Dear Dr. Wang,

Thank you very much for your letter and advice. We revised the manuscript and would like to re-submit it for your consideration. We have processed the comments put forward by reviewers, and the revisions of the manuscript are highlighted in red. The point-by-point responses to the reviewers' comments are listed below.

I'm so glad that the first decision for publication of the current manuscript is available. We hope that the revised version of the manuscript will be finalized for publication.

We would like to express our heartfelt gratitude to you and the reviewers.

Best regards,

Yours sincerely,

Wen-Fu Tang

Replies to reviewer:

Comment 1

'English is generally adequate, but there are sporadic typos across the manuscript - should be re-checked.'

Answer:

We have proofread the manuscript again and have attempted to correct all language errors that we found in this manuscript, while also making several corrections to improve clarity.

Comment 2

'I would NOT agree that using serial Student t-tests (or their non-parametric analogues) is appropriate in these experiments. Both experiments are, generally, settings in which (for each outcome) one-way analysis of variance (parametric or non-parametric) is appropriate.'

Answer:

In terms of the statistical analysis, we have made some adjustments according to the reviewer's suggestions. In the first part of the pharmacokinetic experiment, which involved four groups, we replaced the Student t-tests (or their non-parametric analogues) with one-way analysis of variance (parametric or non-parametric) followed by pairwise comparisons for statistical analysis. The modified results are also adjusted accordingly in table 1.

For the second part of pharmacodynamics experiment, we still performed the Student t-tests, because we did not make comparisons in the three treatment groups (4h-TG, 12h-TG and 24h-TG), we only compared the IL6 and IL-10 and pathological damage of rats treated at the same time point, that is, 4 h-TG (administered DCQD) *vs.* 4 h control group (administered saline), Similarly, 12h-TG *vs.* 12 h control group and 24h-TG *vs.* 24 h control group. In this way, we could see the difference between the DCQD treatment group and the control saline group at each time point, and further deduce the optimal time for administration. In our study, late time (12h/24h after modeling) dosing may result in better pharmacodynamics of anti-inflammation than early-time (4 h after modeling), thereby showing the late time to be the optimal dosing time of DCQD for the protective of extrapancreatic organs in SAP.

Coment 3

'One problem that arises in this very complex work (considering the number of outcomes/analytes) - is the question of the overall type 1 error…It is often forgotten that P-index IS NOT A MEASURE of an effect. and sometimes - like here, at least regarding PK data - the primary interest is getting insight into the

extent of difference between different administration timings. Avoiding focus on p-values in this setting (with so many tests) - I believe it is very important - anyone aware of the multiplicity problem will immediately recognize that at least some null-hypotheses were rejected - simply by chance. and for PK data...one thing is not very clear - how was the "mathematics" done? It is generally accepted that for example - Cmax, AUC and elimination rate constant follow log-normal distribution. This would mean: for each analyte, use ANOVA on ln-transformed data, and compare each group vs. the control.'

Answer:

We really appreciate the constructed comments raised by the reviewer. we have tried our best to make adjustments to improve the precision of the estimate. As mentioned earlier, we've changed the one-way analysis of variance (parametric or non-parametric) followed by pairwise comparisons for statistical analysis in the first part of the pharmacokinetic experiment. In order to avoid excluding the fact that the difference most likely exists, we re-calculated the 90%CIs around the mean difference, rather than focusing only on the p values, thereby reducing the likelihood of overall type 1 error and improving the precision of the estimate. The 90% CI of each comparison are listed at the end of the reply (Supplementary table), through further comparison, it is found that there is no difference with the results in our current manuscript (table 1), so this supplement is not included in the revised manuscript to avoid redundancy.

Additionally, we agree with the reviewer's opinion that C max, AUC and elimination rate constant follow log-normal distribution, and you suggested us use ANOVA on ln-transformed data, and compare each group *vs*. the control. Before we make changes to the analysis of pharmacokinetic statistics, we re-checked pub-med database to get more information and studies concerning the pharmacokinetics of drugs and consulting with experts in this field, we found that the ln-transformed data of C max, AUC and elimination rate are often used in in vitro experiments on the metabolic stability of liver microsomes or other in vitro pharmacokinetic experiments[1-5], however, in almost all the in vivo pharmacokinetic experiments of Chinese Herb Medicine or their monomers, these parameters are usually directly analyzed [6-14] , and our research team have also completed other similar pharmacokinetic studies on Chinese Herb

Medicine (e.g. Shengjiang powder, Liu-He-Dan) in this same way[6, 15]. Hence, we did not change this part in our revised manuscript.

