Video Article

Standardized Colon Ascendens Stent Peritonitis in Rats - a Simple, Feasible Animal Model to Induce Septic Acute Kidney Injury

Wolfgang Baar[1,2], Sven Flemming[3], Nicolas Schlegel[3], Jakob Wollborn[1,2], Reinhard Schneider[4], Robert W Brock[5], Christian Wunder[2], Martin Alexander Schick[1,2]

[1]Department of Anesthesiology and Intensive Care Medicine, University Medical Center

[2]Department of Anesthesia and Critical Care, University of Würzburg

[3]Department of General, Visceral, Vascular and Paediatric Surgery, Department of Surgery I, University of Würzburg

[4]Department of Internal Medicine I, Division of Nephrology, University Hospital Würzburg

[5]Department of Physiology and Pharmacology, West Virginia University School of Medicine

Correspondence to: Wolfgang Baar at wolfgang.baar@gmx.de

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Abstract

AKI in septic patients is associated with increased mortality and poor outcome despite major efforts to refine the understanding of its pathophysiology. Here, an *in vivo* model is presented that combines a standardized septic focus to induce AKI and an intensive care (ICU) setup to provide an advanced hemodynamic monitoring and therapy comparable in human sepsis. Sepsis is induced by standardized colon ascendens stent peritonitis (sCASP). AKI is investigated functionally by measurement of blood and urine samples as well as histologically by evaluation of histopathological scores. Furthermore, the advanced hemodynamic monitoring and the possibility of repetitive blood gas sampling enable a differentiated analysis of severity of induced sepsis.

The sCASP method is a standardized, reliable and reproducible method to induce septic AKI. The intensive care setup, continuous hemodynamic and gas exchange monitoring, low mortality rate as well as the opportunity of detailed analyses of kidney function and impairments are advantages of this setup. Therefore, the described method may serve as a new standard for experimental investigations of septic AKI.

Introduction

Sepsis still remains the leading cause of death on non-cardiac intensive care units (ICU) with mortality rates of ≈ 30 - 50%[1-3]. A hallmark of severe sepsis and septic shock is the acute kidney injury that causes a further increase of mortality rate when it is associated with distant organ dysfunction such as cardiac and respiratory failure[4-6]. The overall incidence of AKI in ICU patients varies from 20 to 50%[7]. Despite the pivotal role of AKI regarding outcome and mortality in sepsis the underlying pathomechanism is still poorly understood.

Overall there are the 3 major components: inflammation, toxic injury, and hemodynamic changes that contribute to AKI development[7]. Hemodynamic changes encompass reduced renal blood flow (RBF) and global or regional renal ischemia. Here, it has to be considered that sepsis can also cause an impairment of renal microcirculation due to systemic hypotension and/or endothelial barrier disruption[8]. Therefore, the study of septic AKI should always include hemodynamic monitoring. Recent *in vivo* studies about AKI used mostly animal models such as renal ischemia-reperfusion injury or bilateral nephrectomy. These studies usually showed a lack of hemodynamic monitoring and intensive care.

The investigation of potential new pathomechanisms and therapies of septic AKI requires an *in vivo* model with a defined septic focus, an intensive care setup, a predictable outcome and an organ injury[9-12]. Here, we describe an innovative rodent model for septic AKI that meets the requirements mentioned before. Septic AKI is induced by standardized colon ascendens stent peritonitis (sCASP). The used sCASP model causes an abdominal sepsis by an intestinal fecal leakage leading to bacterial invasion and multi organ failure[13]. It has been shown that pathophysiological changes after CASP are similar to those in human sepsis and thus CASP represents a clinical relevant model in sepsis research[11,14].

