





When less is more: Experimental Bishop–Koop technique for reduction in the use of laboratory animals for intestinal pathophysiological studies

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Juan Cruz Abate¹ , Natalia Lausada¹, Leandro Vecchio Dezillio² ,
Jeremías Moreira³ , Ivana Ivanoff Marinoff²,
Maria Malena Ferreyra Compagnucci², Ane Miren Andrés Moreno^{5,6},
Carlota Largo⁴, Martín Rumbo², Francisco Hernández Oliveros^{5,6},
David Romanin^{2,*} and Pablo Stringa^{1,2,6*} 

Abstract

The use of animals to gain knowledge and understanding of diseases needs to be reduced and refined. In the field of intestinal research, because of the complexity of the gut immune system, living models testing is mandatory. Based on the 3Rs (replacement, reduction and refinement) principles, we aimed to developed and apply the derived-intestinal surgical procedure described by Bishop and Koop (BK) in rats to refine experimental gastrointestinal procedures and reduce the number of animals used for research employing two models of intestinal inflammation: intestinal ischemia-reperfusion injury and chemical-induced colitis. Our results show the feasibility of the application of the BK technique in rodents, with good success after surgical procedure in both small and large intestine (100% survival, clinical recovery and weight regain). A considerable reduction in the use of the number of rats in both intestinal inflammation models (80% in case of intestinal ischemia-reperfusion damage and 66.6% in chemical-induced colitis in our experimental design) was achieved. Compared with conventional experimental models described by various research groups, we report excellent reproducibility of intestinal damage and functionality, survival rate and clinical status of the animals when BK is applied.

Keywords

Animal model, animal use, ethics and welfare, organisms and models, 3Rs, reduction, surgery, techniques

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Introduction

Since Russell and Burch reported on the concept of the 3Rs (replacement, reduction and refinement) in 1959, researchers have had a moral duty to minimise harm to laboratory animals. Currently, in the area of animal

⁴Department of Experimental Surgery (IdiPaz), La Paz University Hospital, Spain

⁵Department of Pediatric Surgery, La Paz University Hospital, Spain

⁶Transplant Group, La Paz University Hospital Health Research Institute (IdiPAZ), Spain

*Share senior authorship.

¹Organ Transplant Laboratory, School of Medicine, National University of La Plata, Argentina

²Institute for Immunological and Pathophysiological Studies (IIFP), School of Exact Sciences, National University of La Plata, National Council of Scientific and Technical Research (CONICET), Argentina

³Institute of Translational Medicine, Transplantation and Bioengineering (IMETTyB), Favaloro Foundation University Hospital, Argentina

Corresponding author:

Pablo Stringa, UNLP, 60 y 120, La Plata, 1900, Argentina.
Email: pstringa7@gmail.com

research, expectations to work within the principles of the 3Rs are becoming stronger, and oversight of compliance with these principles is getting stricter. It is now being reinforced with the addition of a further concept; responsibility, which is appearing as the fourth R. For this reason, the development of tools and methodologies that allow optimisation of the use of animals for research is in great demand and is a high-value goal for researchers.¹

The study of intestinal physiology, as well as several diseases and treatments involving the small intestine (SI) and large intestine (LI), are of great interest to the human and animal health science community. In relation to the SI, there has been a constant advance in the knowledge of intestinal ischemia-reperfusion injury (IIRI)—a phenomenon that occurs under conditions such as strangulated hernias, necrotising enterocolitis and intestinal transplantation, among others.^{2–10} Regarding the LI, colitis, which has a high prevalence in the human population, is among the topics of greatest interest to researchers. Therefore, the understanding of the pathophysiology and developing strategies to prevent and treat these diseases are relevant to today's health needs.^{11–14}

Rodents (rats and mice) are the animals used most widely in experimental intestinal research. There are numerous studies where, due to a lack of available techniques, one single animal is used for collecting each intestinal sample rather than using the same animal for a series of samples from any of the intestines under study. For example, in the SI, superior mesenteric artery clamping model is currently used to evaluate IIRI, requiring one animal for each sample and study time.^{15–29} The same situation occurs in the field of experimental colitis, where old and recent scientific articles use one animal in each LI sample collection.^{30–37} For the reasons mentioned above, it is a challenge to develop models that enable reducing the number of animals involved in these studies.

The intestinal bypass surgical technique specially considered in paediatric patients with meconium ileus and short bowel syndrome described by Bishop and Koop (BK) appears as a novel surgical tool to transfer from the clinic to the experimental scenario. The BK surgical procedure consists of an entero-anastomosis of the end of the proximal segment of intestine to the side of distal bowel, with exteriorisation of the end of the distal segment as an end-stoma.³⁸

The goal of this study was twofold: first, to evaluate the feasibility of developing the BK surgical procedure in rats to take several intestinal samples from the same animal in both SI and LI. Second, to use the BK procedure in an experimental model of IIRI and colitis as a proof of concept to demonstrate the capacity of the BK technique to considerably reduce the number of

animals used for research and refine experimental models for gastrointestinal (GI) studies.

Methods

Sprague–Dawley rats (4–5 weeks old; weight 221 ± 13 g) were housed in a climate-controlled room ($21^\circ \pm 2^\circ\text{C}$ and relative humidity of $45\% \pm 15\%$) on a 12 h light–dark cycle (lights come on at 8.00 a.m. and then go off at 8.00 p.m.) at the animal facilities in the School of Medicine, National University of La Plata, Argentina. The animals arrived at least 5 days before the experiment to acclimatise to the facility and were housed individually in standard plastic cages ($42.5\text{ cm} \times 26.5\text{ cm} \times 18\text{ cm}$) before any surgical procedure. For enrichment, each cage included a plastic tube, bedding and nesting material. Rodent standard chow and water were provided ad libitum before the experimental procedures. The study protocol was approved by the local Animal Welfare Ethics Committee of the School of Medicine, National University of La Plata, Argentina (Ethics No: P01-03-2021). As we were developing a novel experimental surgical procedure with unknown reproducibility, we divided the workflow into two steps: in the first, we determined the feasibility of establishing this new surgical technique and in the second we compared the results obtained using the new technique with results from standard practice using two different models of intestine biology to analyse the effects on the results introduced by the technique and the impact in reduction on animal use. Since the first step showed high reproducibility, the sample size of animals used for the second step was established as five—a group size used regularly in our GI rat studies.

