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RAPID COMMUNICATION

Curcumin modulates oxidative stress to inhibit pyroptosis and improve the inflammatory microenvironment to treat endometriosis

Endometriosis (EM) is a common disease that affects approximately 10%–15% of women of childbearing age. The pathogenesis of EM is unclear, but studies have shown a strong association between EM and inflammation, as well as oxidative stress.[1] Pyroptosis is also called inflammatory cell death. When pyroptosis occurs, it activates a strong inflammatory response. Pyroptosis is associated with oxidative stress, and ROS act as intermediate triggers to activate pyroptosis, which can exacerbate the subsequent inflammatory cascade.[2] However, it is unknown whether pyroptosis is regulated by oxidative stress during EM. Curcumin (CUR) is one of the world's best-selling natural food colorants and has a variety of pharmacological activities, including anti-inflammatory and antioxidant activities, and the pharmacological effects of CUR are associated with the mechanisms of pyroptosis and EM.[3,4] In the present study, we showed that CUR ameliorated the inflammatory environment of EM by modulating oxidative stress and inhibiting GSDMD-mediated pyroptosis involving the NLRP3 inflammasome. These results further confirm the inflammation and oxidative stress theory of EM and provide new drug options for EM treatment.

First, it was determined that pyroptosis was associated with EM and could interfere with the inflammatory environment. The expression levels of pyroptosis markers and mature inflammatory factors in the ectopic lesions of EM patients were measured by immunohistochemistry, and it was found that pyroptotic markers such as *NLRP3*, *CASPASE-1*, and *GSDMD* were significantly elevated in ectopic lesions compared with normal endothelial tissue, suggesting pyroptosis in ectopic lesions (Fig. 1A). The inflammatory molecules LL-1β and IL-18 were also significantly elevated (Fig. 1B, C). The ELISA results showed that the levels of IL-1β and IL-18 in the peritoneal fluid of EM patients were significantly increased compared with those in control peritoneal fluid; together with the histochemical results, these findings suggested the presence of an abnormal inflammatory environment in EM. These results confirmed that the focal tissues of EM patients underwent pyroptosis and that pyroptosis led to an altered inflammatory environment in the foci and peritoneal fluid. Using network pharmacology and molecular docking techniques, we found that CUR could regulate pyroptosis through *NOD*-like receptors and could dock well with *NLRP3*, *CASPASE-1*, and *GSDMD*, which are key pyroptosis molecules. The computer simulation demonstrated that CUR could treat EM by regulating pyroptosis (Fig. S1, 2).

To clarify whether CUR could resist pyroptosis-related oxidative stress, we used CUR and ATP to treat LPS-induced pyroptotic cells, and the CUR concentration was set at 10 μmol (Fig. S3). After CUR treatment, cellular reactive oxygen species (ROS) levels decreased, malondialdehyde (MDA) levels decreased ($P < 0.05$), and superoxide dismutase (SOD) levels increased ($P < 0.05$), while the effects of the antioxidant NAC were consistent with those of CUR, indicating that they have the same antioxidant capacity. After the reverse addition of the pro-oxidant $COCL_2$ in the CUR group, ROS levels increased, MDA levels increased ($P < 0.05$), SOD levels decreased ($P < 0.05$), and the antioxidant capacity of CUR was offset (Fig. 1D–F). The difference in pyroptosis in each group was observed by scanning electron microscopy, which showed that cells in the LPS-ATP group were significantly swollen compared with those in the CON group, and a large number of pores appeared on the cell membrane. Moreover, pyroptosis was significantly inhibited in the CUR