Furthermore, an intensive care (ICU) setup that comprises an advanced hemodynamic monitoring and ICU therapy is established in the experimental protocol. The ICU setup enables fluid resuscitation, analgesia application intravenously and repetitive blood gas analysis. The kidney function is evaluated by measurement of standard values such as creatinine and by inulin- and p-aminohippuric-acid-(PAH) clearance. Additional information is delivered by pathohistological scores of harvested tissue and organs at the end of the experiment. The sCASP model to induce septic AKI is already evaluated and revealed new insights in renal pathology[15]. Further application of this protocol presented below might help to refine the understanding of septic AKI.

Protocol

All animal procedures were approved by the Laboratory Animal Care and Use Committee of the District of Unterfranken, Germany and carried out according to the Declaration of Helsinki.

1. Surgical Preparation and installation of invasive monitoring and continuous medication

1. Anaesthetize Sprague-Dawley rats by using 5 vol. % isoflurane at 5 L/min O_2 flow for 2 min in an inhalation chamber. Confirm adequate depth of anesthesia by observing breathing rate, which becomes slower and deeper, and verify unresponsiveness after tail/toe pinch. Shave the throat and stomach with a razor.
   Note: Massive reduction of the breathing rate can be an indicator of an overdose of anesthesia and lead to bradypnoe, hypotension and death finally.
2. Place the rat in a supine position on a heating pad. Apply vet ointment to the eyes to avoid eyes drying out. Provide continuous anesthesia with 2.0 vol. % isoflurane with FiO_2 0.28 via a face mask while the animal breathes spontaneously. Place the rat in a supine position on an automated heating pad.
   Note: Hypothermia and thermal injury influence macro- and microhemodynamic parameters and should be avoided to gain reliable result. It is recommended to use a heating pad with control mechanism that is linked to the rat through a flexible rectal probe to maintain body temperature in physiological range.
3. After disinfection of the neck and throat with an 55% alcohol based scrub and 7.5% povidone iodine solution, incise the skin on the throat medial with a scalpel and make an incision of about 2 cm. Turn the rat and perform an incision of about 1 cm with surgical scissors on the neck about 1 cm distal of the occiput.
4. Place the rat back in supine position. Dissect gently the right jugular vein and the left carotid artery with the scissors and cotton buds. Dissect the vessels from the neighboring structures. Avoid too much drag and prepare further with careful spreading movements.
   Note: Keep the vessels always moist by application of pre-warmed sterile saline.
5. Dissect gently and with spreading movements subcutaneously from the vessels to the neck to get a connection between the two incisions. Insert clamps into each formed tunnel.
6. Flush the swivel and the catheters, coming from the swivel device with 0.9% NaCl. Insert the catheters into a stainless steel spring.
   Note: The catheters have to be flushed with sodium chloride before being inserted, as minimal air application can lead to sudden death due to air embolism.
7. Turn the rat and clamp the catheters using the inserted clamps. Slide the catheters from the neck to the throat. Fix the spring with a plastic button tether about 1 cm distal of the occiput with 6 singular sutures (e.g. 4/0) on the neck.
8. Place the rat again in supine position. Lay cotton threads of about 4 cm distal and proximal to each prepared vessel. Clip the artery proximal with a micro-clip, usually used for clipping subarachnoid aneurysms. Alternatively, leave the clip and stop the arterial blood flow by tightening one of the threads.
9. Incise the artery with a surgical micro scissors, keep the incision open with a hook in the one hand and insert the arterial catheter into the vessel by using forceps with the other hand. Open the clip or the pulled thread, push the catheter about 1 cm forward into the vessel towards the heart and fix it with surgical knots using the laid cotton threads.
10. Repeat step 1.8 on the jugular vein and leave the clip, as the vein is not as solid as the artery.
11. Having fixed both catheters straight-line in the vessels, close the wound on the throat with sutures.

