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Abstract:	<p>AKI in septic patients is associated with increased mortality and poor outcome despite major efforts to refine the understanding of its pathophysiology. Here, an 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.</p> <p>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.</p>

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TITLE:

Standardized colon ascendens stent peritonitis in rats – a simple, feasible animal model to induce septic acute kidney injury

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SHORT ABSTRACT:

Acute kidney injury (AKI) is a severe complication in critically ill patients and is related with an increased mortality. Here, we present a reliable and reproducible *in vivo* model to mimic AKI under inflammatory conditions that might contribute towards understanding the pathogenesis of septic AKI.

LONG 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 $\approx 30 - 50\%$ 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 failure⁴⁻⁶. 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 development⁷. 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 disruption⁸. 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 injury⁹⁻¹². 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 failure¹³. 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 pathology¹⁵. 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

1.1) Anaesthetize Sprague-Dawley rats by using 5 vol. % isoflurane at 5 L/min O₂ 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.

1.2) 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 FiO₂ 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.

1.3) 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.

1.4) 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.

1.5) 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.

1.6) 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.

1.7) 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.

1.8) 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.

1.9) 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.

1.10) Repeat step 1.8 on the jugular vein and leave the clip, as the vein is not as solid as the artery.

1.11) Having fixed both catheters straight-line in the vessels, close the wound on the throat with sutures.

2. sCASP-Procedure

2.1) Keep the rat in a supine position, shave the abdomen and disinfect the abdominal region with alcohol and povidone iodine solution.

2.2) 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.

2.3) Identify the caecal pole and pull out the caecum gently by using cotton buds.

2.4) 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.

2.5) 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.

2.6) Put the flap over a 14G needle and puncture the ascending colon with the needle where suture 1 was fixed¹⁵.

2.7) 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.

2.7.1) 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.

2.8) Milk stool from the caecum towards the colonic stent by using cotton buds until stool appears at the outlet of the stent.

2.9) 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.

2.10) 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

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

3.2) 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

4.1) 24 hr after sCASP procedure, apply 0.5-1.0 mg midazolam intravenously. Afterwards use 2.0 Vol. % isoflurane with FiO₂ 0.28 via face mask for sufficient anesthesia.

4.2) 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.

4.2.1) 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.

4.2.2) 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 μ g/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 analyser.

Note: Intravenous anesthesia should start only if the mechanically controlled ventilation is established.

4.3) 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.

4.4) Insert a thermodilution catheter into the femoral artery by opening the artery with a hook and inserting the catheter with forceps.

4.5) 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 function¹⁷

5.1) 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.

5.2) 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.

5.3) Apply a bolus of a mixture of both substances of 75 μ L i.v., followed by constant intravenous infusion of both substances with a rate of 3.7 μ L/hr/300 g BW.

5.4) After having reached a steady state¹⁷ 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.

5.5) Determine inulin concentrations of the urine and the plasma by fluorescence spectrometry and measure PAH by photospectrometry using the anthrone method¹⁷.

6. Ending of the experiments

6.1) Euthanize the rat by applying a lethal dose of barbiturate, i.e. thiopental.

6.2) Withdraw as much blood as possible via the arterial catheter by using syringes.

6.3) 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 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).

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

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

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 \pm standard deviation. * $p < 0.05$ vs. control, § $p < 0.05$ vs. sham.

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 levels¹⁵. It has been postulated previously that there is a need for improved and new animal models to investigate acute kidney injury associated with sepsis¹⁹. 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 performed¹⁴. Furthermore Maier et al. shows that the CLP model represents an intra-abdominal abscess situation rather than a ubiquitous peritonitis with systemic bacteremia¹⁴. 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 inserted¹⁵. 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 PaO₂ in sepsis²². Changes in hemodynamic and metabolic conditions led to tissue hypoxia that influences renal integrity and may affect morbidity and mortality²³. 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 resuscitation²⁴.

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DISCLOSURES:

The authors have nothing to disclose.

