



## Reducing Infanticide in Neonatal Rodents after Surgery by Using Tissue Glue

Xinhao Zeng<sup>1</sup>, Zefeng Lin<sup>2\*</sup>, Huiting Lin<sup>2</sup>, Ming Fu<sup>2</sup>, Yanlu Tong<sup>2</sup>, Hongjiao Chen<sup>2</sup> and Qiuming He<sup>2</sup>

<sup>1</sup>Department of Pediatric Surgery, Affiliated Hospital of Southwest Medical University, Luzhou, Sichuan, China

<sup>2</sup>Department of Pediatric Surgery, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou, Guangdong, China

### Abstract

To establish some special animal model, neonatal rodents must be experienced surgery. However, surgical and even simple invasive procedures in pups often lead to infanticide or maternal rejection by their dam. Tissue glue has been clinically applied to close the human operated incision over four decades. In this study, we present the feasibility of tissue glue in wound's closure and outcomes in neonatal rodent's surgery. One hundred and thirty-two neonatal rodents in experimental group underwent ligation of the common carotid artery (CCA, n=85, small incision) and proper hepatic artery (PHA, n=47, large incision) and their wound were closed by tissue glue while 58 pups in historical control group and their incision were sutured. There were statistically significant differences in the survival rate of CCAL (98.9% vs. 65.7%, P<0.01) and PHAL (95.7% vs. 47.8%, P<0.01). Clearly, high survival rate in the experimental pups suggests that the application of tissue glue can improve the survival rate in neonatal rodent's surgery and reduce the number of laboratory animal.

**Keywords:** Neonatal surgery; Rodents; Infanticide; Survival; Animal welfare

### Introduction

Postoperative survival of neonatal rodents is the primary and critical factor to the success of specific experimental protocols. Simple manipulation or surgical procedures in newborn rodents often leads to maternal neglect or infanticide. It is an extremely distressing issue that experimental pups, received invasive procedures, were killed by their mothers, though infanticide, one of the most incomprehensible behaviors, is quite common in laboratory rodents [1,2]. Ten et al. [3] described the phenomenon that the pup was cannibalized by its mother within the first day after experiencing Hypoxia and Ischemia. Hickman and Swan [4] also reported average 8.45% of litter were rejected by the foster dam, even though the litter was picked up gently and mixed with dirty bedding, nesting material, when they studied 'Effects of Age of Pups and Removal of Existing Litter on Pup Survival during Cross-Fostering between Multiparous Outbred Mice'. Helander et al. [1] reported to carefully wipe the blood of the incision and involution the wound, then sprayed a layer of plastic film to cover the wound, and the postoperative rat survival rate could reach at 99%. However, no detailed description of pictures, method and statistical studies were shown in the literature. On the basis of reducing the ratio of infanticide in neonatal rodents surgery and of complying with the 3R concept proposed by the Russell and Burch in 1959: 'replace, reduce, refine' [5], in our study, we describe a briefness technique for wound in newborn rodents and show that it effectively reduced infanticide.

### Materials and Methods

#### Animals

We used Sprague-Dawley neonatal rats aged 1 hr to 24 hr and Balb/c mice aged 7 day, which were obtained from the Experimental Animal Center of Sun Yat-Sen University (Number of institution license SYXK2011-0029) and cultured in specific pathogen free conditions with 12h light and 12h dark cycles. The protocols of the operation were approved by the Sun Yat-sen medical university. During operation the animals were under anesthesia with isoflurane via inhalation administration. During the postoperative period the pups were place under 37°C heating lamp with full of maternal cages bedding and returned to their cage when recovered. The entire protocol was validated by

### OPEN ACCESS

#### \*Correspondence:

Zefeng Lin, Department of Pediatric Surgery, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou, Guangdong, China,  
E-mail: zefeng-lin@foxmail.com

**Received Date:** 12 Sep 2017

**Accepted Date:** 25 Nov 2017

**Published Date:** 04 Dec 2017

#### Citation:

Zeng X, Lin Z, Lin H, Fu M, Tong Y, Chen H, et al. Reducing Infanticide in Neonatal Rodents after Surgery by Using Tissue Glue. *Clin Surg.* 2017; 2: 1800.

**Copyright** © 2017 Zefeng Lin. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



**Figure 1:** The healing of wound in CCA+TG group. (A) before closure of incision, (B) after closure of incision with tissue glue, (C & D) the image of wound on postoperative 1<sup>st</sup> day, 4<sup>th</sup> day, respectively.