Supplementary table 1: results of multiple comparisons followed by ANOVA.

		Tukey's multiple comparisons test	Mean Diff.	90% CI of diff.	Significant?
Emodin	AUC	MG_1 vs. SOG_1	-1246	-2390 to -101.1	Yes
		MG_1 vs. MG_2	-3.232	-1148 to 1141	No
		MG_1 vs. MG_3	-68.22	-1213 to 1076	No
		MG_2 vs. MG_3	-64.99	-1210 to 1080	No
	t 1/2	MG_1 vs. SOG_1	13.73	1.824 to 30.27	Yes
		MG_1 vs. MG_2	9.891	-6.658 to 26.44	No
		MG_1 vs. MG_3	-3.655	-20.20 to 12.89	No
		MG_2 vs. MG_3	-13.55	-30.10 to 3.003	No
	T max	MG_1 vs. SOG1	2.917	0.5184 to 5.316	Yes
		MG_1 vs. MG_2	0	-2.399 to 2.399	No
		MG_1 vs. MG_3	3.167	0.7684 to 5.566	Yes
		MG_2 vs. MG_3	3.167	0.7684 to 5.566	Yes
	C max	MG_1 vs. SOG_1	-586.6	-1097 to -76.29	Yes
		MG_1 vs. MG_2	4.372	-505.9 to 514.7	No
		MG_1 vs. MG_3	-91.01	-601.3 to -419.3	Yes
		MG_2 vs. MG_3	-95.38	-605.7 to -414.9	Yes
Aloe-emodin	AUC	MG_1 vs. SOG1	-7346	-26301 to 11609	No
		MG_1 vs. MG_2	-8322	-27277 to 10633	No
		MG_1 vs. MG_3	-25443	-44398 to -6488	Yes
		MG_2 vs. MG_3	-17121	-36077 to 1834	No
	t1/2	MG_1 vs. SOG1	-1.596	-8.067 to 4.875	No
		MG_1 vs. MG_2	-0.963	-7.434 to 5.508	No
		MG_1 vs. MG_3	-2.683	-9.154 to 3.788	No
		MG_2 vs. MG_3	-1.72	-8.191 to 4.751	No
	T max	MG_1 vs. SOG1	-0.166	-2.393 to 2.061	No
		MG_1 vs. MG_2	-1.583	-3.810 to 0.6440	No
		MG_1 vs. MG_3	0.75	-1.477 to 2.977	No
		MG_2 vs. MG_3	2.333	0.1060 to 4.560	Yes
	C max	MG_1 vs. SOG1	-1593	-6062 to 2876	No
		MG_1 vs. MG_2	-194.5	-4663 to 4274	No
		MG_1 vs. MG_3	-6146	-10614 to -1677	Yes
		MG_2 vs. MG_3	-5951	-10420 to -1482	Yes
Rhein	AUC	MG_1 vs. SOG1	-18263	-55559 to -19033	Yes
		MG_1 vs. MG_2	-48658	-85954 to -11362	Yes
		MG_1 vs. MG_3	-24722	-62017 to -12574	Yes

