RESEARCH LETTER

Human Colonic Organ Culture Reveals XPO1-Independent Ability of Salvianolic Acid to Reduce Ulcerative Colitis–Related Inflammation

Ulcerative colitis (UC) is a chronic and idiopathic disease characterized by ulceration of the colon and/or rectum, with severe loss of mucosal and submucosal tissue.[1] Although it is known that genetics, the microbiome composition, and the immune system are the main factors involved in its development,[2] the exact molecular mechanisms leading to UC are still unknown. Bowel inflammation is the main cause of mucosal destruction in UC, so current therapeutic options are mostly based on anti-inflammatory treatments such as corticosteroids, which have serious side effects.[3] For this reason, an increasing amount of research is being focused on the discovery of new therapies for this inflammatory disease, such as Janus kinase inhibitors which dampen inflammatory pathways[4] or natural compounds from traditional Chinese medicine that are able to alleviate disease-related symptoms.[5]

Salvianolic acid (SAC) is widely used in traditional Chinese medicine and its ability to reduce UC–related inflammation has been previously tested using *in vivo* models. Specifically, SAC A was evaluated in a rat model of acute colitis, demonstrating a reduction in the levels of inflammatory cytokines interleukin (IL) 1β and IL6, while concurrently increasing the expression of genes involved in tight junction.[6] In addition, SAC B has been shown to decrease *Il1β* RNA expression in a recurrent colitis mice model.[7] Interestingly, SAC has been recently identified as a specific inhibitor of the m^{6}A reader protein YTH domain familiy member 1 (YTHDF1),[8] linking the anti-inflammatory ability of this natural compound with epitranscriptomic modifications.

The m^{6}A-XPO1-NFκB pathway has been recently identified to be involved in intestinal inflammation in the context of celiac disease (CeD), another inflammatory disorder of the gastrointestinal tract. In the context of CeD, YTHDF1 has the ability to bind the methylated 5′ untranslated region of exportin 1 gene (*XPO1*), inducing protein translation and activating nuclear factor kappa B (NFκB) and downstream cytokine expression. Moreover, SAC A and C (YTHDF1 inhibitors, referred to as Y20 and Y22, respectively) have been found to inhibit this pathway, ameliorating gluten-induced intestinal inflammation in a mouse model of gluten exposure and a human organ culture system developed from CeD patients' biopsies.[9]

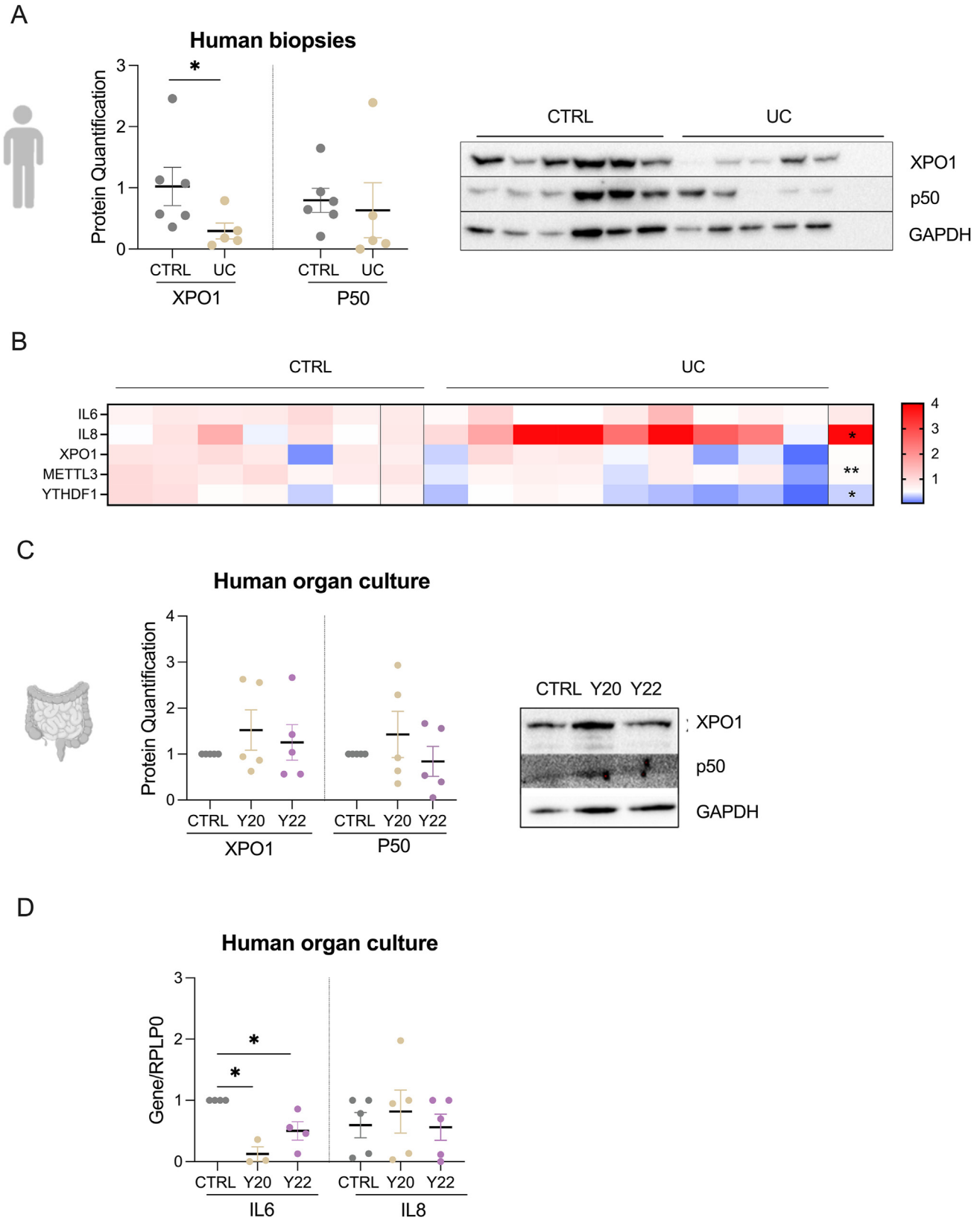
With this background, the aim of this study was to evaluate the alterations of the m^{6}A-XPO1-NFkB pathway in intestinal biopsies from UC patients and to test the ability of Y20 and Y22 to reduce UC–related intestinal inflammation using an organ culture model derived from UC patient biopsies.

