

Point-by-point response

We thank the reviewer and science editor very much for the excellent and encouraging comments and review of our manuscript. We have done our utmost to accommodate the requested corrections and modification in the manuscript and the answers below. All changes can be found below and are marked red in the document Revised_manuscript. Our changes to your suggestions are marked in red below.

Reviewer #1

- 1) Scientific Quality: Grade C (Good). Language Quality: Grade A (Priority publishing). Conclusion: Minor revision. Specific Comments to Authors: Dear Authors, Thank you. I read the manuscript with high interest. However, the novelty of the findings is limited. Certainly, an intestinal anastomosis creates ischemic microenvironment, thus increases lactate and glycerol, decreases glucose. The measurement of cefuroxime levels has added some novelty. The Title reflects the main hypothesis presented in the manuscript. Methods section is detailed and clear. I understand each pig was used as its control.

Answer: We thank the reviewer for her/his interest for the manuscript, and can confirm that each pig was used as its own control.

- 2) The results should be presented better though. Table 1 should be showing anastomosis and non-anastomosis levels of the ischemic metabolites but, I cannot see these two presented separately. Same for Figure 1.

Answer: We agree. As such, we have added a new Table 2 presenting the ischemic metabolites for both the anastomosis and non-anastomosis ileum and colon. However, it should be kept in mind, that the ischemic metabolites concentrations do not reflect the total tissue concentration as the relative recovery for the ischemic metabolites was not determined. This is noted on page 7.

- 3) On another note, I have detected 10 self citations among 32 references. I understand the need for citing previous research however, I found this a bit high compared to other articles I have reviewed.

Answer: We thank the reviewer for detecting this issue. We have removed the following four self-citations from the manuscript:

Bue M, Tøttrup M, Hanberg P, Langhoff O, Birke-Sørensen H, Thillemann TM, Andersson TL, Søballe K. Bone and subcutaneous adipose tissue pharmacokinetics of vancomycin in total knee replacement patients. Acta Orthop 2018; 89(1): 95-100 [PMID: 28914105 PMCID: PMC5810840 DOI: 10.1080/17453674.2017.1373497]

Bue M, Sou T, Okkels ASL, Hanberg P, Thorsted A, Friberg LE, Andersson TL, Öbrink-Hansen K, Christensen S. Population pharmacokinetics of piperacillin in plasma and subcutaneous tissue in patients on continuous renal replacement therapy. Int J Infect Dis 2020; 92: 133-140 [PMID: 31978581 DOI: 10.1016/j.ijid.2020.01.010]

Hanberg P, Bue M, Birke Sørensen H, Søballe K, Tøttrup M. Pharmacokinetics of single-dose cefuroxime in porcine intervertebral disc and vertebral cancellous bone determined by microdialysis. Spine J 2016; 16(3): 432-438 [PMID: 26620946 DOI: 10.1016/j.spinee.2015.11.031]

Hanberg P, Bue M, Jørgensen AR, Thomassen M, Öbrink-Hansen K, Søballe K, Stilling M. Pharmacokinetics of double-dose cefuroxime in porcine intervertebral disc and vertebral cancellous bone-a randomized microdialysis study. Spine J 2020 [PMID: 32194245 DOI: 10.1016/j.spinee.2020.03.006]

Science editor:

- 4) 1 Scientific quality: The manuscript describes a basic study of the influence of anastomoses on intestine ischemia and cefuroxime concentrations. The topic is within the scope of the WJG. (1) Classification: Grade C; (2) Summary of the Peer-Review Report: The authors found that an intestinal anastomosis creates ischemic microenvironment, thus increases lactate and glycerol, decreases glucose. The measurement of cefuroxime levels has added some novelty.

Answer: We thank the science editor for her/his interest for the manuscript.

- 5) The results should be presented better though. Table 1 should be showing anastomosis and non-anastomosis levels of the ischemic metabolites.

Answer: We agree. Please find the answer to the Reviewers point two.

- 6) (3) Format: There are 3 tables and 3 figures. A total of 32 references are cited, including 14 references published in the last 3 years. There are 10 self-citations. 2 Language evaluation: Classification: Grade A. 3 Academic norms and rules: The authors provided the Biostatistics Review Certificate, Institutional Animal Care and Use Committee Approval Form, the Institutional Review Board Approval Form, and The ARRIVE Guidelines.

Answer: Four self-citations have been removed. Please find the answer to the Reviewers point three.

- 7) The authors need to provide the signed Conflict-of-Interest Disclosure Form and Copyright License Agreement. No academic misconduct was found in the CrossCheck detection and Bing search. 4 Supplementary comments: This is an unsolicited manuscript. The study was supported by Orthopaedic Research in Aarhus Foundation. The topic has not previously been published in the WJG. The corresponding author has not published articles in the BPG. 5 Issues raised: (1) I found the authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s)

Answer: We thank the scientific editor for detecting this lack of information. We have added a "signed Conflict-of-Interest Disclosure Form". However, we were not able to find the "approved grant application form". We have written to WPG main office for guidance but haven received an answer yet. If there is a form to be filled out, we are happy to do provide you with that.