2. sCASP-Procedure

1. Keep the rat in a supine position, shave the abdomen and disinfect the abdominal region with alcohol and povidone iodine solution.
2. Perform an abdominal midline incision of the skin of about 2 cm length with surgical scissors and afterwards again along the linea alba to open the peritoneal cavern.
3. Identify the caecal pole and pull out the caecum gently by using cotton buds.
4. Pierce the ascending colon around 2 cm distal from the ileocaecal valve with a suture (6/0) [suture 1] at the anti-mesenteric side and fix it with two surgical knots. Avoid lesions of any colon vessels.
5. Cut off the distal end - about 1.5 cm - of a 10 FR suction catheter. Cut this piece of the catheter with a scalpel to form a rectangular flap with a length of about 0.5 cm. Prepare the flap with a suture (6/0) [suture 2] in the middle of the flap.
6. Put the flap over a 14G needle and puncture the ascending colon with the needle where suture 1 was fixed[15].
7. Insert the prepared plastic tube into the colon over the needle. Simultaneously remove the needle gently. Position the tube so that the flap lays outside of the colon and the rest of the tube inside the colon.
   1. After exact positioning of the plastic tube fix the flap with the already existing suture 2 by stitching the colonic wall and surgical knots. Avoid again any lesion of colon vessels. Put the free ends of suture 1 around the rest of plastic tube that leads outside of the colon and perform 2 surgical knots.
8. Milk stool from the caecum towards the colonic stent by using cotton buds until stool appears at the outlet of the stent.
9. Put the small intestine and colon back into the abdominal cavern. Hereby the plastic tube with the stool should be in contact with the peritoneum. Flush the stent with 2 mL 0.9% NaCl to distribute the feces into the peritoneal cavern.
10. Close the abdominal cavern with a continuous suture (4/0) of the peritoneum and afterwards with a continuous suture (4/0) of the skin.

3. Postoperative Procedure

1. Stop the inhalation of isoflurane and put the rat back into its cage.

2. Start the intravenous analgesia via the venous line using Fentanyl (2.0 µg/100 g bodyweight/hr).

4. Preparing the measurements on the second day

1. 24 hr after sCASP procedure, apply 0.5-1.0 mg midazolam intravenously. Afterwards use 2.0 Vol. % isoflurane with FiO_2 0.28 via face mask for sufficient anesthesia.
2. Having disinfected with alcohol and povidone iodine solution again, perform a tracheotomy with the plastic tube of a 14 G venous cannula to provide sufficient oxygenation and ventilation.
    1. Therefore, open the suture on the throat with surgical scissors and open gently from the midline to the trachea. Incise the trachea vertically between two cartilages of a length of about 2 mm with surgical scissors - just enough to insert surgical scissors. Widen the incision bluntly to about half of the diameter of the trachea with the scissors. Insert 1 cm of a 14 G plastic tube and fix it with 2 sutures.[16]
    Note: If the incision of the trachea is bigger than half of the circumference, vessels going alongside the trachea can be harmed resulting in severe hemorrhage, blood aspiration or lethal bradycardia.
    2. Start mechanical ventilation with a rodent ventilator with a F_iO_2 of 0.28 and 0.7 Vol. % isoflurane and start intravenous anesthesia with midazolam (0.7 mg/100 g BW/hr) and fentanyl (7 µg/100 g BW/hr). Perform blood gas analyses to ensure sufficient ventilation and oxygenation. Therefore, withdraw about 0.7 mL of blood via the arterial catheter and measure it with a blood gas analysator.
    Note: Intravenous anesthesia should start only if the mechanically controlled ventilation is established.
3. After having shaved and disinfected in a similar manner as in 2.1 perform a 1 cm longitudinal incision of the skin of the right leg 0.5 cm proximal of the knee. Dissect the artery from the neighboring structures using surgical scissors and cotton buds bluntly.
Note: If femoral artery is teared, an insertion of catheter in a proximal position is still possible. Thus, it is recommended to start the implantation as far distal as possible.
4. Insert a thermodilution catheter into the femoral artery by opening the artery with a hook and inserting the catheter with forceps.
5. Start measuring the cardiac output by thermodilution method. Therefore, infuse 1 mL of chilled NaCl rapidly manually via the venous catheter after having started the measurement of a cardiac index measuring software. Perform this measurement twice.