		MG_2 vs. MG_3	23936	13360 to 61232	Yes
	t 1/2	MG_1 vs. SOG1	2.418	-8.490 to 13.33	No
		MG_1 vs. MG_2	1.667	-9.241 to 12.57	No
		MG_1 vs. MG_3	-14.29	-25.19 to -3.377	Yes
		MG_2 vs. MG_3	-15.95	-26.86 to -5.044	Yes
	T max	MG_1 vs. SOG1	5.654	2.614 to 8.694	Yes
		MG_1 vs. MG_2	4.82	1.780 to 7.860	Yes
		MG_1 vs. MG_3	4.904	1.864 to 7.944	Yes
		MG_2 vs. MG_3	0.084	-2.956 to 3.124	No
	Cmax	MG_1 vs. SOG1	-9603	-16073 to -3133	Yes
		MG_1 vs. MG_2	-7041	-13512 to -571.3	Yes
		MG_1 vs. MG_3	-8909	-15379 to -2439	Yes
		MG_2 vs. MG_3	-1867	-8338 to 4603	No
Chrysophanol	AUC	MG_1 vs. SOG1	-14295	-28542 to -47.91	Yes
		MG_1 vs. MG_2	-12655	-26902 to -1592	Yes
		MG_1 vs. MG_3	-10676	-24923 to -3571	Yes
		MG_2 vs. MG_3	1979	-12268 to 16226	No
	t 1/2	MG_1 vs. SOG1	-0.922	-4.170 to -2.326	Yes
		MG_1 vs. MG_2	0.112	-3.136 to 3.360	No
		MG_1 vs. MG_3	-2.148	-5.396 to 1.100	No
		MG_2 vs. MG_3	-2.26	-5.508 to 0.9882	No
	T max	MG_1 vs. SOG1	1.833	0.1062 to 3.560	Yes
		MG_1 vs. MG_2	1.75	0.02325 to 3.477	Yes
		MG_1 vs. MG_3	1.5	-0.2268 to 3.227	No
		MG_2 vs. MG_3	-0.25	-1.977 to 1.477	No
	C max	MG_1 vs. SOG1	-5747	-10698 to -796.8	Yes
		MG_1 vs. MG_2	-2135	-7085 to 2816	No
		MG_1 vs. MG_3	-2299	-7250 to 2651	No
		MG_2 vs. MG_3	-164.4	-5115 to 4786	No
Rheochrysidin	AUC	MG_1 vs. SOG1	2798	1175 to 4421	Yes
		MG_1 vs. MG_2	633.6	-989.4 to 2257	No
		MG_1 vs. MG_3	2155	532.3 to 3778	Yes
		MG_2 vs. MG_3	1522	-101.3 to 3145	No
	t 1/2	MG_1 vs. SOG1	-1.795	-4.594 to 1.004	No
		MG_1 vs. MG_2	0.035	-2.764 to 2.834	No
		MG_1 vs. MG_3	-5.073	-7.872 to -2.274	Yes
		MG_2 vs. MG_3	-5.108	-7.907 to -2.309	Yes
	T max	MG_1 vs. SOG1	0.917	-1.428 to 3.262	No
		MG_1 vs. MG_2	-0.25	-2.595 to 2.095	No
		MG_1 vs. MG_3	0.417	-1.928 to 2.762	No
		MG_2 vs. MG_3	0.667	-1.678 to 3.012	No
	C max	MG_1 vs. SOG1	531.3	64.82 to 997.8	Yes
		MG_1 vs. MG_2	239	-227.5 to 705.5	No

		MG_1 vs. MG_3	555.9	89.41 to 1022	Yes
		MG_2 vs. MG_3	316.9	-149.6 to 783.3	No
Naringin	AUC	MG_1 vs. SOG1	5943	3163 to 8723	Yes
		MG_1 vs. MG_2	847	-1933 to 3627	No
		MG_1 vs. MG_3	4107	1327 to 6888	Yes
		MG_2 vs. MG_3	3261	480.3 to 6041	Yes
	t 1/2	MG_1 vs. SOG1	-2.257	-4.186 to -0.3284	Yes
		MG_1 vs. MG_2	-1.242	-3.171 to 0.6866	No
		MG_1 vs. MG_3	-1.618	-3.547 to 0.3106	No
		MG_2 vs. MG_3	-0.376	-2.305 to 1.553	No
	T max	MG_1 vs. SOG1	1	-1.435 to 3.435	No
		MG_1 vs. MG_2	-0.5	-2.935 to 1.935	No
		MG_1 vs. MG_3	-0.75	-3.185 to 1.685	No
		MG_2 vs. MG_3	-0.25	-2.685 to 2.185	No
	C max	MG_1 vs. SOG1	680.6	194.0 to 1555	Yes
		MG_1 vs. MG_2	123.8	-750.8 to 998.4	No
		MG_1 vs. MG_3	548.7	325.9 to 1423	Yes
		MG_2 vs. MG_3	424.9	-449.7 to 1299	No
Naringenin	AUC	MG_1 vs. SOG1	3281	1175 to 5386	Yes
		MG_1 vs. MG_2	3293	1187 to 5399	Yes
		MG_1 vs. MG_3	2419	312.8 to 4525	Yes
		MG_2 vs. MG_3	-874.5	-2980 to 1231	No
	t 1/2	MG_1 vs. SOG1	-2.914	-7.453 to 1.625	No
		MG_1 vs. MG_2	-6.415	-10.95 to -1.876	Yes
		MG_1 vs. MG_3	-3.34	-7.879 to 1.199	No
		MG_2 vs. MG_3	3.075	-1.464 to 7.614	No
	T max	MG_1 vs. SOG1	1.333	-0.1414 to 2.807	No
		MG_1 vs. MG_2	0.667	-0.8074 to 2.141	No
		MG_1 vs. MG_3	1.667	0.1926 to 3.141	Yes
		MG_2 vs. MG_3	1	-0.4744 to 2.474	No
	C max	MG_1 vs. SOG1	171.1	-288.4 to 630.6	No
		MG_1 vs. MG_2	316.4	-143.2 to 775.9	No
		MG_1 vs. MG_3	224.2	-235.3 to 683.8	No
		MG_2 vs. MG_3	-92.14	-551.7 to 367.4	No
Magnolol	AUC	MG_1 vs. SOG1	-109	-151.3 to -66.71	Yes
		MG_1 vs. MG_2	-34.53	-76.83 to 7.767	No
		MG_1 vs. MG_3	-29.34	-71.64 to 12.96	No
		MG_2 vs. MG_3	5.195	-37.10 to 47.49	No
	t 1/2	MG_1 vs. SOG1	1.625	-10.34 to 13.59	No
		MG_1 vs. MG_2	-3.266	-15.23 to 8.703	No
		MG_1 vs. MG_3	-13.87	-25.84 to -1.903	Yes
		MG_2 vs. MG_3	-10.61	-22.57 to 1.363	No
	T max	MG_1 vs. SOG1	0.083	-1.356 to 1.522	No