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Figure 1: Macrohemodynamics and sepsis parameters. * p<0.05 vs. control, § p<0.05 vs. sham

	HR [beats/min]	MAP [mmHg]	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§

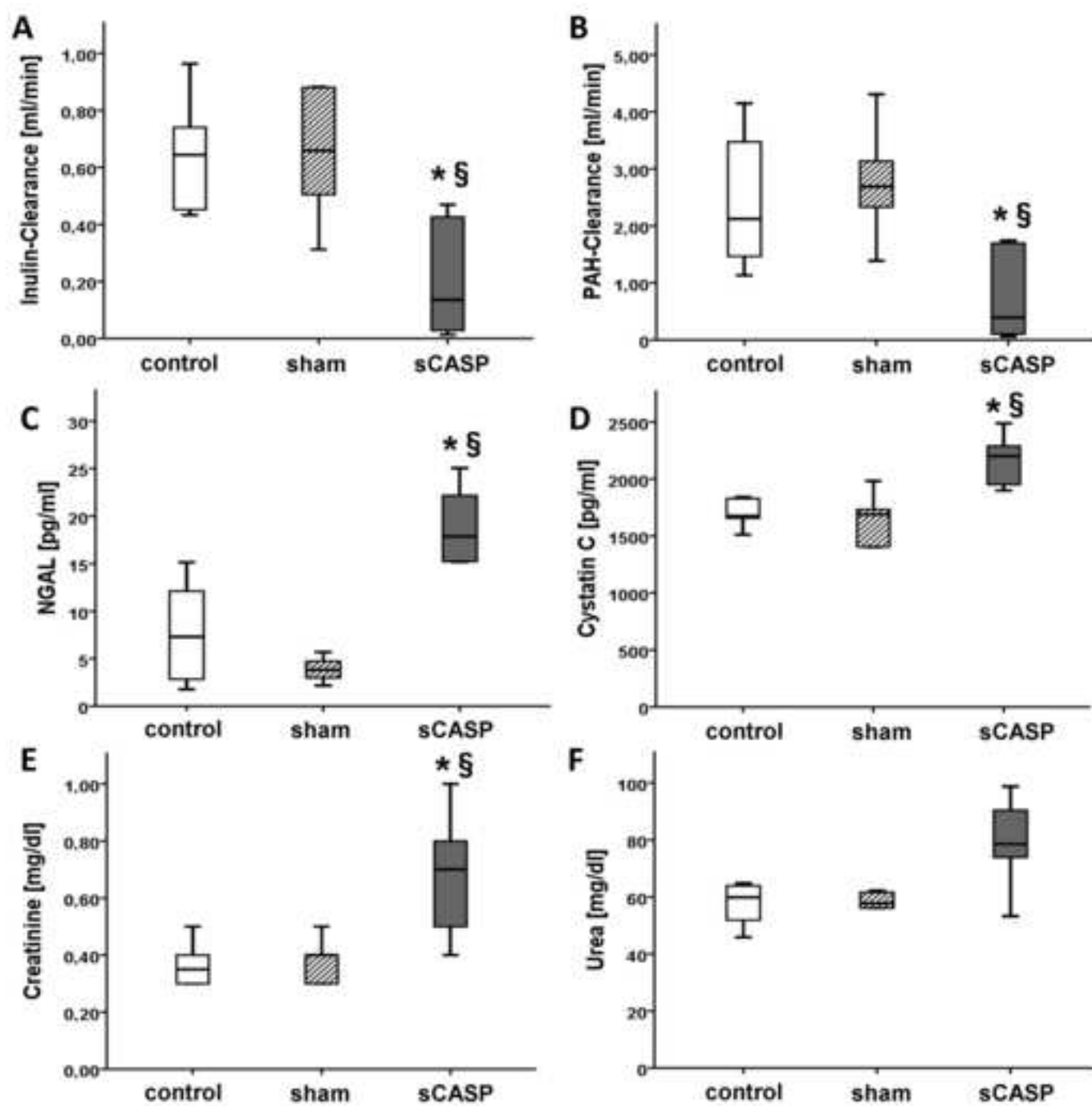
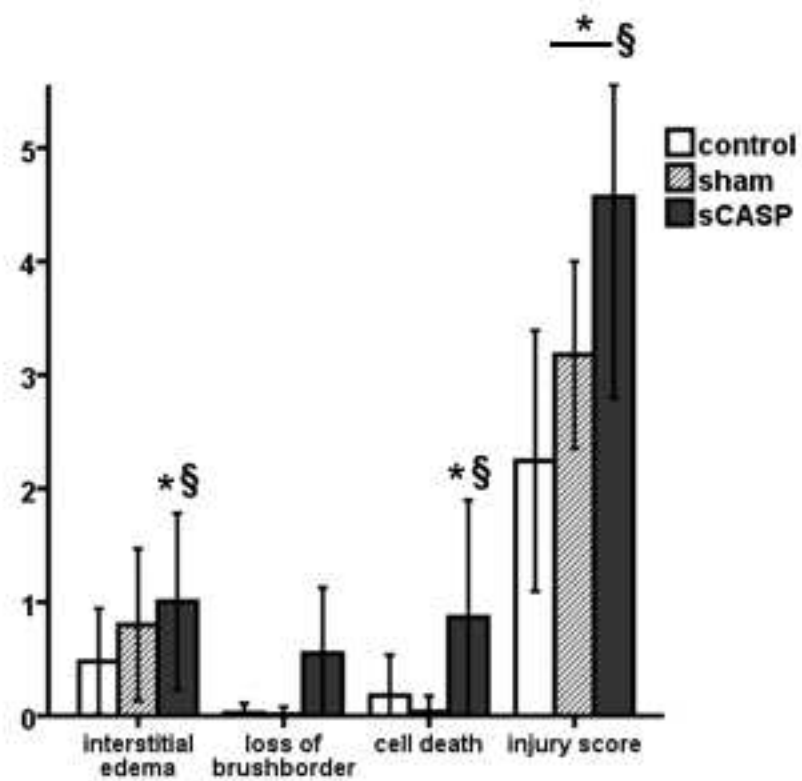