Step 1. Feasibility of BK technique in rats

To achieve the first aim of the study, animals were divided into two randomized groups: (a) BK SI and (b) BK LI bypass (five animals per group).

SI BK procedure. Rats were placed on a thermal blanket from the start of the procedure and until postoperative recovery to prevent hypothermia. Inhalation anaesthesia, isoflurane 5 and 2%–2.5% (oxygen 1 l/h) for induction and maintenance, respectively, were used. Subcutaneous buprenorphine (0.04 mg/kg) was administered as analgesic before starting the procedure. Also, local anaesthesia (lidocaine 2%) was subcutaneously administered in the area of surgical incision and stoma. After corresponding skin antisepsis, the abdomen was approached through a xiphoid–pubic incision. A quick exploration of the abdominal cavity was performed to identify the thin jejunum loops. After coagulating the mesenteric vessels of the area,

jejunal transection was performed 15 cm from the ileocecal junction. Next, the distal bowel was anastomosed side-to-end into the proximal bowel and fashioned into the stoma. Entero-anastomosis was performed using a 7-0 polypropylene continuous transmural single-layer suture technique with a taper point needle (Figure 1a). The end-stoma was made by placing cardinal suture points of 7-0 polypropylene that included the skin, the aponeurosis, and entire thickness of the intestinal wall. Finally, the abdomen was closed in layers with 4-0 nylon.

In addition, the Santulli technique was performed on a group of animals ($N = 3$). This procedure is similar to the BK procedure, with the difference being that, after intestinal transection, the proximal intestinal portion is fixed to the outside by means of a stoma, and a side-to-end anastomosis with the distal bowel is performed.³⁹ A representative drawing of the Santulli technique and its differences with BK are shown in Figure 2c. Based on the similarity of both surgical procedures, and given the lack of scientific literature regarding these experimental surgeries, we decided to perform the Santulli procedure to compare the outcome versus the BK technique.

LI BK procedure. The procedure for the LI followed the same initial steps. Thin loops were mobilised to the

right of the animal, and the descending colon was located. The fascia was incised all the way from the rectum to a few centimetres beyond the splenic flexure to achieve better mobilisation. At this angle, the bowel was transected after coagulation of the mesenteric blood vessels. Approximately 5 cm was measured from the transection site distally to then perform the end-to-side colo-colonic anastomosis with the same technique used for the SI (Figure 1b). The space in the abdominal wall necessary for the colostomy was left 1 or 2 cm from the edge of the incision. Finally, colostomy was performed using the same technique as described for SI.

Post-surgical animal care. Rats were placed on a thermal blanket during recovery to prevent hypothermia. After surgical recovery, the animals were housed individually. Subcutaneous ceftriaxone (65 mg/kg) and buprenorphine (0.04 mg/kg) were administered as antibiotic prophylaxis and analgesics every 24 and 12 h respectively, immediately following the surgical procedure until the 5th postoperative day (POD). A soft diet (Gelatin) was provided in the cage for the first 48 h, after which the usual solid diet was resumed with standard rodent chow ad libitum. The animals were not restricted from access to water.