5. Evaluation of kidney function[17]

1. Perform a laparotomy by opening the sutures of the skin and the peritoneum with scissors. Incise the urinary bladder with surgical scissors just enough to catheterize it with a small plastic catheter. Having laid a cotton thread of about 7 cm around the bladder, fasten the thread, fix the catheter with knots, and finally collect as much urine as possible via the catheter.
2. Dissolve fluorescein-isothiocyanate-inulin (FITC-Inulin) in 0.9% NaCl and p-aminohippuric acid sodium salt (PAH) in 0.9% NaCl to get concentrations of 2 mg/mL inulin, 5 mg/mL PAH.
3. Apply a bolus of a mixture of both substances of 75 µL i.v., followed by constant intravenous infusion of both substances with a rate of 3.7 µL/hr/300 g BW.
4. After having reached a steady state[17] with the infusion of FITC-Inulin and PAH collect the urine for 20 min. Take blood samples via the arterial catheter as described under 4.2.1 regarding performing a blood gas analysis.
5. Determine inulin concentrations of the urine and the plasma by fluorescence spectrometry and measure PAH by photospectrometry using the anthrone method[17].

6. Ending of the experiments

1. Euthanize the rat by applying a lethal dose of barbiturate, i.e. thiopental.
2. Withdraw as much blood as possible via the arterial catheter by using syringes.
3. Harvest the small and large intestine by cutting the peritoneal fixation of the duodenum and colon, take out some parts of these by using surgical scissors. Prepare and harvest the kidneys by preparing retroperitoneal and release them of their fatty capsule and cut through the ureter and the attached vessels. Perform a thoracotomy and cut out the lungs and the heart. Fix all organs in formaldehyde and perform histological stainings as previously published.[15,18]

Representative Results

As previously published by Schick et al.[8], we demonstrate the following results.

Induction of sepsis without mortality
In the CASP model, sepsis is induced by a continuous leakage of intraluminal located bacteria of the colon ascendens into the abdominal cavity resulting in fecal peritonitis and bacteremia. Hereby, the size of the implanted catheter regulates the output of faeces and thus the severity of peritonitis and sepsis. In the experimental protocol presented above a 14G needle combined with the tip of the specially prepared 10 FR suction catheter was used to have a sufficient peritonitis. Smaller catheters caused only abscesses with local inflammation as the seminal vesicle mostly occluded the smaller stents and prevented a continuous fecal output.

Control and sCASP animals survived the experiment, but in sCASP-group a fluid resuscitation was necessary to maintain mean arterial pressure (MAP) ≥ 70 mmHg. sCASP-treated animals showed ubiquitous faecal peritonitis without abscess formations and exhibited clinical signs of severe sepsis revealed by decreased activity, reduced alertness, ruffled fur and hunched posture. This clinical status deteriorated continuously over time.

The fluid resuscitated sCASP rats showed significant differences in MAP and heart rate but no differences in cardiac output compared to control animals (Figure 1). Blood analyses after 24 hours revealed signs of sepsis in sCASP-treated animals with increased lactate, increased IL-6 and a typical leucopenia for these animals compared to control (Figure 1).

Determination of sepsis-induced AKI

The inulin clearance, described as the gold standard to evaluate kidney function, was significantly decreased in sCASP animals compared to the control after 24 hours (Figure 2 A). Furthermore, urine output (Figure 1) and PAH clearance were significantly reduced in sCASP animals (Figure 2 B). NGAL (Figure 2 C) and Cystatin C (Figure 2 D) were increased in sepsis compared to the control group. The standard parameters for AKI such as urea and creatinine confirmed the impaired kidney function in the sCASP group by elevated levels of both parameters measured in blood serum (Figure 2 E and F).