Figure 3



Name of Material/ Equipment	Company	Catalog Number
Sprague-Dawley rats	Janvier Labs, France	
Isoflurane CP	cp-pharma, Burgdorf, Germany	
polyethylen catheter PE 10; 30m	A. Hartenstein, Wuerzburg, Germany	
Swivel (375/D/20)	Instech, Plymouth Meeting, PA, USA	
plastic button tethers	Instech, Plymouth Meeting, PA, USA	
Perfusor	B. Braun; Melsungen, Germany	
suction catheter ch. 10	B.Braun Melsungen AG, Germany	
suture	Syneture; USA	
suture	Ethicon; Scotland	
14G-i.v. catheter	BD Insynte; BD Vialon; Madrid; Spain	
	NOBA Verbandmittel	
cotton buds	Danz GmbH u Co KG; Wetter; Germany	
rodent respirator	Hugo Sachs Elektronik KG, Germany	
Midazolam	Ratiopharm, Germany	
	ADInstruments,	
Thermodilutioncatheter	Spechbach, Germany	
	Sigma-Aldrich; St. Louis; USA	
p-Aminohippuric acid		

Inulin	Sigma-Aldrich; St. Louis; USA
Formaldehyde	Otto Fischar GmbH & CoKG; Saarbrücken, Germany
Cyclopentan	Merck; Darmstadt; Germany
alcohol based scrub	Schülke & Mayr GmbH, Norderstedt; Germany
povidone iodine solution	B.Braun Melsungen AG, Germany

Comments/Description

0.58x0.96 mm

(375/D/20)

LW105S

Perfusor fm

suction catheter typy „Ideal“; ch. 10
Surgipro; Monofilament Polypropylen 4-0
Prolene; Polypropylen 5-0

14GA i.v. catheter

rodent respirator, Type:7025
Midazolam

p-Aminohippuric acid sodium salt; A3759-25G

Inulin-FITC; F3272-1G

Formaldehyde 3.5%

Uvasol: 2-Methylbutan

kodan Tinktur forte; 45g 2-Propanol, 10g 1-Propanol per 100g

Braunol, 7.5g Povidone Iodine per 100g



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Standardized colon ascendens stent peritonitis in rats - a simple, feasible animal model to induce septic acute kidney injury

Author(s):

Baur, Wolfgang; Fleckner, Sven; Schlegel, Nicolas; Vollmar, Jakob; Schneider, Reinhold; Bruch, Robert; Wunder, Christian; Schich, Martin Alexander

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CORRESPONDING AUTHOR:

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Institution:

University Medical Center Freiburg / University of Würzburg

Article Title:

Standardized colon ascendens stent peritonitis in rats - a simple, feasible animal model to induce septic AKI

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Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

-Thank you for your advice, we have corrected the spelling or grammar issues we have found.