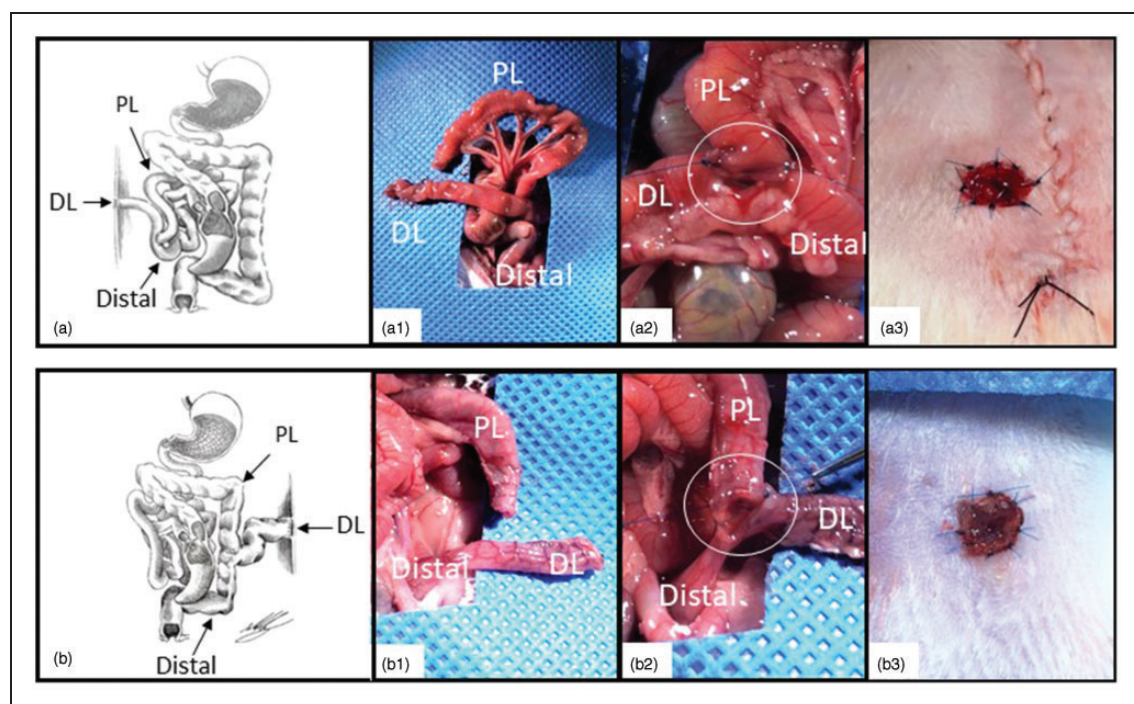


Figure 1. (a) Scheme of BK technique in SI. (a1) Intraoperative image of BK surgery in the SI of the rat. (a2) SI entero-anastomosis. (a3) Macroscopic appearance of end-ileostomy and (b) Scheme of BK technique in rat LI. (b1) Image of intraoperative BK procedure in LI of the rat. (b2) LI entero-anastomosis. (b3) Macroscopic appearance of end-colon stoma. BK, Bishop-Koop; Distal, distal loop; DL, derived loop; LI, large intestine; PL, proximal loop; SI, small intestine.

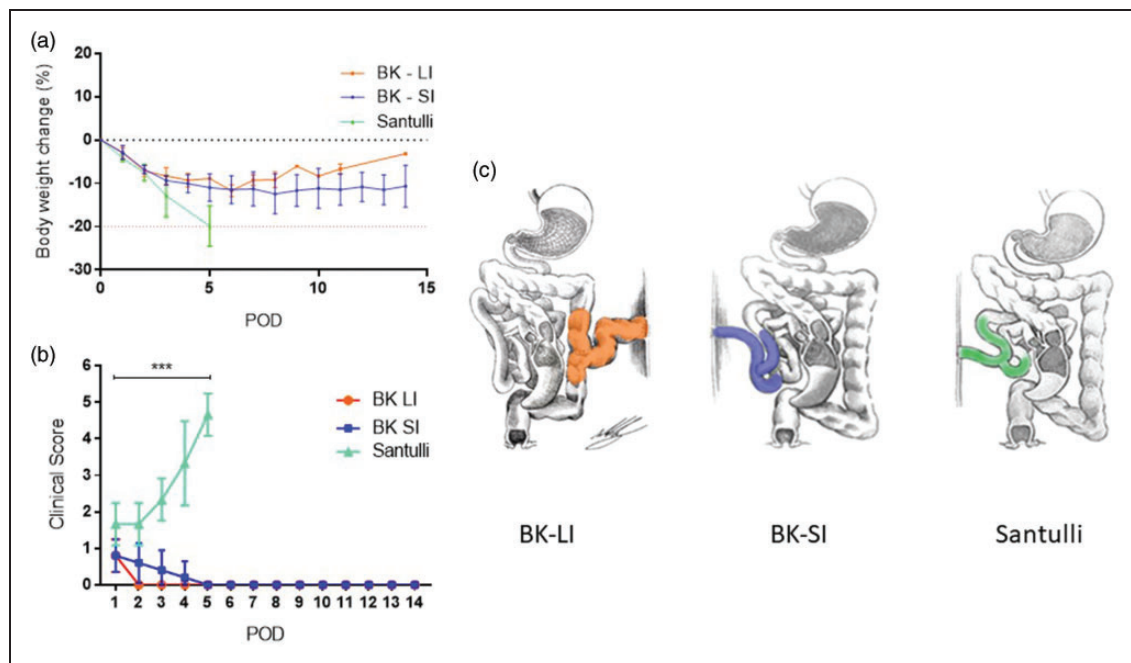


Figure 2. (a) Percentage bodyweight change after surgical procedures in experimental groups: LI, SI and Santulli. (b) Clinical status of rats subjected to BK or Santulli procedures (statistically significant differences were observed between BK vs Santulli) and (c) Schematic representation of BK-LI (orange), BK-SI (blue) and Santulli (green) procedures. BK, Bishop-Koop; LI, large intestine; POD, postoperative day; SI, small intestine.

To determine the success of the novel BK experimental procedure, post-surgical survival and clinical status of the animals was considered as a main measure. Twice-daily clinical monitoring was performed after the BK procedure, including for manifestations of pain and/or discomfort. The signs that were taken into account in each evaluation included the presence or absence of piloerection, presence of clinical signs of dehydration, ocular discharge, animal posture, lethargy attitude, diuresis and catharsis. In turn, the viability of the end-stoma and scar were evaluated, as well as the state of the abdomen by means of palpation. A value (0 = normal, 1 = mild, 2 = moderate, 3 = severe) was assigned to each parameter to establish a semi-quantitative clinical score. Rats received a general score resulting from adding evaluated parameters. Human end-point was applied in animals with a clinical value of >4 .⁴⁰

To avoid potential confounding factors, all surgeries and sampling were performed by the same surgeon—an MD and general surgeon with approximately five years of experience in microsurgery. Likewise, postoperative management was identical for all the animals involved in the study.

Intestinal biopsy and histological evaluation

Small bowel microscopic analysis. Intestinal samples of the derived loop (approximately 1–1.5 cm in

length) were taken from the end-stoma at PODs 0, 7 and 14. The animal was anaesthetised with isoflurane as previously described, the holding points were released and the intestinal stump pulled with a delicate maneuver. The small bowel mesenteric blood vessels were coagulated, and intestinal sectioning was performed. After sampling, a new ostomy was performed. As we previously described, local anaesthesia (Lidocaine) and subcutaneous buprenorphine (1 dose at sampling time and one dose 12 h after sampling procedure) were administered.