**Figure 2:** The recovery of wound in PHA+TG group. (A) before closure of incision, (B) after closure of incision with tissue glue, (C & D) the image of wound on postoperative 1<sup>st</sup> day, 4<sup>th</sup> day, respectively.

the local ethics committee under the authority of the Sun Yat-Sen University in accordance with animal welfare guidelines.

There were two experimental groups, including CCAL with tissue glue (CCAL+TG, n=85) and PHAL with tissue glue (PHAL+TG, n=47), while compared to historical groups, CCAL without tissue glue (CCAL-TG, n=35) and PHAL without tissue glue (PHAL-TG, n=23).

### Operation procedure

The surgical procedures were performed under sterile conditions, using surgical micro-instruments and stereomicroscopy (magnification 8x), and the operation table temperature was maintained at 37°C by used electric blanket under it. In both experiment groups, during the surgical procedure meticulous care was taken to ensure that portal vein, bile ducts, jugular vein, trachea and vagus nerve were not injured.

**PHAL:** Mice of 7-day-old were placed in a supine position. After laparotomy through a transverse incision (1.0-cm length) in the upper abdomen under the xiphoid process 0.2 cm. The liver lobes were gently mobilized by used saline gauze, the proper hepatic artery (PHA) was exposed from the common hepatic artery by snipping the ligamentum hepatogastricum and uplifting the hepatic caudate lobe, ligated with a 9-0 silk suture and divided twice.

**CCAL:** A 24-hr-old SD newborn were placed on the operating table. The surgery was carried out through a longitudinal incision (0.3-cm length) on the slightly left of middle of the cervical region upper thorax. The common carotid artery (CCA) was separated from the vagus nerve and the jugular vein beside the trachea by a glass minute hand, then ligated with 5-0 silk suture and sectioned.

### Management of wound

After checking no significant active bleeding, use 6-0 absorbable

suture thread for the subcutaneous, the wound bloodstain must be carefully wiped out by iodophor cotton swab, then use sterile dry cotton to ensure the wound dry. Pups' wounds in CCAL+TG and PHAL+TG groups were closed by tissue glue (Histoacryl<sup>®</sup>, B. Braun Medical Supplies, Inc.) while CCAL-TG and PHAL-TG were sutured 6-0 suture material. After perfectly involution the skin wound, tissue glue directly bond the skin wound.

### Postoperative observation

The pups were place under 37°C heating lamp with full of maternal cages bedding after surgical procedure and returned to their cage when recovered. The mice were observed daily after surgery, the condition of wound and the health of mice were photographed and recorded.

### Statistical analysis

Statistical analysis was treated with the Fisher's exact test and  $P < 0.05$  was considered to be statistically significant.

## Results

In historical groups, the dam always took pups in her mouth, licked their wound, removed the stitches, and even ate the contents of the abdominal cavity once the pups were back.

As shown in Table 1, the survival of postoperative rodents in neonates in CCAL was significant improved after application of tissue glue (98.9%) while compared to pups without tissue glue (65.7%) ( $P < 0.0001$ ). The similar improvement of survival was observed in PHAL+TG group (95.7%) while PHAL-TG group (47.8%) ( $P < 0.0001$ ). The wound healing was shown in Figure 1 and Figure 2, respectively.

## Discussion

Surgical neonatal rodent's models were widely used to research some aspects infant disease, for example, in order to study perinatal

**Table 1:** Survival of neonatal rodents after surgery.

	+TG		-TG		
	Number	Survival	Number	Survival	P value
CCAL	85	84	35	23	<0.0001
PHAL	47	45	23	11	<0.0001

brain injury to establish animal models of acute neurological injuries via cerebral hypoxia-ischemia in Neonatal Rats or Mice [6]. Survival of neonatal rodents following surgical procedures may be vital to the success of specific experimental protocols. As the Natural Selection, "survival of the fittest" will be inevitable problems between offsprings, as a result, these pups which was stillborn, listless or weakened would be abandoned, even consumed by the dams, to guarantee other pups survival [7]. In our previous experimental, the high cannibalism rate (34.3% in CCAL and 52.2% in PHAL) always obstructed the studies progress and violates the principles of "3R" in experimental animal. This phenomenon is very common in laboratory rodents. We summarized the possible cause: the blood of operation incision, and with sutures, as the foreign bodies. Some laboratory methods were used to prevent maternal infanticide: 1) grabbing the mother rat to make them struggle to pee, then, smearing the urine on their young; 2) covering postoperative rodents with the cage padding, both of methods were to make rat recognize the pups with mothers odour; 3) using acepromazine maleateto sedata the dam [8]; 4) Libbin et al. [9] adopted "hand gentling" to prevent postoperative pups from maternal cannibalism. Though these methods could improve the survival of pups, few details of pictures, protocols and statistical studies were demonstrated in the literature.