2. Please note that if you want steps 4.2, 4.3, and 4.4 to be filmed, more explicit step-wise details will be needed.

4.2: You cannot reference out the details on how to perform the tracheotomy if this is to be filmed.

4.3: Incise where exactly? How big is the incision? Dissect how?

4.4: Catheterize how? How is the cardiac output measured?

-The required details were integrated to the steps.

3. Animal Care Note: In order to pass vet review, sites for survival surgeries should be disinfected with both an alcohol and an iodine-based or chlorhexidine-based scrub. In addition, the initial incision should be made with a scalpel (1.3). Finally, all surgical sites should be shaved/clipped and disinfected in a similar manner (2.2), and vet ointment should be applied to the eyes to prevent dryness during anesthesia (1.1).

-Thank you for your advice, we have added the shown notes to the protocol.

4. Formatting: Please remove copyright/trademark symbols from Materials/Equipment list.

-All copyright and trademark symbols have been removed from the materials list.

5. The manuscript should be copyedited for typographical and grammatical errors.

There are also several instances where language can be clarified. For example:

-Long Abstract: Control animals received intensive care setup, hemodynamic variables and blood gases were monitored 24 hr continuously.

-Long Abstract: After 24 h, animals were re-anaesthetized, cardiac output (CO), inulin-and p-aminohippuric-acid-(PAH) clearances were measured.

-Introduction: changes with hypotension, reduced renal blood flow (RBF) following global or regional renal ischemia.

-Introduction: An ideal AKI animal sepsis model should base upon...

-Discussion-because to much mechanical irritation can induce

-There is purple text in the Introduction: "Compared to each other, the"

-The sentences that were criticized above are now clarified and integrated to the text. The purple text is not purple any more.

6. Additional detail is required

-1.1-What is the initial amount of isoflurane used for the anesthesia, and how long

are rats left in the chamber? How is anesthesia confirmed (also applies to 4.1)?

- 1.4-Prepare more what? How does one "prepare bluntly down"? Please clarify.
- 1.5-Does the swivel device need to be readied/prepared prior to this step?
- 1.7-Are the threads mentioned here synonymous with the sutures from the previous step? Are they part of the swivel device/catheters? Please clarify.
- 1.10-Hasn't the swivel already been attached to the neck with sutures (in 1.6)?
- 2.5-How is the flap "prepared" with sutures? If this step is to be filmed, additional detail is required (the superscript reference is not sufficient).
- 4.2-If this step is to be filmed, additional detail is required as to how to perform the tracheotomy. The superscript reference is not sufficient. In addition, how is this site disinfected (also applies to 4.3-4)?
- 4.4-How/with what is cardiac output measured? Please specify.
- 6.1-What (general) type of barbiturate? How is euthanasia confirmed?
- 6.3-If possible, please provide superscript references for the dissections/fixation.

-The required details are added to the protocol, where it had been possible.

7. Results

- Please include parenthetical references to Figure 1-specifically panels in this figure- in the Results section.
- If possible, please provide a figure supporting the statement that "The kidneys of the sCASP group showed significantly more dead cells when compared to control."

-Parenthetical references have been included. Due to reviewer #2 we changed the illustration of exact values in the text to graphics and statistics for improved readability of the manuscript.

Reviewers' comments:

Reviewer #1:

Minor Concerns:

The manuscript would be improved by considering the following aspects:

The introduction should include a short paragraph on the sham-procedure: is there a stent placed on the colon wall fixed by sutures? Or did the authors use simply laparotomised animals without manipulation of the intestines as a control group?

-We thank the reviewer for this statement. We included a short paragraph to the introduction.

In line 128 reference nr 8 is given to cite the CASPI procedure (stent removal following CASP). As this technique was first described by Zantl et al (reference nr. 7) this item should be corrected.

-We thank the reviewer for this comment and corrected and added the reference.

In 2010 Traeger et al have already published a similar manuscript in the Journal of Visualized Experiments: "Colon Ascendens Stent Peritonitis (CASP)- a standardized model for polymicrobial abdominal sepsis". In this manuscript the CASP procedure was performed on mice. Therefore, it is necessary to complete the present study by

including this reference into introduction and discussion.