Tissue samples were fixed in 10% formalin, embedded in paraffin and 5 μ m sections were stained with haematoxylin and eosin for histological evaluation. The intestinal architecture was evaluated double-blind, according to the Park/Chiu Score (score: 0, normal mucosa; 1, subepithelial space at villus tip; 2, more extended subepithelial space; 3, epithelial lifting along the villus side; 4, denuded villi; 5, loss of villus tissue; 6, crypt layer infarction; 7, mucosal infarction; and 8, transmural infarction).^{41,42} The intestinal villus/crypt ratio was assessed using FIJI software.⁴³

Assessment of colonic epithelial damage and inflammation in colon. Colonic samples (approximately 1 cm in length) were taken from the ostomy at PODs 0, 1 and 2. Histopathological damage was determined according to previously described methods.⁴⁴

This system records two separate scores for evaluating epithelial damage and infiltration. Briefly, epithelial damage was scored as 0 for none, 1 for a minimal loss of goblet cells, 2 for extensive loss of goblet cells, 3 for a minimal loss of crypts and extensive loss of goblet cells and 4 points for extensive loss of crypts; infiltration was scored as 0 for none, 1 for an infiltration around crypt bases, 2 for an infiltration in muscularis mucosa, 3 for extensive infiltration in muscularis mucosa with oedema and 4 points for the infiltration of submucosa. Preparations were assessed in a double-blind manner, and the histopathological activity index (HAI) was calculated as the sum of the epithelial damage and the infiltration score, ranging from 0 to 8 points from unaffected to severe colitis.

Step 2. BK model application

As previously stated, we evaluate the BK technique to reduce the number of animals used in the study of intestinal pathologies. Among experimental studies focused on gastrointestinal diseases found in the literature, IIRI- and 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis in rats appears as a current model used by many authors and research groups around the world.^{20–37} For this reason, we selected these two relevant pathologies to evaluate the experimental BK procedure in an intestinal injury scenario. IIRI was used for simulating a model of intestinal intussusception, strangulated hernia or intestinal transplantation. TNBS-induced colitis was performed as a model of inflammatory large bowel disease.

BK for the study of IIRI. For the induction of IIRI, immediately after the SI BK procedure as previously

described, selective vascular and intestinal clamping of the derived intestinal loop was performed for 60 min, followed by reperfusion (BK + IIRI, $N=5$) (Figure 3). We ensured complete ischemia of the derived loop in a uniform manner, which was corroborated by the change to a pale colouration. After the stipulated ischemia time (60 min), we unclamped (Figure 3d) and observed the return to intestinal physiological colouration (intense pink) confirming the reestablishment of blood flow. All rats received a dose of 50 IU intraperitoneal heparin before and after vascular clamping.

Two control groups were also assessed: (a) Control BK group (CT-BK, $N=5$): BK without IIRI (only BK procedure without clamping of the derived loop) and (b) Conventional IIRI by occlusion of superior mesenteric artery (OSMA) without BK procedure ($N=5$). Briefly, as described by our group and others, after laparotomy, superior mesenteric artery was identified and occluded with a vascular clamp for 60 min followed by different times of reperfusion, depending on stipulated sampling design.^{8,20–22,29}

Small bowel sampling and morphological analysis.

A series of tissue samples of approximately 1 cm were obtained from the intestinal-derived loop in the BK+IIRI and BK without IIRI groups: during surgery, when making the derived loop (T0), 30 min (T1), 60 min (T2), 1 day (T3) and 7 days after reperfusion (T4). Tissues were sectioned, fixed, embedded, stained (haematoxylin–eosin) and scored using the Park/Chiu score as previously described. In the OSMA group, five animals were used for each sampling time (0, 30 min, 60 min, 1 day and 7 days after IIRI).

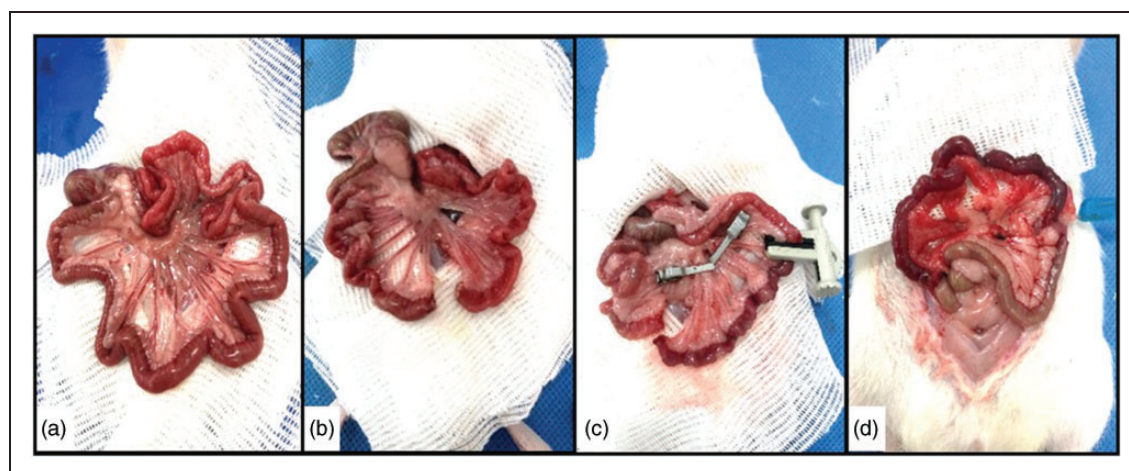


Figure 3. Step by step surgical procedure for IIRI by clamping the derived loop and BK surgery. (a) Macroscopic appearance of normal SI of the rat. (b) Section of the SI to create the derived loop. (c) Clamping of the derived loop including mesentery and intestinal lumen at the beginning and (d) Immediate reperfusion of the derived loop after 60 min of ischemia. BK, Bishop-Koop; IIRI, intestinal ischemia reperfusion injury; LI, large intestine; SI, small intestine.