Acute kidney injury in sCASP group was not only be determined by functional parameters but also by an increased histo-pathological injury score (Figure 3). This score is based on morphological alterations including formation of edema, cellular edema, detachment of tubular epithelium from the basement membrane, loss of the brush border of the proximal tubular cells, cell death and vacuolisation. sCASP group showed an increased histo-pathological score of kidneys 24 hours after sepsis induction, whereby interstitial edema and impaired tubules´ brush border were the leading factors for the higher score compared to control animals (Figure 3). Furthermore, rate of dead cells and events of detached basement membrane were significantly increased in sCASP animals (Figure 3).

	HR [beats/min]	MAP [mm Hg]	CI [mL/min/kg]	lactate [mmol/L]	leukocyte [Thsd /µL]	IL 6 [pg/mL]	urine output [mL/20min/100g]
control	378±35	92±9	379±55	1.7±0.2	3.00±1.06	41.4±20	0.10±0.05
sham	383±48	98±17	447±112	1.5±0.3	2.17±1.34	39.1±23.1	0.19±0.14
sCASP	446±56*§	80±11§	453±72	3.0±1.5§	1.35±0.59*	251.4±239	0.05±0.03§

Figure 1: Macrohemodynamics and sepsis parameters. Following values confirm presence of sepsis in sCASP operated animals: Heart rate (HR), mean arterial pressure (MAP), coronary index (CI), lactate, leukocytes, interleukin 6 (IL 6) and urine output. * p<0.05 vs. control, § p<0.05 vs. sham. Data are already published in ICMex by Schick et al.[15] Please click here to view a larger version of this figure.

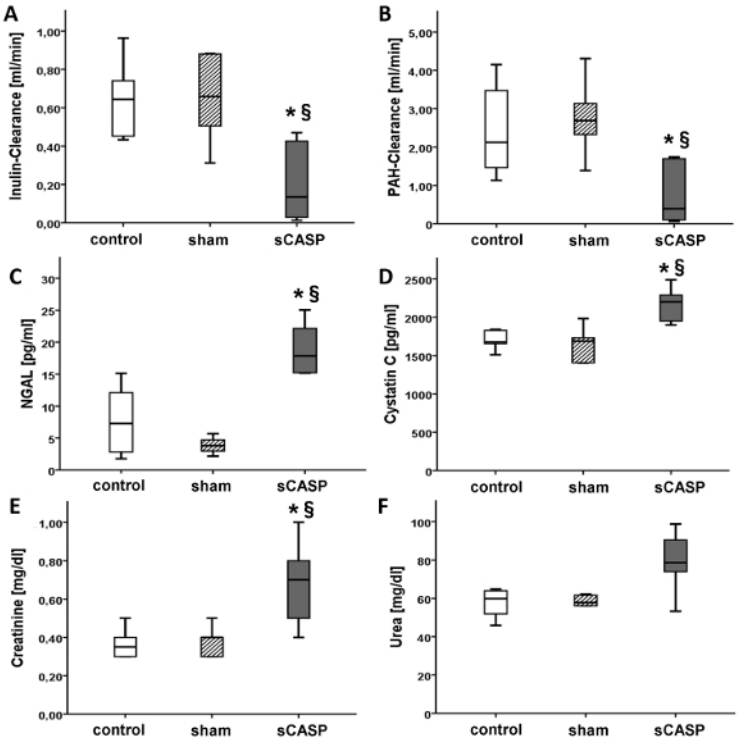

Figure 2: Kidney function parameters. The parameters inulin- (A) and PAH- clearance (B) [mL/min], described as the gold standard to measure kidney function were significantly decreased in the sCASP group. In contrast NGAL (C) and cystatin C (D) [pg/mL] were increased as a further sign of septic AKI. The clinical standard parameters such as creatinine (E) and urea (F) [mg/dL] showed also elevated levels in septic animals. * p< 0.05 vs. control, § p<0.05 vs. sham. Data are already published in ICMex by Schick et al.[15] Please click here to view a larger version of this figure.