-The authors thank the reviewer for this hint. The reference is added in both mentioned parts of the manuscripts.

Reviewer #2:

Major Concerns:

The main modifications of the CASP procedure are technical variation of the stent material, insertion of a venous and arterial catheter for applying medication and continuous observation of MAP to exclude pre-renal failure (= "intensive care setup"). The modifications are important for AKI studies, but certainly this "standardized CASP (sCASP)" model can be used for other sepsis-related research as well, e.g. studies of the immune dysregulation. In this regard, the title (and the abstract) unnecessarily narrow the importance of the published method. The authors should at least include other potential applications for the method/protocol in the discussion.

-We thank the reviewer for this statement and modifications in the discussion were included.

However, with regard to the proposed target (AKI studies) the protocol has actually some weaknesses; in particular: sham operated animals also show elevated kidney injury markers due to the invasiveness of the methods. As a consequence, no "healthy" control is available, which limits the usefulness of the model. In the worst case, therapeutic studies to reduce/prevent septic AKI can fail, because of the small differences between treated and untreated animals. The authors should carefully discuss those limitations.

-The authors agree with the reviewer's comment. Data of control animals are available in the papers:

1) Balanced Hydroxyethylstarch (HES 130/0.4) Impairs Kidney Function In-Vivo without Inflammation.

Schick MA, Baar W, Bruno RR, Wollborn J, Held C, Schneider R, Flemming S, Schlegel N, Roewer N, Neuhaus W, Wunder C.

PLoS One. 2015 Sep 4;10(9):e0137247. doi: 10.1371/journal.pone.0137247.

eCollection 2015, PMID: 26340751

2) Sepsis-induced acute kidney injury by standardized colon ascendens stent peritonitis in rats - a simple, reproducible animal model.

Schick MA, Baar W, Flemming S, Schlegel N, Wollborn J, Held C, Schneider R, Brock RW, Roewer N, Wunder C.

Intensive Care Med Exp. 2014 Dec;2(1):34. doi: 10.1186/s40635-014-0034-x. Epub 2014 Dec 9. PMID: 26266931

In the PLoS One paper we tested hydroxyethylstarch in our animal model. We were able to show - with marginal or even no changes in routine blood AKI parameters - an HES induced AKI by Inulin/PAH changes and histopathology.

It is possible to adapt our set up (easily) to the point of research interest. As described in ICMex the trial can be prolonged up to 10 days. However, stent enlargement will also worsen sepsis, and differences of sepsis induced AKI parameter will increase compared to sham.

However, the aim of this setup was to reduce sepsis mortality to reduce numbers of

animals, time and costs. Again we thank the reviewer for this hint and discussed it in the paper.

Minor Concerns:

Protocol: Since the modification of the stent is important as stated by the authors, they should exactly describe the dimensions (inner and outer diameter) of the used commercial suction catheter in case the reader has no access to this brand.

-The stent –as mentioned in the protocol- is the tip of a suction catheter of 10Fr. The outer diameter is 0.4mm, the inner diameter 0.25mm.

Results: The results sections has exact numerical values for blood pressure, heart rate, cardiac output, lactate, WBC etc. The reviewer would prefer graphical presentations instead to improve readability of the manuscript. Previously published figures should have permission by the publisher. Also, the histopathological score is neither explained nor are histologies shown.

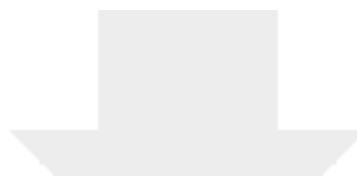
-The authors thank the reviewer and inserted a second figure (new “Figure 1”) with the exact numerical values. Therefore, the readability should be improved. Also we tried to explain the histopathological score. The histological sections are shown in the previous publication and do not lead to better understanding of the method.

Discussion: In addition to the issues outlined above, there is no need to mention the author's HES130/0.4 study in the concluding paragraph unless it is important to understand the method.

-We agree with the reviewer and removed the sentence.

Table of materials: Fentanyl is not mentioned in the table. Cyclopentan is not mentioned in the manuscript.

-Changes were included in the table.



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