Functional analysis of the reperfused intestines. To assess the absorptive capacity of the derived loop, a glucose absorption test was performed on POD 7. A concentrated glucose solution (2 g/kg) diluted in saline solution was administered through the ostomy of the intestinal-derived loop in BK and BK+IIRI groups. The OSMA group received intraluminal glucose solution. Blood glucose values were established using an Accu-Chek blood glucose meter (Roche) immediately before administration (baseline), and then again at 15 and 30 min. Blood droplets were obtained by puncturing the tail.⁴⁰

BK in a colitis model induced by TNBS. The BK technique was used in the study model in which colitis was induced by TNBS in the LI. Immediately after the BK procedure in the distal portion of the LI, a single dose of a 200 µl solution of 2.5% TNBS (diluted in 50% ethanol) was administered through the colostomy (group TNBS). In the control group, 200 µl of a 50% ethanol solution (vehicle group) was administered through the colostomy. The third group (sham) consisted of animals in which BK technique was performed and PBS solution was administered through the colostomy.

The daily clinical score was recorded as described previously, paying special attention to the presence of anal and stomal discharge and the presence of anal blood.

Sampling from the derived loop was performed at the time of anastomosis (T0) and subsequently through the ostomy at 24 h (T1) and 48 h (T2). At animal euthanasia, a sample was taken from both the derived loop as well as the loops proximal and distal to the entero-anastomosis.

Intestinal biopsies were stained with haematoxylin–eosin for colonic histopathological analysis as previously reported.

Statistical methods

Data were analysed using GraphPad Prism 8 (San Diego, CA). Kruskal–Wallis test followed by Dunn's multiple comparison test were used for clinical score and histopathological SI and LI analysis. Data were presented as means and standard deviations. Statistical significant differences was determined and reported by a *P* value <0.05.

Results

Experimental BK surgical technique is feasible in both SI and LI

Rat survival for the BK technique in both SI and LI was 100%. The maximum weight loss was observed on

POD 10 (10.6% ± 3.9% for the SI group; 11.17% ± 1.47% for the LI group), after which the rats regained their original body weight (Figure 2a). No complications associated with the surgical procedure were observed and an acceptable clinical score was obtained in all cases. Animals showed mild piloerection during the first 48 h after BK surgery, with an excellent recovery until 3 POD (Figure 2b).

Interestingly, the animals that received the Santulli technique worsened their clinical score from the surgical procedure and lost considerable weight on the first 5 PODs, at which time the humane endpoint criteria were applied. These rats showed moderate piloerection, mild ocular discharge, mild lethargy, antalgic posture and excessive loss of intestinal content (Figure 2b).

Histopathological analysis of the SI did not show changes in the villi/crypt ratio, suggesting the absence of major alterations in terms of morphology (Figure 4b). The same was evident in the Park/Chiu score, which presented an average value of 1 with a maximum value of 3 on its scale in the derived loop, as well proximal and distal to the anastomosis (Figure 4c). Histopathological evaluation of the LI samples showed no alteration in any of the sections (proximal loop, distal loop and derived loop) in samples taken 48 h after anastomosis compared with animals with no intervention.

Applications of the BK technique for animal use reduction

Kinetic study of IIRI after BK procedure. As proposed in the objectives, five samples for the IIRI study were taken from the same SI in each animal (Figure 5a), showing considerable potential for reducing the number of rats used. As shown in Figure 5b, histopathological analyses of the BK + IIRI group (total use of animals in this group = 5) revealed a basal Park/Chiu score before the small bowel-derived loop IIRI ≤ 1, which reached a maximum of 4.0 ± 0.5 at 30 min and 60 min post-reperfusion, with a posterior decrease in intestinal damage, which reached a minimum value of Park/Chiu score = 0.66 ± 0.8 at 7 POD in the derived loop, showing good regeneration after ischemic injury. The evolution of these animals were absolutely identical to the group subjected to the conventional methodology (Figure 5b). In the OSMA, one animal was used for each time point, with a total usage of 5 animals per time point and a total use of 25 animals in this group. Comparison of these two groups showed that the use of the BK technique allows reduction of 80% in the use of animals while not changing the biological outcome of the IIRI event (Supplementary Material). Furthermore, we included a group subjected to BK technique without ischemic challenge to determine the impact of the

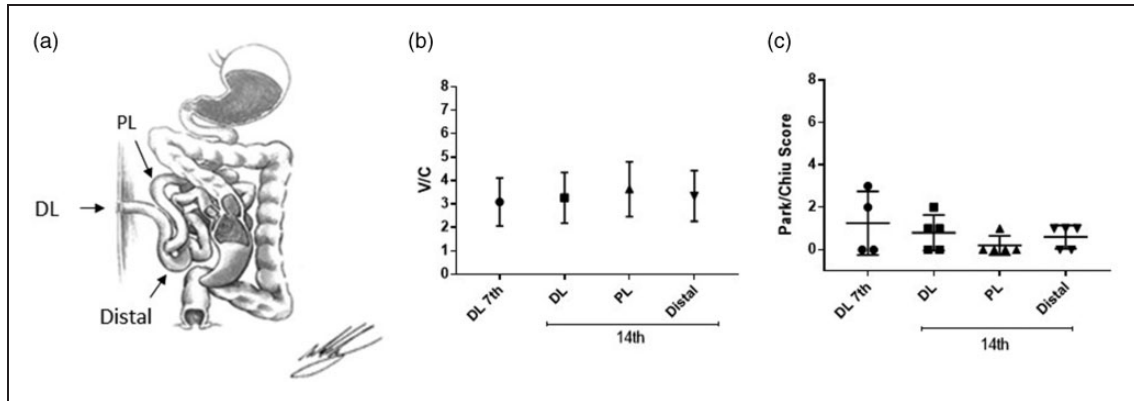


Figure 4. (a) SI BK sampling locations in DL, PL and Distal. (c, b) Villus/crypt ratio (b) and Park/Chiu score (c) at 7 and 14 postoperative days. No statistically significant differences were observed between sampling sites and study times in any case. BK, Bishop-Koop; Distal, distal loop; DL, derived loop; PL, proximal loop; SI, small intestine.

