Author's response to reviews

Title: Anti-inflammatory and anti-oxidative effects of corilagin in a rat model of acute cholestasis

Authors:

Feng Jin (jinfengsdjin@sina.com)
Du Cheng (476687947@qq.com)
Jun-Yan Tao (Junyan.Tao2@ucsf.edu)
Ran Pang (pangran05@163.com)
Yuan-Jin Guo (sophiaguot1028@yahoo.com.cn)
Pian Ye (122454993@qq.com)
Ji-Hua Dong (djh7045@126.com)
Shu-Ling Zhang (chinesemod@ yahoo.com)
Lei Zhao (chinesemag@hotmail.com)

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Author's response to reviews: see over
Dear editor,

According to the reviewer’s comment, we revised the manuscript carefully. The revisions in detail are listed as follows.

1. From Introduction part, p. 6:
Recent research indicated that corilagin has multiple activities including antioxidative, anti-inflammatory, antiapoptotic, hepatoprotective and others. It was reported that corilagin could attenuate tert-butyl hydroperoxide-induced oxidative stress injury in microglial cells, which suggests that corilagin should be a potential candidate for the treatment of oxidative stress-induced neurodegenerative diseases [10]. It has been shown that corilagin has the potential to reduce HSV-1-induced inflammatory insult to the brain [11] and an anti-inflammatory activity in a cellular model [12]. Furthermore, it was confirmed that corilagin is an inhibitor of TNF-α [13] and can restrain radiation-induced microglia activation via suppression of the NF-κB pathway [14], and corilagin is protective against GalN/LPS-induced liver injury through suppression of oxidative stress and apoptosis [15]. Our recent research showed that corilagin can alleviate the hepatic fibrosis caused by egg granuloma in Schistosoma japonicum infection [16].

Response: We revised the expression as reviewer pointed out.

2. From Methods part, pp. 7-10:
All chemicals were purchased from Gibco (Invitrogen, city, country) or HyClone (Thermo Scientific, city, country) (like PBS and other basic stuff – this was an explanation for the authors and should not appear in the text) unless indicated otherwise.

The rats were maintained under standard laboratory conditions at a temperature of 25 ± 2°C, a relative humidity of 50 ± 15% and normal circadian rhythm (12-h dark/12-h light).

90 rats were equally divided into 5 groups i.e. corilagin, UDCA, dexamethasone, model and blank control groups.

Corilagin was prepared as 1.6% suspension; UDCA (Dr. Falk Pharma GmbH, Freiburg, Germany) was prepared as 0.6% suspension with water; dexamethasone (Zhejiang XianJu Pharmaceutical Company Ltd., Zhejiang, China) was dissolved in water at a concentration of 0.045%; ANIT (Sigma, St. Louis, MO) was dissolved in Sesame Oil at a concentration of 1%. Following anesthesia with 6% chloral hydrate by intraperitoneal injection, 3ml arterial blood was collected in coagulant test tube. Blood serum was obtained after 3250g centrifugation and stored at -20°C until testing.

After washing with normal saline, the whole hepatic tissue was divided into two parts: one was sheared and stored at -80°C, the other was fixed in 10% formalin for 48h and then...
dehydrated, followed by imbedding in paraffin and slicing. Following normal dehydration, lucidification and mounting the slides were analysed under microscope (model – here the model should have been entered. Like CX51 or whatever was used) (Olympus, Tokyo, Japan) as specified in our previous studies [19, 20, 21, 22] and digital images were captured with camera (enter model, type or name of the camera) (Olympus, Tokyo, Japan).

Response: We corrected the mistakes in related sentences accordingly.

3. (Liver function tests) → Serum markers of liver damage
Liver function tests should be replaced in the Methods and in the Results part as these biomarkers give information about the extent of damage or injury in the liver, but not about liver function. Liver function tests comprise galactose elimination capacity, lidocain metabolism, indocyanine green clearance or $^{14}$C exhalation. Although these terms were mixed in normal clinical practice, a scientific publication should be precise.