		MG_1 vs. MG_2	-2.75	-4.189 to -1.311	Yes
		MG_1 vs. MG_3	0.25	-1.189 to 1.689	No
		MG_2 vs. MG_3	3	1.561 to 4.439	Yes
	C max	MG_1 vs. SOG1	-25.33	-46.53 to -4.138	Yes
		MG_1 vs. MG_2	-0.22	-21.41 to 20.97	No
		MG_1 vs. MG_3	-7.525	-28.72 to 13.67	No
		MG_2 vs. MG_3	-7.305	-28.50 to 13.89	No

Rats were randomly divided into SOG1 and three model groups (MG1, MG2 and MG3), orally dosed with DCQD) (10 g/kg). Blood samples were collected via the tail vein at 10 min, 20 min, 40 min, 1 h, 2 h, 4 h, 8 h, 12 h and 24 h after a single dose of DCQD to detect its main components. The pharmacokinetic parameters were calculated by pharmacokinetic statistic software DAS2.0.1. SOG1: the sham-operated group with the dosing time at 4 h after operation. MG1, MG2 and MG3: rats were dosed orally with DCQD at 4 h, 12 h and 24 h after AP induction, respectively. Data are presented as the mean ± SD (n=6).

References

1 Ahn SH, Heo TH, Jun HS, Choi Y. In vitro and in vivo pharmacokinetic characterization of LMT-28 as a novel small molecular IL-6 inhibitor. *Asian-Australasian journal of animal sciences* 2019 [PMID: 31480155 DOI: 10.5713/ajas.19.0463]

2 Huang J, Hu L, Xu L, Sun M, Fan Z, Qiu J, Li G, Si L. In vitro enantioselective metabolism of TJ0711 hydrochloride by human liver microsomes using a novel chiral liquid chromatography-tandem mass spectrometry method. *Journal of chromatography B, Analytical technologies in the biomedical and life sciences* 2012; **891-892**: 98-101 [PMID: 22406105 DOI: 10.1016/j.jchromb.2012.02.018]

3 Ma S, Wang L, Ouyang B, Bai X, Ji Q, Yao L. The Design, Synthesis and Preliminary Pharmacokinetic Evaluation of d3-Poziotinib Hydrochloride. *Biological & pharmaceutical bulletin* 2019; **42**(6): 873-876 [PMID: 31155586 DOI: 10.1248/bpb.b19-00153]

4 Xue Y, Liu J, Fu R, Gao L, Yang X, Zhang Z, Song R. In vitro studies on the metabolism of saikogenins and the detection of their metabolites in authentic biosamples. *Journal of pharmaceutical and biomedical analysis* 2019; **172**: 295-301 [PMID: 31079023 DOI: 10.1016/j.jpba.2019.04.051]