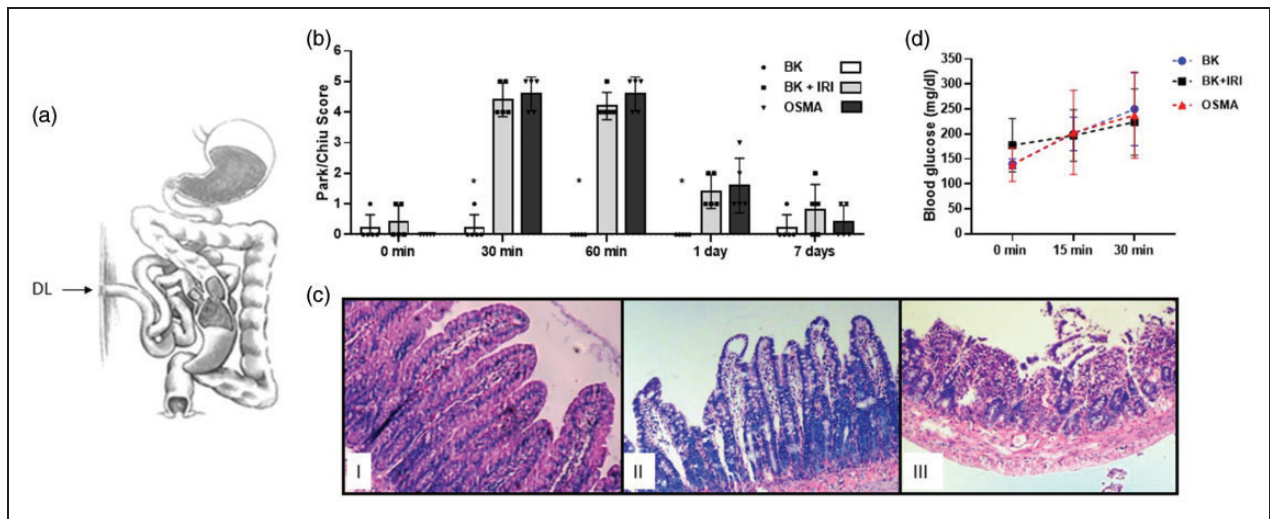


Figure 5. (a) Scheme of small bowel BK procedure. Serial intestinal samples were taken from DL. (b) Microscopic IIRI analysis using the Park/Chiu score in the different experimental groups and sampling times. Statistically significant differences (BK vs BK + IIRI and OSMA) were observed at 30, 60 min and 1 day after the corresponding surgical procedure. (c) Representative microscopic images (20×) showing a Park/Chiu Score of 0, 1 and 5 (I, II and III, respectively) and (d) Glucose intestinal absorption test showed a good functionality in the three experimental groups. No statistically significant differences between groups through the study were observed. BK, Bishop-Koop; DL, derived loop; IIRI, intestinal ischemia reperfusion injury; OSMA, occlusion of superior mesenteric artery.

technique itself in small intestinal parameters, showing no changes generated by this intervention (Figure 5b). Besides, a favourable glucose absorption test result through the stoma 1 week after surgery was observed in all groups, with an ascending curve of blood glucose values that reached a maximum value 30 min after its administration, indicating normal absorptive function in all groups at 7 POD (Figure 5d).

Colitis evaluation in the experimental BK model of the LI. From the histopathological analysis of the colon samples in the TNBS model (Figure 6c), the

progressive increase in lesions in the HAI from 0 to 48 h after administration stands out. A minimal loss of goblet cells and cellular infiltrates predominate around the base of the crypts at the time of anastomosis. At 48 h, there was an extensive loss of crypts and infiltrates in the submucosa (Figure 6b).

Animals that received TNBS from the colostomy, showed significantly more damage, as measured by the HAI score than the vehicle and sham groups (sham 1.9 ± 1.10 ; vehicle 2.87 ± 1.12 ; TNBS 5.55 ± 2.24) (Figure 6b). Serial sampling of the LI from the colostomy of a single rat (Figure 6a) indicate that BK

