Science Editor
World Journal of Gastroenterology

June 27, 2020

Re: **Feasibility and efficacy evaluation of metallic biliary stents eluting gemcitabine and cisplatin for extrahepatic cholangiocarcinoma.**
Manuscript NO.: 57275

Dear Editor,

Thank you for your careful evaluation and the reviewers' comments on our previously submitted manuscript. All your suggestions were valuable and we deeply appreciate the opportunity to revise and improve our manuscript. We have considered them carefully and made corresponding changes in the revised manuscript (highlighted in red). Attached please kindly find the point-by-point response to the reviewers' comments.

We hope that the revised manuscript is now suitable and acceptable for publication in *World Journal of Gastroenterology*.

We look forward to hearing from you soon.

Sincerely,
Xinjian Wan

Reviewer #1:
Scientific Quality: Grade C (Good)
Language Quality: Grade A (Priority publishing)
Conclusion: Minor revision
Specific Comments to Authors: The authors performed a study to evaluate the drug release property, anti-tumor activity, and biocompatibility of gemcitabine and/or cisplatin eluting stents for the treatment of extrahepatic cholangiocellular carcinoma. In vivo part was performed on 27 pigs which were divided into 5 groups according to the drugs used. The study is informative and well-written.
Reply: Thank you. We are grateful for your comments and appreciation.

Below, are my comments about the manuscript.
1. The number of pigs included to the study is too low (6 in each group) to make a conclusion.
Reply: We should acknowledge that the sample size of our *in vivo* study of the stent in the porcine biliary tract was really small, which might decrease the statistical power of our results. In the past, researchers considered six animals per group as an adequate sample size, but recently there has been a new notion that six animals per group has little scientific and statistical basis[1].

However, the experimental difficulties concerning large animals (pigs, dogs, monkeys, etc.) are rather conspicuous due to the capacity of animal Lab, the shortage of fund budget, and the complexity and time constraint of surgical procedures and follow-ups. Thus, the sample sizes of similar studies reported so far were commonly small, for example, Chung *et al*'s study about the gemcitabine-eluting stents in a porcine biliary model, n = 2[2]; Lee *et al*'s study about the paclitaxel-eluting stents in a canine biliary model, n = 5[3].

We added it as one of the limitations in the discussion part (as illustrated in the line 15-19, page 26), and we'll adjust our sample size using an appropriate calculation approach in our further study[4].

Reference:

[1] Charan J, Kantharia ND. How to calculate sample size in animal studies? *J Pharmacol Pharmacother* 2013, 4: 303-306 [PMID: 24250214 DOI: 10.1186/1471-230X-11-76]

[2] Chung MJ, Kim H, Kim KS, Park SS, Chung JB, Park SW. Safety evaluation of self‐expanding metallic biliary stents eluting gemcitabine in a porcine model. *J Gastroenterol Hepatol* 2012; 27: 261-267 [PMID: 21793905 DOI: 10.1111/j.1440-1746.2011.06866.x]

[3] Lee SS, Shin J H, Han J M, Cho CH, Kim MH, Lee SK, Kim JH, Kim KR, Shin KM, Won YH, Song HY. Histologic influence of paclitaxel-eluting covered metallic stents in a canine biliary model. *Gastrointest Endosc* 2009; 69: 1140-1147 [PMID: 19243763 DOI: 10.1016/j.gie.2008.08.005]

[4] Arifin WN, Zahiruddin WM. Sample size calculation in animal studies using resource equation approach. *Malays J Med Sci* 2017, 24: 101-105 [PMID: 29386977 DOI: 10.21315/mjms2017.24.5.11]

2. As the author stated, endoscopic stent placement is associated with high rates of stent occlusion. The aim of local inhibition of tumor proliferation is to prevent stent obstruction due to tumor ingrowth. However, the authors euthanized pigs only 4 weeks after stent insertion, which is a very short time to make a conclusion about the efficacy of drug eluting.