Tissue glue was clinically used to close the fresh, clean, dry and tension-free skin wound over decades. The component of tissue glue is n-2-butyl cyanoacrylate monomer, and once contact with human tissue fluid will react into a polymer, to form a layer of film, and bond together the wound edge tightly. As a consequence, it is no need to suture skin wound, shorten operation time [10], and alleviate sufferings of the pups which was caused by suturing, reduce the risk of dams biting the sutures of the wound to lead wound dehiscence, then the postoperative pups being eaten by dams. The polymer film can also isolate the tissue from germ, have antimicrobial effect [11], have more effective reduce inflammatory reaction of wound in comparison with traditional silk suture [12], and can spur wound healing. Howard et al. [13] also reported that application of glue does not increase the risk of wound infection.

The application of tissue glue can shorten the operation time of experimental animal, reduce the pain of pups, and so reflect the animal welfare about the pups. This simple technology solves the problem of the survival of post-operation rodents, improves the success ratio of animal models, quickens experimental process, reduces the number of laboratory animals used, and it can provide technical support to build other neonatal animal model by surgery.

## Acknowledgment

We thank Prof. Yan Chen, Professor in University of Hong Kong for the manuscript preparation. Moreover, we thank Yanlu Tong, Huiting Lin and Hongjiao Chen for their assistance in experimental information collection. E-mail addresses: zefeng-lin@foxmail.com (Z. Lin) or xinhaozeng@foxmail.com (X. Zeng).

## References

1. Helander HF, Bergh A. How to avoid maternal cannibalism after neonatal surgery in rats. *Experientia*. 1980;36(11):1295-96.
2. DeSantis DT, Schmaltz LW. The mother-litter relationship in developmental rat studies: cannibalism vs. caring. *Dev Psychobiol*. 1984;17(3):255-62.
3. Ten VS, Wu EX, Tang H, Bradley-Moore M, Fedarau MV, Ratner VI, et al. Late measures of brain injury after neonatal hypoxia-ischemia in mice. *Stroke*. 2004;35(9):2183-8.
4. Hickman DL, Swan MP. Effects of age of pups and removal of existing litter on pup survival during cross-fostering between multiparous outbred mice. *J Am Assoc Lab Anim Sci*. 2011;50(5):641-6.
5. Russell WMS, Burch RL. *The Principles of Humane Experimental Technique*. Universities Federation for Animal Welfare Wheathampstead, England.1959.
6. Changlian Zhu, Xiaoyang Wang, Klas Blomgren. Cerebral Hypoxia—Ischemia in Neonatal Rats or Mice: A Model of Perinatal Brain Injury. *Animal Models of Acute Neurological Injuries*. 2009;221-30.
7. Reynolds RD. Preventing maternal cannibalism in rats. *Science*. 1981;213(4512):1146.
8. Hayek A, Kuehn C. A method for preventing maternal cannibalism after neonatal rat surgery. *Lab Anim Sci*. 1982;32(2):171-2.
9. Libbin RM, Person P. Neonatal rat surgery: avoiding maternal cannibalism. *Science*. 1979;206(4414):66.
10. Suriano MM, Stirbu O, Pérez DM, Serra Segarra M. Blepharoplasty: to suture or to use cyanoacrylate? *Arch Soc Esp Oftalmol*. 2011;86(3):81-4.
11. Wilkinson JN, Chikhani M, Mortimer K, Gill SJ. The antimicrobial effect of Histoacryl skin adhesive. *Anaesthesia*. 2008;63(12):1382-4.
12. Kumar MS, Natta S, Shankar G, Reddy SH, Visalakshi D, Seshiah GV. Comparison between Silk Sutures and Cyanoacrylate Adhesive in Human Mucosa- A Clinical and Histological Study. *J Int Oral Health*. 2013;5(5):95-100.
13. Howard BM, Eshraghi SR, Holland CM, Refai D. Octyl-cyanoacrylate skin adhesive is effective for wound closure in posterior spinal surgery without increased risk of wound complications. *Clin Neurol Neurosurg*. 2014;125:137-42.