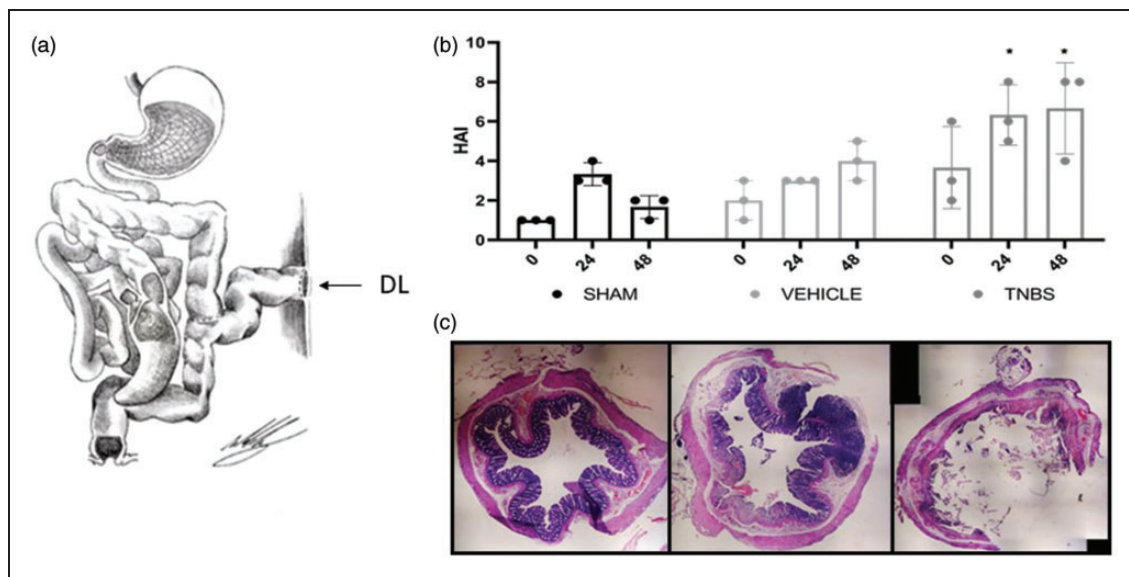


Figure 6. (a) Schematic drawing of the BK procedure in the LI. Serial colon samples were taken from the loop (DL). (b) Different degrees of HAI evidenced in the histopathological samples of the LI stained with haematoxylin-eosin. Significant differences were observed in TNBS group (24 and 48 h) versus Sham group ($P < 0.05$) and (c) Representative microscopic images (10 \times) of Sham/Vehicle/and TNBS at 48 h after TNBS-colitis induction. BK, Bishop-Koop; DL, derived loop; HAI, histopathological activity index; LI, large intestine; TNBS, 2,4,6-trinitrobenzenesulfonic acid.

procedure could be used to reduce the number of animals required for colitis studies, compared with conventional sampling of one time, one sample, one animal. Reduction in the use of animals in the colitis model for the present study was 66.6%.

Discussion

The commitment to reducing the use of experimental animals is an ethical obligation that is incumbent on researchers. Therefore, it is necessary to develop methods for achieving this goal. In this study, we demonstrate the feasibility of performing the BK technique in rats for the study of intestinal pathophysiology using fewer animals. The BK procedure consists of a surgical technique that can be reproduced by a surgeon experienced in microsurgery with the ability to handle rat abdominal organ anatomy and approach, and intestine suture techniques with the use of a surgical microscope. Our experience shows that the animals subjected to this surgery in both SI and LI tolerate it adequately. Their weights decreased by approximately 10% at 7 POD, with no negative incidence in terms of survival rate and an acceptable severity in terms of the clinical condition of the animal (Figure 2). As we postulate in our main objectives, the feasibility of BK allows us to obtain several samples from the same intestine of each animal, thereby reducing the number of rats used and not adversely affecting their clinical status despite repetitive sampling (Figure 2a,b).

Interestingly, the animals subjected to the Santulli technique decreased considerably in weight and worsened their clinical status (value of ≥ 4 in all cases) and, for this reason, humane endpoint criteria had to be applied. These results suggest that the intestinal loop exteriorised through the abdominal wall should be considered, since the alteration of intestinal transit in the Santulli technique has repercussions that manifest in an excessive loss of jejunal content and, consequently, a clinical deterioration of the animals. Considering that the Santulli technique showed a strong negative impact on the severity of the procedure, we decided to consider an N of only three instead of five animals.

In the case of SI, the search for strategies to attenuate IIRI is an area of interest, and several articles that use rodents have been published in recent times. All of these studies required the use of one animal per sampling incident, which highlights the importance of techniques that optimise the use of experimental animals.^{20–29} In our selective clamping IIRI-BK model, we obtained five samples from the same injured intestine, considerably reducing the number of rats and allowing kinetic studies. In addition, SI morphological damage and villus recovery were comparable with several previous results published by our group and others.^{15–19,40} In the present study, we observed the greatest damage at 30–60 min post-reperfusion in both the BK-IIRI and OSMA groups, with denuded villi and loss of villi (scores 4 and 5, respectively, on the Park/Chiu score) as the main characteristic lesions. The kinetic study

facilitated the recovery of the intestinal mucosa, with edema (Park/Chiu score 1–2) as the predominant lesion at 24 h post-reperfusion and slight intestinal damage at 1 week after IIRI (Figure 5c). The presence of greater damage at 30 and 60 min after reperfusion and post-surgical intestinal recovery over time was similar in BK-IIRI and OSMA, demonstrating the possibility of producing intestinal injury in the derived loop and obtaining serial sampling of the same intestine and from the same rat if BK is performed.

Interestingly, we demonstrated the feasibility of performing glucose absorption tests through the end-stoma with results equal to the group without BK (Figure 5d). This study reinforces the model by assessing functional tests of the damaged intestinal loop.

The results of TNBS-induced colitis in the BK model also show a viable way to study this inflammatory disorder in rats without generating the same degree of effect for the entire length of the LI. Animal number reduction was achieved by sampling the tissue at different time points after induced-colitis. The results in the sham group indicate that there were no histological changes caused by the surgical intervention. This demonstrates a novel opportunity to test treatments for intestinal inflammation and while being able to easily access the intestinal lumen from the colostomy to further characterise the aetiology of the pathology. Using BK to apply local treatments to the derived loop without substantial changes in general clinical conditions is a refinement in the use of rats for these specific diseases.

In summary, this study, which provides examples of IIRI in the SI and colitis in the LI, was carried out with regard to the concept of the 3Rs. It demonstrates that the BK technique applied in rats could be a highly useful tool to reduce the number of animals in experimental designs focused on intestinal pathophysiology without severe impact on the clinical status of the rats subjected to BK, IIRI and/or induced colitis. The scientific community must continue to strive to promote the 3Rs to optimise the use of laboratory animals.

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Data availability statement


The data that support the findings of this study are available from the corresponding author (pstringa7@gmail.com) upon reasonable request.


Declaration of conflicting interests


The authors declare that there is no conflict of interest.