Reply: We'd like to make an explanation here for the setting of observation period after stent insertion in the porcine biliary tract. Firstly, according to the *in vitro* drug release study, our stent was able to maintain an effective drug release for 30 days; however, the release of gemcitabine was nearly complete at this point (≥95%). Secondly, the drug-loaded nanofilms could effectively inhibit the growth of EGI-1 cells *in vitro* and xenograft tumors of nude mice *in vivo* in 30 days; specifically, the PLCL-GEM&CIS loaded nanofilm could almost inhibit tumor growth in mouse xenograft models completely after 4 weeks. Based on these results, we then decided to euthanize the pigs 4 weeks after stent insertion, and our main purpose was to evaluate the histologic influence of the stent in the porcine biliary tract. According to previous studies, the histologic changes caused by the loaded drugs were usually analyzed in the early stage, the mean observation period was 4-6 weeks[2,5-6]. Besides, drug related lumen stenosis, ulceration, or necrosis was more likely to occur due to the toxicity of released drugs at a relative high concentration in early stage. So we thought it was reasonable to set 4 weeks as the last follow-up time,

The conclusion about the efficacy of stent was made based on the result of mouse xenograft model, since corresponding large animal model of cholangiocarcinoma in porcine biliary tract is still not available so far[7].

Thanks for your notice, we think it's worthwhile to prolong the observation time to evaluate long-term influence of the stent in our further study and we also added it into the limitation discussion part (as illustrated in the line 15-19, page 26).

Reference:
[2] Chung MJ, Kim H, Kim KS, Park SS, Chung JB, Park SW. Safety evaluation of self‐expanding metallic biliary stents eluting gemcitabine in a porcine model. *J Gastroenterol Hepatol* 2012; 27: 261-267 [PMID: 21793905 DOI: 10.1111/j.1440-1746.2011.06866.x]
[5] Bakhru MR, Foley PL, Gatesman J, Schmitt T, Moskaluk CA, Kahaleh M. Fully covered self-expanding metal stents placed temporarily in the bile duct: safety profile and histologic classification in a porcine model. *BMC gastroenterol* 2011; 11: 76. [PMID: 21689439 DOI: 10.1186/1471-230X-11-76]
[6] Wang ZM, Liu JY, Wu KQ, Shen YY, Mao AW, Li J, Chen ZJ, Guo SR. Nitinol stents loaded with a high dose of anti-tumor 5-fluorouracil or paclitaxel: esophageal tissue responses in a porcine model. *Gastrointest Endosc* 2015; 82: 153-160 [PMID: 25936448 DOI: 10.1016/j.gie.2015.02.034]
[7] Shatzel J, Kim J, Sampath K, Syed S, Saad J, Hussain ZH, Mody K, Pipas JM, Gordon S, Gardner T, Rothstein RI. Drug eluting biliary stents to decrease stent failure rates: A review of the literature. *World J Gastrointest Endosc* 2016; 8: 77-85 [PMID: 26839648 DOI: 10.4253/wjge.v8.i2.77]

3. Based on these results, it is not possible to make a conclusion that DES may be considered as an alternative strategy for the palliative therapy of ECC patients and help improve their survival and quality of life.

Reply: Thank you for your kindly notice and we've modified our conclusion accordingly (as illustrated in the abstract part, line 17-18, page 4; also the discussion part, line 4-7, page 27). Our study was just a basic, pre-clinical research, it was surely impossible and inadequate to make a rash judgment.

4. The authors used FC stents and fixed them on the bile duct wall with 5.0 suture to avoid stent migration. However, this does not correspond to clinical practice since non-covered stents are used to prevent migration and acute cholecystitis.

Reply: Thank you for your kindly notice about the choice of fully covered self-expandable metal stents (FCSEMSs) versus uncovered self-expandable metal stents (USEMSs) in patients with malignant biliary stricture.