- 8) (2) I found the authors did not provide the original figures. Please provide the original figure documents. Please prepare and arrange the figures using

PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor

Answer: The original figures have now been uploaded in the resubmission.

- 9) (3) I found the authors did not write the “article highlight” section. Please write the “article highlights” section at the end of the main text. 6 Re-Review: Required. 7 Recommendation: Conditionally accepted.

Answer: The Article highlight section have now been added to the manuscript as seen below. Furthermore, to meet the requirements for the manuscript format, the abstract background, method, and result section have been elaborated as seen below. All changes and additions have been marked in red.

Page 13 in the revised manuscript:

Article highlights

Research background

Anastomotic leakage is a serious complication to gastrointestinal surgery associated with increased morbidity and mortality. The etiology of anastomotic leakage is multifactorial, and to some extent not fully understood.

Research motivation

Previous studies have suggested to divide the etiology into three main factors: healing disturbances, communication between intra and extra luminal compartments, and infection. However, no studies have previously investigated ischemic metabolites in anastomotic intestine tissue and the intestine antimicrobial concentrations.

Research objectives

To evaluate ischemic metabolites and cefuroxime concentrations in both anastomosis and non-anastomosis ileum and colon in a porcine model.

Research methods

Eight healthy female pigs were included. Microdialysis catheters were placed for sampling of the ischemic metabolites and cefuroxime concentrations in both anastomosis and non-anastomosis ileum and colon. Cefuroxime 1.5 g was administered as intravenous infusion over 15 min.

Research results

*Only the colon anastomosis induced mean ischemic lactate/pyruvate ratios above 25 (ischemic cut-off) throughout the entire sampling interval, and simultaneous decreased glucose concentrations. The mean time for which the cefuroxime concentrations were maintained above the clinical breakpoint minimal inhibitory concentration for *Escherichia coli* (8 µg/mL) ranged between 116-128 min across all the investigated compartments, and was similar between the anastomosis and non-anastomosis ileum and colon. For all pigs and in all the investigated compartments, a cefuroxime concentration of 8 µg/mL was reached within 10 min from administration.*

Research conclusions

Administering 1.5 g cefuroxime 10 min prior to intestine surgery seems sufficient, and effective concentrations are sustained for approximately 2 hours. Only colon anastomosis was locally vulnerable to ischemia.

Research perspectives

The present study demonstrates that microdialysis can be used to investigate ischemic metabolites and cefuroxime concentrations in both anastomosis and

non-anastomosis intestines. This method may therefore have the potential generate knowledge for a better future understanding of anastomotic leakage.

Page 3 in the revised manuscript:

Abstract

Background

Anastomotic leakage is a serious complication to gastrointestinal surgery associated with increased morbidity and mortality. The incidence of anastomotic leakage is determined by anatomy and reported between 4-33% for colon anastomosis and 1-3% for small intestine anastomosis. The etiology of anastomotic leakage of the intestine has been divided into three main factors: healing disturbances, communication between intra and extra luminal compartments, and infection. All three factors interact, and one factor will inevitably lead to the two other factors ending in tissue ischemia, tissue necrosis, and anastomotic leakage.

Aim

To evaluate ischemic metabolites and cefuroxime concentrations in both anastomosis and non-anastomosis ileum and colon in a porcine model.

Methods

Eight healthy female pigs (Danish Landrace breed, weight 58-62 kg) were included. Microdialysis catheters were placed for sampling of the ischemic metabolites (glucose, lactate, glycerol, and pyruvate) and cefuroxime concentrations in both anastomosis and non-anastomosis ileum and colon. Cefuroxime 1.5 g was administered as intravenous infusion over 15 min.

Subsequently, dialysates and blood samples were collected over eight-hours and the ischemic metabolites and cefuroxime concentrations were quantified in all samples. The concentrations of glucose, lactate, glycerol and pyruvate were determined using the CMA 600 Microdialysis Analyzer with Reagent Set A (M Dialysis AB, Sweden), and the concentrations of cefuroxime and meropenem were quantified using a validated ultra-high-performance liquid chromatography assay.

Results

Only the colon anastomosis induced mean ischemic lactate/pyruvate ratios above 25 (ischemic cut-off) throughout the entire sampling interval, and simultaneous decreased glucose concentrations. The mean time for which the cefuroxime concentrations were maintained above the clinical breakpoint minimal inhibitory concentration for Escherichia coli (8 µg/mL) ranged between 116-128 min across all the investigated compartments, and was similar between the anastomosis and non-anastomosis ileum and colon. For all pigs and in all the investigated compartments, a cefuroxime concentration of 8 µg/mL was reached within 10 min from administration. When comparing the pharmacokinetic parameters between the anastomosis and non-anastomosis sites for both ileum and colon, only colon T_{max} and half-life differed between anastomosis and non-anastomosis ($p < 0.03$). Incomplete tissue penetrations were found to all tissues except for the non-anastomosis colon.

Conclusion

Administering 1.5 g cefuroxime 10 min prior to intestine surgery seems sufficient, and effective concentrations are sustained for approximately 2 hours. Only colon anastomosis was locally vulnerable to ischemia.