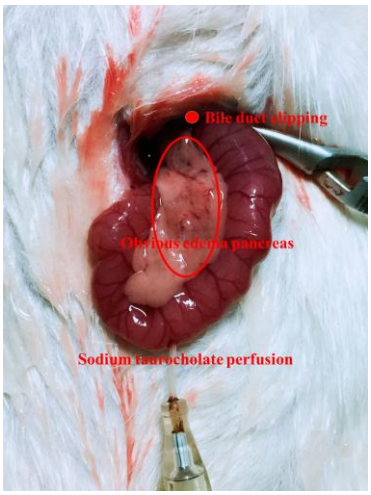
35253-The response letter

The response to the comments of reviewer 1

1. AP model: AP models were induced by retrograde perfusion of 3% sodium taurocholate (1 mL/kg body weight) into the biliopancreatic duct at a rate of 6 mL/h with a micro-infusion pump after anesthetization with 10% chloral hydrate (3 mL/kg body weight) injected into the abdominal cavity. **Is the method for AP induction original? It would be useful authors to provide more information on the timing and extent of pancreatitis in experimental animals.**

   This method was not original. It was cited Bettina Rau's study in 2000 [1]. In his study, acute necrotizing pancreatitis was induced by standardized retrograde infusion of 0.1 mL/100g body weight of 3% sodium taurocholate solution into the biliopancreatic duct [1]. When sodium taurocholate was perfused, we could see the obvious edema pancreas immediately (the picture attached). Wang SF's study [2] showed that AP (induced by 3% sodium taurocholate solution) develop in the rats to the most severe at 24h but tended to self-restore at 36 and 48h.



[1] Bettina Rau, Bertram Poch, Frank Gansauge, Annette Bauer, Andreas K. Nussler, Timo Nevalainen, Michael H. Schoenberg, and Hans G. Beger. Pathophysiologic Role of Oxygen Free Radicals in Acute Pancreatitis: initiating event or mediator of tissue damage? Ann Surg. 2000, 231 (3): 352-360.

[2] Hu YY, Zhou CH, Dou WH, Tang W, Hu CY, Hu DM, Feng H, Wang JZ, Qian MJ, Cheng GL, Wang SF. Improved autophagic flux is correlated with mTOR activation in the later recovery stage of experimental acute pancreatitis. Pancreatology 2015, 15：470-477.

2. **Hesperidin and honokiol were not detected in the rat plasma? How to explain that Hesperidin pancreatic tissue concentrations were very high in NG group!**

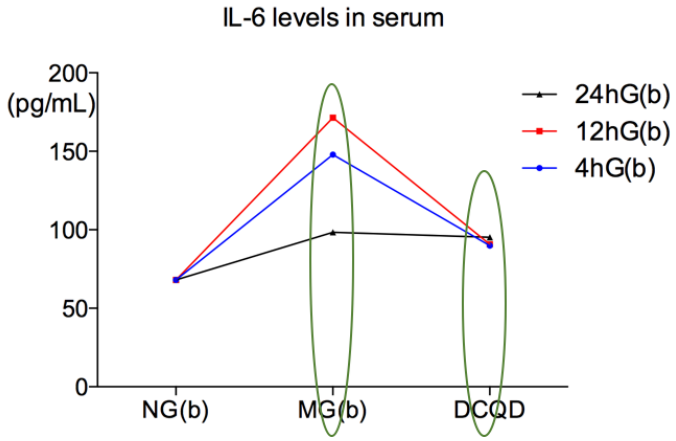
The two components were detected unsuccessfully in some point-in-time. It may be related with insufficient plasma or the content of components in some point-in-time. So the incomplete data couldn't be fitted curve.

3. **Tmax was delayed in all AP groups practically for all components.**

   Not exactly. The Tmax of emodin and chrysophanol was 0.33 h in the 24hG(a). That of rheochrysidin and magnolol in the 4hG(a) and naringenin in the 24hG(a) were 0.67 h as well as the NG(a). We will upload the original data of Figure 1 *via* the F6Publishing system.

4. **IL-6 in serum was decreased in the same extent in all AP groups!**

   The decreased extent was different in all AP groups. The IL-6 levels in MG(b) in different dosing time groups were 147.91±4.36, 171.44±13.43, 98.48±2.7. However, those in DCQD treatment groups were 89.99±4.61, 90.82±5.34, 95.17 ±3.98.



5. **Please, describe in more details the histopathological scores method? Histilogical slides on figure 3A: visually there are differences between groups 4h and 24h, but no differences in scores on figure 3B? And why the score for NG group is zero?**

   The method has been added in details in "Collection and Measurement of Samples" section. The scores in the 4hG(b) and 24hG(b) were 2.63±0.4, 2.5± 0.3 ($P > 0.05$). The picture of the 24hG(b) on figure 3A was slighter in the group and we have selected again. The score for NG group was not zero and it was 0.7, however, we had input 0.07 when plotted. We have corrected.