Similar to your comment, Jang[8] demonstrated covered stents were associated with increased rates of migration and cholecystitis. Conio[9] also claimed FCSEMSs had a significantly higher rate of migration than USEMSs, and stent occlusion occurred earlier. However, a related trial by Isayama[10] demonstrated a higher patency rate favoring FCSEMSs over USEMSs, and no statistically significant difference was seen in migration rates between them, as was reported in another study[11] that assessed the same type of FCSEMS (Comvi) used in the trial by Conio *et al*[9]. A recently published meta-analysis on this topic reported a risk reduction of approximately 32% for stent failure and an 11% increase in patient survival with FCSEMS, although this difference was not statistically significant[12]. In other words, this debate has been protracted in the medical literature and still goes on[13]. Thus, in our opinion, there are no final conclusions on the better choice between FCSEMSs and USEMSs, and the FC design enables us to achieve the goal of drug eluting.

We mentioned in the discussion part (as illustrated in the line 11-20, page 23) why we didn't use the partially covered-design. Though it may be helpful for preventing stent migration, the histological changes were more severe in the biliary mucosa in contact with the bare ends of the stent[3]. Besides,

partially covered stent hasn't yet been proved to be effective in the prevention of stent migration for malignant biliary stricture and still needs to be further evaluated in clinical trials[14-15].

The reason why we fixed the stent on the bile duct wall with 5.0 suture was based on our previous experience. We've carried out several experiments about biliary stents in porcine model[16-18], and we found that pig sphincter of Oddi was not so sufficient to prevent stent migration, so we took the suture measure to decrease the rates of migration after stent insertion since our sample size was already small. Just like your comment, this does not correspond to clinical practice and we also added it into the limitation discussion part (as illustrated in the line 19-22, page 26).

Above all, thanks again for your patience and useful comments.

Reference:

[3] Lee SS, Shin J H, Han J M, Cho CH, Kim MH, Lee SK, Kim JH, Kim KR, Shin KM, Won YH, Song HY. Histologic influence of paclitaxel-eluting covered metallic stents in a canine biliary model. *Gastrointest Endosc* 2009; 69: 1140-1147 [PMID: 19243763 DOI: 10.1016/j.gie.2008.08.005]

[8] Jang S, Stevens T, Parsi M, Lopez R, Zuccaro G, Dumot J, Vargo JJ. Association of covered metallic stents with cholecystitis and stent migration in malignant biliary stricture. *Gastrointest Endosc* 2018, 87: 1061-1070. [PMID: 28867074 DOI: 10.1016/j.gie.2017.08.024]

[9] Conio M, Mangiavillano B, Caruso A, Filiberti RA, Baron TH, Luca LD, Signorelli S, Crespi M, Marini M, Ravelli P, Conigliaro R, Ceglie AD. Covered versus uncovered self-expandable metal stent for palliation of primary malignant extrahepatic biliary strictures: a randomized multicenter study. *Gastrointest Endosc* 2018, 88: 283-291 [PMID: 29653120 DOI: 10.1016/j.gie.2018.03.029]

[10] Isayama H, Komatsu Y, Tsujino T, Sasahira N, Hirano K, Toda N, Nakai Y, Yamamoto N, Tada M, Yoshida H, Shiratori Y, Kawabe T, Omata M. A prospective randomised study of 'covered' versus 'uncovered' diamond stents for the management of distal malignant biliary obstruction. *Gut* 2004, 53: 729-734 [PMID: 15082593 DOI: 10.1136/gut.2003.018945]

[11] Isayama H, Kawabe T, Nakai Y, Ito Y, Togawa O, Kogure H, Yashima Y, Yagioka H, Matsubara S, Sasaki T, Sasahira N, Hirano K, Tsujino T, Tada M, Omata M. Management of distal malignant biliary obstruction with the ComVi stent, a new covered metallic stent. *Surg Endosc* 2010, 24: 131-137 [PMID: 19517184 DOI: 10.1007/s00464-009-0537-9]

[12] Tringali A, Hassan C, Rota M, Rossi M, Mutignani M, Aabakken L. Covered vs. uncovered self-expandable metal stents for malignant distal biliary strictures: a systematic review and meta-analysis. *Endoscopy* 2018, 50: 631-641 [PMID: 29342491 DOI: 10.1055/s-0043-125062]

