceuticals Inc.
Femoral Vein Catheter: Polyurethane catheter with Outer Diameter: 0.040” and Inner Diameter: 0.025”.

Animals

Sixty male CD rats (Crl:CD®(SD)IGS BR) produced by Charles River Laboratories (Raleigh, N.C.) weighing between 225 and 250 grams were used. They were maintained in polycarbonate cages in a dedicated rodent surgical complex that was kept at 21 ± 2ºC with a relative humidity of 60 ± 5% and a 12/12 hour light/dark cycle. Commercially produced, sterilized feed, bedding and water were provided ad libitum. All conditions of animal preparation and use were in accordance with recommendations set forth in the Guide for the Care and Use of Laboratory Animals. The animals were of a VAF® health status.

Surgical Procedures

The animals were anesthetized with ketamine (75 mg/kg) and xylazine (6.0 mg/kg) administered intraperitoneally. The left caudal abdominal area, thigh and intrascapular areas were shaved and the skin prepared using chlorhexidine and alcohol. A 2.0 centimeter cranial-caudal incision was made to expose the femoral vein. The vein was isolated and tied off distally using non-absorbable suture material. A small incision was made in the femoral vein and a polyurethane catheter was inserted into the vein and a ligature subsequently tied around the cannulated vessel to fix the catheter in place. The catheter was locked with one of the lock solutions under study. The end of the catheter was sealed with a metal plug. The extravascular portion of the catheter was tunneled beginning in the inguinal area and extending subcutaneously and exiting at the inter-scapula region and the metal pin was secured using wound clip.

Experimental Design

The 60 rats were randomly allocated into 3 groups consisting of 20 rats each for LOCK1, LOCK2 and LOCK3. Patency of the catheter was checked for five animals within each lock solution group at 7, 14, 21 and 28 days post-implantation. Prior to patency checks the external portion of the catheter was examined for reflux of blood and for any visible evidence of clotting. A 1 cc syringe with a blunted 23 gauge needle filled with 0.5 cc of saline was attached to the catheter after the plug was removed.
The catheter was aspirated to determine the ability to remove the lock solution and withdraw blood. If the first aspiration failed, then an attempt was made to inject saline into the catheter. If flush solution could be infused, a second aspiration was then made to determine if blood could be withdrawn.
Patency of the catheter was classified in to the following categories:
Patent = Total number of catheters that were patent (fully and on flush).
Fully = Successful blood withdrawal on first attempt.
On Flush = Successful blood withdrawal after flushing solution into catheter.
Infuse only = Unsuccessful blood withdrawal but patent for infusion.
Non-patent = Unsuccessful blood withdrawal and infusion.

Table 1: Experimental Design

Group		Number tested for patency at time point post-op			
		Day 7	Day 14	Day 21	Day 28
1	a	5			
	b		5		
	c			5	
	d				5
2	a	5			
	b		5		
	c			5	
	d				4†
3	a	5			
	b		5		
	c			5	
	d				5

† One animal was excluded from the study in Group2d due to error made in patency test schedule.

Results

General observations

- All animals remained clinically healthy throughout the study.
- All the animals showed similar weight gains after surgery (i.e. no difference between treatment groups, p > 0.05).

Catheter Patency

Heparinized Dextrose (LOCK1)	7 Days	14 Days	21 Days	28 Days	Total
Patent	5/5	5/5	5/5	2/5	17/20
Fully	(5/5)	(4/5)	(5/5)	(0/5)	(14/20)
On Flush	(0/5)	(1/5)	(0/5)	(2/5)	(3/20)
Infuse only	(0/5)	(0/5)	(0/5)	(2/5)	(2/20)
Non-Patent	(0/5)	(0/5)	(0/5)	(1/5)	(1/20)
Heparinized Glycerol (LOCK2)	**7 Days**	**14 Days**	**21 Days**	**28 Days**	**Total**
Patent	4/5	5/5	5/5	1/4	15/19
Fully	(3/5)	(5/5)	(5/5)	(1/4)	(14/19)
On Flush	(1/5)	(0/5)	(0/5)	(0/4)	(1/19)
Infuse only	(1/5)	(0/5)	(0/5)	(2/4)	(3/19)
Non-Patent	(0/5)	(0/5)	(0/5)	(1/4)	(1/19)
Sodium Citrate glycerol (LOCK 3)	**7 Days**	**14 Days**	**21 Days**	**28 Days**	**Total**
Patent	4/5	2/5	2/5	3/5	11/20
Fully	(2/5)	(1/5)	(1/5)	(1/5)	(5/20)
On Flush	(2/5)	(1/5)	(1/5)	(2/5)	(6/20)
Infuse only	(1/5)	(3/5)	(3/5)	(2/5)	(9/20)
Non-Patent	(0/5)	(0/5)	(0/5)	(0/5)	(0/20)

Patent = Total number of catheters that were patent (fully and on flush).
Fully = Successful blood withdrawal on first attempt.
On Flush = Successful blood withdrawal after flushing solution into catheter.
Infuse only = Unsuccessful blood withdrawal but patent for infusion.
Non-patent = Unsuccessful blood withdrawal and infusion.

Discussion

Heparinized dextrose (LOCK 1) and Heparinized glycerol (LOCK 2) had 100% full patency or patency after flush up to Day 21 (3 weeks). The patency rate after Day 21 fell down to 40% and to 20% by day 28. However, Sodium Citrate glycerol (LOCK 3) had 80% full patency or patency after flush up to Day 7 and then decreased to 40%-60% for the remaining time points. The patency rate of LOCK1 and LOCK2 are better than LOCK3 ($p < 0.05$). This data supports earlier findings (Luo, et.al 2000), that Heparinized dextrose and Heparinized glycerol appear to be preferred catheter locking solutions to maintain patency in the rat. Sodium citrate locking solution may be used as an alternative, at a lower patency rate, where heparin is contraindicated or unavailable.

Ref: Y Luo et. al. AALAS 2000, Comparison of catheter lock solutions in rats.

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