6. **Figure 3C and D: please correct the figures titles.**
   We have corrected.

7. **Figure 3D: the claim for serum amylase levels after treatment: "The AP model was successful. That in the 12hG(b) group was reduced compared to its control group (*P* < 0.05)" was not true!! There are no statistical differences between the groups!**

   Compared to the NG(b), the amylase levels of the 4hG(b), 12hG(b) and 24hG(b) were increased (P <0.05, respectively). So AP model was successful. The original data were as follows:

	4hG(b)	12hG(b)	24hG(b)
NG(b)	389.00±98		
MG(b)	718.65±51.04	711.68±55.37	666.40±73.41
DCQD	701.05±26.35	649.00±131.69*	636.80±112.48

*vs MG(b), P < 0.05.

8(a). "Their absorption may be better if the oral administration time of DCQD is approximately 12 hours after the onset of AP". This claim does not apply for all active components. The tissue pancreatic levels do not directly correspond to absorption bioavailability parameters.

This result was obtained according to the parameters of most active components. It's a conjecture. The objective was to identify the optimal oral dosing time of DCQD in rats with AP based on parameters. That wasn't involved with bioavailability. We agreed with the reviewer's opinion and have corrected.

8(b). Increased t1/2 levels of active components in AP animals should not be due to differences in absorption rate or amount absorbed.

We agreed with it.

8(c). "In this study, the pathological damage to pancreatic tissues had no obvious improvement. This observation may be related to the dosage of DCQD being less than in our previous study". **Correct conclusion which was in some discrepancy with the claims when describing the results.**

According to the result, the tissue damage was improved only in the 12hG(b) group. We have corrected.

9. Conclusion: The article needs a more accurate description of the results to assess whether the conclusion for the optimal time of DCQD application for treatment of pancreatitis is credible and applicable in clinical practice.

   According to the data of animal experiment, we could only obtain a conjecture. In clinical practice, orally dosing or coloclysis of DCQD are performed immediately

when patients with AP are admitted to hospital. Whether these approaches could increase secretion of gastrointestinal tract remain unclear. We hope that we can find an optimal dosing time of DCQD without increasing the severity of AP. Our results need further clinical studies to be confirmed.

The response to the comments of reviewer 2

1. **Data on the secretion of pro- vs anti-inflammatory cytokines add very little to what is already published.**

   The inflammatory cytokines were not the key point in this study. It's a reference value to our objective.

2. **The data shown regarding damage to pancreatic tissue are not consistent with previous data showing that a similar dose of intragastrically administered DCQD 2 hours after AP induction confers some protection against pancreatic tissue damage (Refs. 4 & 5). The discrepancy should be discussed.**

   In previous study (Refs. 4 & 5), the treatment groups were dosed orally with DCQD of 6, 12 and 24 g/kg.BW（LDG, MDG, and HDG, respectively）at 2 h after operation. That result showed the pancreatic tissue damages were not improved in LDG, but improved in MDG and HDG. In this study, rats were dosed with DCQD of 10g/kg.BW at 4 h, 12 h and 24 h. The damages were improved in the 12hG(b) group compared to its MG(b). However, the data showed the standard deviation was large or close to the mean, that is, the difference was large in the 4hG(b) and 24hG(b). Although the weight of rats had no difference, it may be related with different experimenter.

3. **Materials and Methods: The composition of the internal working solution and the composition of the "double-solvents" should be provided.**

   That has been added in "Equipment and Conditions" section in page 8 &9.

4. **It is correct to provide the concentration of the DCQD components extracted from blood or serum (?) in μg/mL. However, for solid tissues it is counterintuitive to provide a concentration in ng/mL (table 1). The data should be normalized according to protein content or tissue weight.**

   We detected the components from plasma and pancreatic tissues. The unit has been corrected.

5. **The circadian clock can influence the absorption and the effects of drugs. Was DCQD intragastrically administered to groups of rats at the same time of day?**

The AP models were induced from 8:00 to 9:30. The dosing time were 4 h, 12 h and 24 h after AP induction. The dosing time to every group was at the same time of day.

6. **The authors recently published two studies showing that DCQD (12 mg/kg) orally administered 2 hours after induction of AP could reduce pathological scores in the pancreas. Here, it is shown that DCQD (12 mg/kg) orally administered 4 hours after induction of AP has no effect on pancreatic tissue damage. These discrepancies should be discussed.**

   The original data showed the standard deviation was large or close to the mean, that is, the difference was large in the 4hG(b) and 24hG(b). Although the weight of rats had no difference, it may be related with different experimenter.