5 Alrabiah H, Kadi AA, Attwa MW, Mostafa GAE. Development and validation of an HPLC-MS/MS method for the determination of arginine-vasopressin receptor blocker conivaptan in human plasma and rat liver microsomes: application to a metabolic stability study. *Chemistry Central journal* 2018; **12**(1): 47 [PMID: 29717376 PMCID: PMC5930294 DOI: 10.1186/s13065-018-0414-5]

6 Zhu L, Li JY, Zhang YM, Kang HX, Chen H, Su H, Li J, Tang WF. Pharmacokinetics and pharmacodynamics of Shengjiang decoction in rats with acute pancreatitis for protecting against

multiple organ injury. *World journal of gastroenterology* 2017; **23**(46): 8169-8181 [PMID: 29290653 PMCID: PMC5739923 DOI: 10.3748/wjg.v23.i46.8169]

7 Gong HL, Tang WF, Yu Q, Xiang J, Xia Q, Chen GY, Huang X, Liang MZ. Effect of severe acute pancreatitis on pharmacokinetics of Da-Cheng-Qi Decoction components. *World journal of gastroenterology* 2009; **15**(47): 5992-5999 [PMID: 20014465 PMCID: PMC2795188 DOI: 10.3748/wjg.15.5992]

8 Tang W, Wan M, Zhu Z, Chen G, Huang X. Simultaneous determination of eight major bioactive compounds in Dachengqi Tang (DT) by high-performance liquid chromatography. *Chinese medicine* 2008; **3**: 5 [PMID: 18445276 PMCID: PMC2383911 DOI: 10.1186/1749-8546-3-5]

9 Zhao J, Tang W, Wang J, Xiang J, Gong H, Chen G. Pharmacokinetic and pharmacodynamic studies of four major phytochemical components of Da-Cheng-Qi decoction to treat acute pancreatitis. *Journal of pharmacological sciences* 2013; **122**(2): 118-127 [PMID: 23739595 DOI: 10.1254/jphs.13037fp]

10 Yu Q, Xiang J, Tang W, Liang M, Qin Y, Nan F. Simultaneous determination of the 10 major components of Da-Cheng-Qi decoction in dog plasma by liquid chromatography tandem mass spectrometry. *Journal of chromatography B, Analytical technologies in the biomedical and life sciences* 2009; **877**(22): 2025-2031 [PMID: 19523886 DOI: 10.1016/j.jchromb.2009.05.030]

11 Zhao X, Yu L, Chen Y, Wang Y, Wan H, Yang J. Comparative Pharmacokinetics of Hydrophilic Components in Salvia miltiorrhiza Bge. and Carthamus tinctorius L. in Rats That Underwent Cerebral Ischemia Reperfusion Using an HPLC-DAD Method. *Frontiers in pharmacology* 2019; **10**: 1598 [PMID: 32038261 PMCID: PMC6992657 DOI: 10.3389/fphar.2019.01598]

12 Guo Y, Lu S, Yang B, Li G, Ma W, Guo Q, Wang Q, Kuang H. HPLC-MS/MS method for the determination and pharmacokinetic study of six compounds against rheumatoid arthritis in rat plasma after oral administration of the extract of Caulophyllum robustum Maxim. *Journal of pharmaceutical and biomedical analysis* 2020; **181**: 112923 [PMID: 32029347 DOI: 10.1016/j.jpba.2019.112923]

13 Ning Y, Pei K, Cao G, Cai H, Liu X, Cao L, Zhang S, Cai B. Comparative Study on Pharmacokinetics of Four Active Compounds in Rat Plasma after Oral Administration of Raw and Wine Processed Chuanxiong Rhizoma. *Molecules (Basel, Switzerland)* 2019; **25**(1) [PMID: 31881790 PMCID: PMC6982718 DOI: 10.3390/molecules25010093]

14 Zhai S, Deng X, Zhang C, Zhou Y, Xie H, Jiang Z, Jia L. A novel UPLC/MS/MS method for rapid determination of murrayone in rat plasma and its pharmacokinetics. *Journal of pharmaceutical and biomedical analysis* 2020; **180**: 113046 [PMID: 31874311 DOI: 10.1016/j.jpba.2019.113046]

15 Zhao XL, Xiang J, Wan MH, Yu Q, Chen WW, Chen GY, Tang WF. Effect of acute pancreatitis on the pharmacokinetics of Chinese herbal ointment Liu-He-Dan in anaesthetized rats. *Journal of ethnopharmacology* 2013; **145**(1): 94-99 [PMID: 23127650 DOI: 10.1016/j.jep.2012.10.036]