ORCID iDs

Juan Cruz Abate  <https://orcid.org/0000-0001-6363-9264>

Leandro Vecchio  <https://orcid.org/0000-0001-9858-5307>

Jeremías Moreira  <https://orcid.org/0000-0002-6120-6067>

Pablo Stringa  <https://orcid.org/0000-0002-6557-2160>

Supplemental material

Summary graph showing the collection of several intestinal samples from the same animal using the BK technique compared with conventional models (one animal, one sample, one sampling time). In this example, the IIRI assay reported in the manuscript is outlined. In one animal where BK + IIRI was performed, samples were obtained at time 0, 30 minutes, 60 minutes, one day and one week after the ischemia reperfusion injury, reaching five samples from the same animal. To obtain the same number of samples in the case of the OSMA group, five animals were needed (one rat for each observation time). This example allows us to observe a reduction in the use of animals of 80% in the BK group.

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Lorsque moins s'avère plus: technique expérimentale de Bishop-Koop pour réduire l'utilisation d'animaux de laboratoire dans les études physiopathologiques intestinales

Résumé

L'utilisation des animaux pour acquérir des connaissances et une compréhension des maladies doit être réduite et raffinée. Dans le domaine de la recherche intestinale, en raison de la complexité du système immunitaire intestinal, le test sur modèles vivants est obligatoire.

Sur la base du principe des 3R (remplacement, réduction et raffinement), nous avons cherché à développer et à appliquer la procédure chirurgicale dérivée-intestinale décrite par Bishop et Koop (BK) chez les rats afin d'affiner les procédures gastro-intestinales expérimentales et de réduire le nombre d'animaux utilisés pour la recherche en utilisant deux modèles d'inflammation intestinale: lésion intestinale d'ischémie-reperfusion et colite induite par les produits chimiques. Nos résultats qu'il est faisable d'appliquer la technique BK chez les rongeurs, avec un bon succès après l'intervention chirurgicale dans le petit intestin et le gros intestin (100% de survie, récupération clinique et récupération de poids). Une réduction considérable de l'utilisation du nombre de rats a été réalisée dans les deux modèles d'inflammation intestinale (80% pour les lésions dues à une ischémie-reperfusion intestinale et 66,6% pour la colite induite par les produits chimiques dans notre conception expérimentale). Par rapport aux modèles expérimentaux conventionnels décrits par divers groupes de recherche, une excellente reproductibilité des lésions et de la fonctionnalité intestinales, du taux de survie et de l'état clinique des animaux ont été observés au moment de l'application de la BK.

Wenn weniger mehr ist: Experimentelle Bishop-Koop-Technik zur Verringerung des Einsatzes von Labortieren für pathophysiologische Darmuntersuchungen

Abstract

Die Verwendung von Tieren zur Gewinnung von Erkenntnissen und zum Verständnis von Krankheiten muss reduziert und optimiert werden. Auf dem Gebiet der Darmforschung sind aufgrund der Komplexität des Immunsystems des Darms Tests an lebenden Modellen zwingend erforderlich.

Auf der Grundlage der 3R-Prinzipien (Replacement, Reduction und Refinement) wollten wir das von Bishop und Koop (BK) beschriebene Verfahren der modifizierten Darmchirurgie bei Ratten entwickeln und anwenden, um experimentelle gastrointestinale Verfahren zu verbessern und die Anzahl der für die Forschung verwendeten Tiere zu reduzieren. Dabei verwendeten wir zwei Modelle für Darmentzündungen: intestinale Ischämie-Reperfusionsschäden und chemisch induzierte Kolitis. Unsere Ergebnisse belegen die Durchführbarkeit der Anwendung der BK-Technik bei Nagetieren mit gutem Erfolg nach dem chirurgischen Eingriff sowohl im Dünne- als auch im Dickdarm (100% Überleben, klinische Erholung und Gewichtszunahme). Bei beiden Darmentzündungsmodellen konnte eine erhebliche Reduzierung der Anzahl der Ratten erreicht werden (80% bei intestinalen Ischämie-Reperfusionsschäden und 66,6% bei chemisch induzierter Kolitis in unserem Versuchsdesign). Im Vergleich zu konventionellen, von verschiedenen Forschergruppen beschriebenen Versuchsmodellen wurde eine ausgezeichnete Reproduzierbarkeit der Darmschädigung und -funktionalität, der Überlebensrate und des klinischen Zustands der Tiere bei Anwendung von BK festgestellt.

Cuando menos es más: Técnica experimental Bishop-Koop para la reducción del uso de animales de laboratorio en estudios fisiopatológicos intestinales

Resumen

Es necesario reducir y perfeccionar el uso de animales para obtener conocimientos y comprensión de las enfermedades. En el campo de la investigación intestinal, debido a la complejidad del sistema inmunitario del intestino, es imperativo realizar pruebas con modelos vivos.

Basándonos en los principios de las 3R (reemplazo, reducción y refinamiento), nos propusimos desarrollar y aplicar el procedimiento quirúrgico intestinal derivado descrito por Bishop y Koop (BK) en ratas para refinar los procedimientos gastrointestinales experimentales y reducir así el número de animales utilizados para la investigación empleando dos modelos de inflamación intestinal: la lesión por isquemia-reperfusión intestinal y la colitis inducida por sustancias químicas. Nuestros resultados muestran la viabilidad de la aplicación de la técnica BK en roedores, con un buen éxito tras el procedimiento quirúrgico tanto en intestino delgado como grueso (100% de supervivencia, recuperación clínica y recuperación de peso). Se consiguió, asimismo, una reducción considerable en el uso del número de ratas en ambos modelos de inflamación intestinal (80% en el caso del daño por isquemia-reperfusión intestinal y 66,6% en la colitis inducida por sustancias químicas en nuestro diseño experimental). En comparación con los modelos experimentales convencionales descritos por varios grupos de investigación, se registró una excelente reproducibilidad del daño y la funcionalidad intestinales, la tasa de supervivencia y el estado clínico de los animales al aplicárseles la técnica BK.