[13] Tringali A, Mutignani M, Adler DG. Fully covered self-expandable metal stents versus uncovered self-expandable metal stents in patients with distal malignant biliary stricture: Is this the right question? Gastrointest Endosc 2019, 89: 897-898 [PMID: 30902212 DOI: 10.1016/j.gie.2018.09.031]

[14] Kim JY, Ko GB, Lee TH, Park SH, Lee YN, Cho YS, Jung Y, Chung IK, Choi HJ, Cha SW, Moon JH, Cho YD, Kim SJ. Partially covered metal stents may not prolong stent patency compared to uncovered stents in unresectable malignant distal biliary obstruction. *Gut and liver* 2017; 11: 440-446 [PMID: 28208003 DOI: 10.5009/gnl16245]

[15] Beyna T, Neuhaus H. Self-expandable metal stents in malignant biliary obstruction: Back to the roots with uncovered stents as the 'new' standard? *Gastrointest Endosc* 2018; 87: 1071-1073 [PMID: 29571772 DOI: 10.1016/j.gie.2017.11.022]

[16] Huang C, Cai XB, Guo LL, Qi XS, Gao Q, Wan XJ. Drug-eluting fully covered self-expanding metal stent for dissolution of bile duct stones in vitro. *World J Gastroenterol* 2019, 25: 3370-3379

[PMID: 31341362 DOI: 10.3748/wjg.v25.i26.3370]
[17] Gao Q, Huang C, Sun B, B Aqeel BM, Wang J, Chen WM, Mo XM, Wan XJ. Fabrication and characterization of metal stent coating with drug-loaded nanofiber film for gallstone dissolution. *J Biomater Appl* 2016, 31: 784-796 [PMID: 27698255 DOI: 10.1177/0885328216671239]
[18] Cai XB, Zhang WX, Zhang RL, Yuan XD, Yang Q, Qi XS, Li BW, Qian YQ, Wang XP, Lu LG, Xu ZJ, Wan XJ. Safety and efficacy of a novel plastic stent coated with stone-dissolving agents for the treatment of biliary stones in a porcine model. *Endoscopy* 2015, 47: 457-461 [PMID: 25479561 DOI: 10.1055/s-0034-1390772]

Reviewer #2:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: Comments to the Author I congratulate the authors for carrying out this experimental study, which is of great clinical importance. I also congratulate you on the reflection made at the end of page 26, showing the limitations of this article and this type of study. I hope you understand that the evaluations carried out are aimed at making the study less biased and divergent, in addition to making it more interesting for your readers.

Reply: Thank you. We are grateful for your comments and appreciation.

About the language quality, we have proofread the manuscript after revision and have attempted to correct all language errors we found. Besides, we invited Dr. David Krason, who is a native-English speaker and works at both Massachusetts General Hospital and Shanghai Jiahui International Hospital as a hospitalist, to edit our revised manuscript for grammar/readability.

We realized some biases that we didn't fully describe in our previous manuscript, for example, small sample size, relatively short observation time, additional suture procedure to prevent stent migration in porcine *in vivo* experiment, etc. We also learned that our previous conclusion was inadequate and biased. We made adjustment and modification accordingly in our revised manuscript, especially in the discussion part.

Editorial Office's comments

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);

Reply: The approved grant application forms are now uploaded.

(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;

Reply: The original figures in the format of PowerPoint are now included.

(3) Please don't include any *, #, †, §, ‡, ¥, @….in your manuscript; Please use superscript numbers

for illustration; and for statistical significance, please use superscript letters. Statistical significance is expressed as $^{a}P < 0.05$, $^{b}P < 0.01$ ($P > 0.05$ usually does not need to be denoted). If there are other series of P values, $^{c}P < 0.05$ and $^{d}P < 0.01$ are used, and a third series of P values is expressed as $^{e}P < 0.05$ and $^{f}P < 0.01$;
Reply: We've made the modifications accordingly.

(4) The authors need to provide the Institutional Review Board Approval Form, complete the Conflict-of-Interest Disclosure Form, and fill out the ARRIVE checklist form with page numbers.
Reply: These request documents are now uploaded.