Response: We respected the reviewer’s advice and changed Liver function tests into Serum markers of liver damage. However, it should be pointed out that Liver function tests refer to those biomarkers mentioned in our article, not galactose elimination capacity, lidocain metabolism, indocyanine green clearance or $^{14}$C exhalation. The explanation of Liver function tests can be found in Wikipedia. http://en.wikipedia.org/wiki/Liver_function_tests

4. From Results part, pp. 12-15:
The liver tissue showed plenty of punctiform or focused necrotic zones and proliferation of Kupffer cells and bile duct epithelial cells all over the visual field. The bile duct exhibited a constrictive canal with necrotic cells and bile thrombus. Many necrotic hepatocytes and infiltrated neutrophils were present around the bile duct. At 72h, the pathological changes were recovered slightly but still featured with necrotic regions and neutrophil infiltration. In the corilagin group the pathological changes were significantly lower than in the model group. The manifestations in the UDCA group were a little severer than in the corilagin group while in the dexamethasone group the pathological impairment appeared more aggravated. As shown in Fig 5, the level of MPO and MDA significantly raised in the model group (P<0.01). At all time points the levels of the two enzymes were notably down-regulated by corilagin treatment and even lower than the normal level. As control, UDCA had no similar effect as corilagin, and dexamethasone only had an effect on MDA at 72h (P<0.05). In contrast, the levels of SOD and NO in tissue decreased notably after ANIT stimulation (P<0.05 or P<0.01, respectively), while in the corilagin group the levels of SOD and NO were remarkably elevated (P<0.05 or P<0.01, respectively). UDCA had not the same effects except for SOD at 48h. Dexamethasone had an up-regulating effect on SOD at 48h, and on NO at 24h and 72h (P<0.05 or P<0.01, respectively).
Response: We modified the expression accordingly.

5. From Discussion part, pp. 15-19:
In current clinical practice, initial assessment of hepatobiliary diseases is accomplished by measuring serum concentrations of bile acids and bilirubin as well as serum activities of liver-associated enzymes which reveal information about the state of liver. Our previous study showed that the indexes of liver damage and pathological changes start to rise at 24h after ANIT treatment, reach a maximum at 48h and trend to restore at 72h [18]. The data of the present study imply that the most effective change after corilagin treatment refers to bilirubin, both total and direct. Compared to UDCA and dexamethasone, corilagin showed a protective effect on the elevation of serum bilirubin after hepatic injury. However, there is no obvious evidence of corilagin to alleviate aminotransferases, which indicates that corilagin has a stronger effect on relieving bile or bile duct disorders.
ANIT-evoked hepatotoxins cause severe neutrophilic inflammation around portal tracts and bile ducts and induce significant inflammatory reactions in the liver, which suggests that the inflammation plays a central role in cholestatic hepatitis. Nuclear factor-kappa B (NF-κB) is a pivotal factor that transfers inflammatory signals from cytoplasm into the nucleus and induces a series of inflammatory responses in the cell [31, 32]. In our present study, it was found that NF-κB was notably suppressed in the corilagin group, which demonstrates that corilagin has the efficacy to control inflammatory injuries during cholestasis in the liver. In conclusion, we found that corilagin has the capability to ensure hepatic protection, to block NF-κB pathway, to provide anti-oxidative effects and to improve hepatic circulation in experimental intrahepatic cholestasis, which suggests that corilagin is a putative medication to treat cholestasis. It is shown that corilagin has the potential to cure inflammation-related and oxidation-related diseases. The further proceeding might be to investigate how corilagin interacts with the inflammatory and oxidative signal pathways in cell and animal models.

Response: We changed the expression as the reviewer’s suggestion.

Editorial Board Comments:
Please discuss current findings on the use of UDCA in cholestatic diseases (PMID: 22948036; Reviews in PMID: 22948005 and PMID: 21304237).

Response: We discussed the findings in the text.

Maybe a native speaker could re-check the manuscript.

Response: We re-check the manuscript carefully and changed some sentences in the article.

Please also ensure that your revised manuscript conforms to the journal style.
Response: We rectified the article format as the journal requirement.

If you have any further requirement, please let us know. Thank you very much.

Lei Zhao