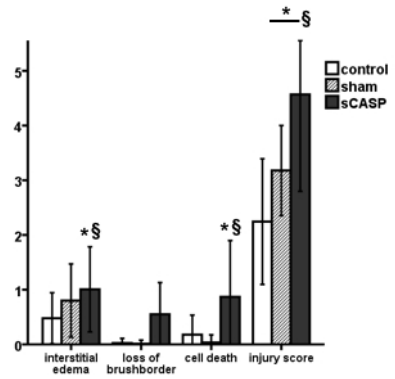

Figure 3: Histological Injury Scores. Showing the differences between control, sham and sCASP regarding interstitial edema, loss of the brush border of the proximal tubular cells, cell death and the total injury score. Bars show mean ± standard deviation. * p< 0.05 vs. control, § p<0.05 vs. sham. Please click here to view a larger version of this figure.

Discussion

The pathophysiology of septic AKI still remains unknown in its complexity. Clinical research and trials in patients will not enable gains of new insights with respect to histopathology changes, microcirculation disturbances or drug interactions on cellular levels[15]. It has been postulated previously that there is a need for improved and new animal models to investigate acute kidney injury associated with sepsis[19]. Therefore, we established a new animal model for septic AKI induced by colon ascendens stent peritonitis.

The CASP model presents a clinical relevant model to mimic sepsis in humans started with faecal peritonitis seen in surgical patients. The major advantage of CASP is that it does not start with an ischemic hit like in the widely used CLP model where a ligation of the caecum is performed[14]. Furthermore Maier et al. shows that the CLP model represents an intra-abdominal abscess situation rather than a ubiquitous peritonitis with systemic bacteremia[14]. By using different sizes of the stent implanted in the colon ascendens, the severity of sepsis and the survival rate can be affected as shown in previous publications[13,14,20]. To avoid an occlusion of stent by omentum or seminal vesicles a modified stent with a diameter of 10 FR was inserted[15]. In the animal model described above only male rats were used to exclude hormone variation instead of female animals as in the original CASP model by Zantl et al. and Traeger et al.[13,21].

It has to be considered that even after performance the experimental setup presented above in a standardized manner, variability can occur based on different mouse strains, animal facility, gender and operator. Therefore, it can be necessary to adjust the size of the stent and thus the severity of peritonitis and sepsis. Furthermore, it is recommended that the same operator performs both the sCASP and the control on the same day to reduce variability.

An acute kidney injury can occur due to hypoxia and ischemia caused by hypotension or respiratory failure with decreased PaO_2 in sepsis[22]. Changes in hemodynamic and metabolic conditions led to tissue hypoxia that influences renal integrity and may affect morbidity and mortality[23]. Therefore, a continuous evaluation of hemodynamic and respiratory values should be available in an *in vivo* model that is used to investigate septic AKI. Here, we presented an *in vivo* model that is characterized by an ICU setup comparable to patients. It enables a continuous measurement of hemodynamic values such as heart rate and arterial blood pressure and evaluation of respiratory status by taking blood for blood gas analyses. Furthermore, these data gained by continuous monitoring are necessary and helpful for fluid resuscitation and adaptation of anesthesia protocol.

The evaluation of kidney function by measurement of inulin and PAH clearance is only possible at the end of the experiment as a re-laparotomy and incision of the urine bladder is necessary. If an investigation of kidney function during the experiment is required, blood samples can be taken to measure standard parameters such as creatinine and urea. Hereby, it has to be considered, that a fluid replacement should be conducted to avoid a pre-renal failure due to hypovolemia.

The purpose of this model was to establish a stable rodent setup of septic AKI with clinical relevance that enables further studies in pathophysiology and treatment of renal failure. This model can also be used to investigate different therapy strategies of abdominal sepsis and septic AKI. Therefore, this model can serve as a stable rodent setup of septic AKI being close to the clinical setting of an intensive care unit. Furthermore, the described model can be modified and successfully used for a wide spectrum of *in vivo* sepsis research, e.g. immune dysregulation, endothelial breakdown, coagulopathy, microcirculation disturbance, pharmacotherapy and resuscitation[24].

Disclosures

The authors have nothing to disclose.

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