Analysis of the levels of the key components of the m^{6}A-XPO1-NFκB pathway using colon biopsies from both, control subjects and patients diagnosed with UC, showed that XPO1 protein levels decreased significantly, and there is no significant alteration on NFκB-p50 protein amounts (Figure A). Even if the expression of *IL6* and *IL8* cytokines, related with intestinal inflammation and located downstream NFκB, is increased in UC patients (Figure B), genes involved in m^{6}A methylation are downregulated in UC patients (Figure B) showing an opposite pattern to that seen in CeD. These results suggest that the induction of inflammation in UC is not mediated by the m^{6}A-XPO1-NFkB pathway previously described in CeD.

In order to analyze if SAC is able to also revert UC–related inflammation in human samples, we used our recently developed organ culture model using biopsies from UC patients.[8] Concordant with the results observed in colon biopsy samples, SAC did not induce changes at XPO1 protein level. Additionally, no significant reduction was observed in p50 protein amounts, confirming the absence of implication of the m^{6}A-XPO1-NFκB axis in UC.

In accordance with these results, there is no decrease in the levels of *IL8* after SAC treatment (Figure D). However, and as previously observed in mouse models, *IL6* levels were significantly reduced in our intestinal biopsy organ cultures after the incubation with SAC (Figure D), confirming the ability of SAC to reduce colitis-related inflammation.

The results from this study discard the involvement of CeD–related m^{6}A-XPO1-NFkB pathway in the development of UC–related inflammation. Nonetheless, our data show the ability of Y20 and Y22 inhibitors to reduce *IL6* levels in the UC–derived intestinal epithelia *ex vivo*. Thus, further investigation evaluating SAC as a therapeutic adjuvant for the reduction of inflammation in the intestine of UC patients opens exciting avenues for future research, offering the prospect of more effective and targeted treatments


A
Human biopsies
Protein Quantification
CTRL
UC
XPO1
P50
GAPDH
B
IL6
IL8
XPO1
METTL3
YTHDF1
C
Human organ culture
Y20
Y22
D
Gene/RPLP0
IL6
IL8

for this intestinal inflammatory condition, which will ultimately improve patient outcomes.

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Supplementary Materials

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Abbreviations used in this paper: **CeD, celiac disease; IL, interleukin; NFkB, nuclear factor kappa b; SAC, salvianolic acid; UC, ulcerative colitis; XPO1, exportin 1; *XPO1*, exportin 1 gene; YTHDF1, YTH domain family member 1**

Most current article

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Ethical Statement:
This study was approved by the Basque Country Clinical Research Ethics Board (CEIC-E ref. PI2019133).

Data Transparency Statement:
All the data supporting the findings of this study are available within the article and/or its supplementary materials.

Reporting Guidelines:
Not applicable for this article type.

Figure. YTHDF1 inhibitors reduce *IL6* levels *ex vivo* by a m^{6}A-XPO1-NFκB independent pathway. (A) XPO1 and p50 protein levels were quantified by western blot using GAPDH as endogenous control in human intestinal biopsies from controls (CTRL, n = 6) and ulcerative colitis patients (UC, n = 6) (left). Immunoblot image of 6 colon samples per condition (right). (B) Heatmap showing the RNA expression levels of *IL6*, *IL8, XPO1*, *METTL3*, and *YTHDF1* genes. Expression was quantified by RT-qPCR in human colonic biopsies from CTRL (n = 6) and UC (n = 9) patients. Individual and mean values (last row of each group) are represented in the heatmap. (C and D) Human colonic biopsies from UC patients (n = 5) were incubated *ex vivo* without (CTRL) or with YTDHF1 inhibitors (Y20 and Y22). (C) XPO1 and p50 protein levels quantified by western blot using GAPDH as endogenous control (left) and representative immunoblot (right). (D) *IL6* and *IL8* RNA expression levels quantified by RT-qPCR. Data are represented as the mean and standard error. All *P* values were determined by Mann-Whitney test, **P* < 0,5, ***P* < .01. GAPDH, glyceraldehyde 3-phosphate dehydrogenase; METTL3, m*ethyltransferase 3*; RT-qPCR, reverse transcription quantitative polimerase chain reaction.