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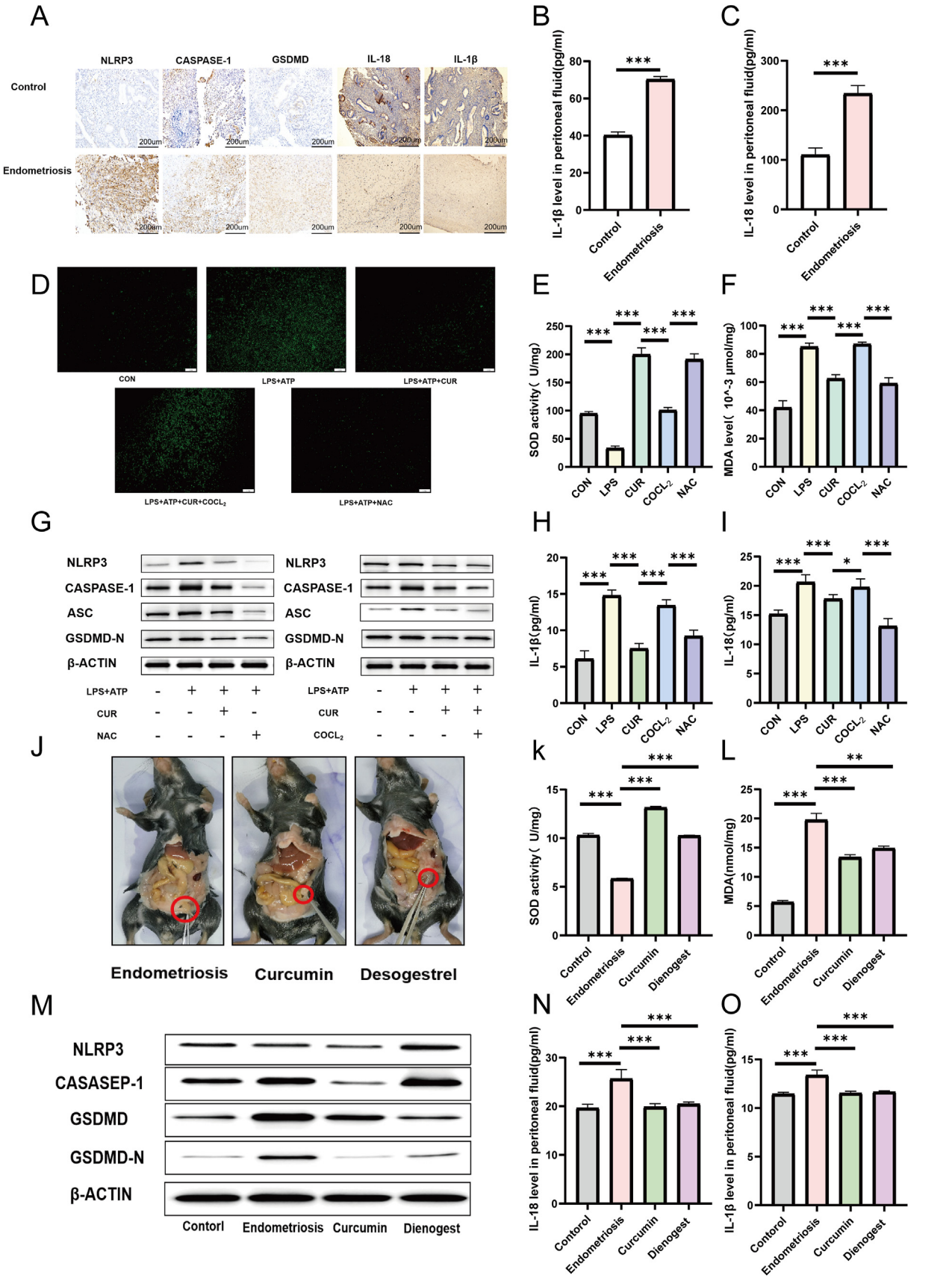
A
NLRP3
CASPASE-1
GSDMD
IL-18
IL-1β
Control
Endometriosis
200um
B
IL-1β level in peritoneal fluid(pg/ml)
***
Control
Endometriosis
C
IL-18 level in peritoneal fluid(pg/ml)
***
Control
Endometriosis
D
CON
LPS+ATP
LPS+ATP+CUR
LPS+ATP+CUR+COCL2
LPS+ATP+NAC
E
SOD activity(U/mg)
CON
LPS
CUR
COCL2
NAC
F
MDA level(10^-3 μmol/mg)
G
NLRP3
CASPASE-1
ASC
GSDMD-N
β-ACTIN
LPS+ATP
CUR
NAC
COCL2
H
IL-1β(pg/ml)
I
IL-18(pg/ml)
J
Endometriosis
Curcumin
Desogestrel
k
SOD activity(U/mg)
Control
Endometriosis
Curcumin
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L
MDA(nmol/mg)
M
NLRP3
CASASEP-1
GSDMD
GSDMD-N
β-ACTIN
Contorl
Endometriosis
Curcumin
Dienogest
N
IL-18 level in peritoneal fluid(pg/ml)
O
IL-1β level in peritoneal fluid(pg/ml)

and NAC groups, with less swelling and fewer pores on the cell membrane, which proved that both treatments could inhibit the occurrence of pyroptosis. In the CUR group, a large number of pores were found on the cells after the addition of $COCL_2$, indicating that $COCL_2$ could counteract the antioxidant effect of CUR, causing the cells to undergo pyroptosis (Fig. S4).

The mRNA and protein expression levels of *NLRP3*, *CASPASE-1*, *ASC*, and *GSDMD-N* were significantly decreased in the CUR group compared with the LPS-ATP group ($P < 0.05$). NAC also decreased the protein and mRNA levels of these genes compared with those in the LPS-ATP group ($P < 0.05$), while *GSDMD-N* was increased after the reverse addition of $COCL_2$ in the CUR and $COCL_2$ groups ($P < 0.05$) (Fig. 1G; Fig. S5, S6). The ELISA results showed that the levels of IL-1β and IL-18 in the culture supernatant of the CUR group were significantly decreased compared with those of the LPS-ATP group ($P < 0.05$), suggesting that CUR could inhibit LPS-ATP-induced pyroptosis. The NAC group did not differ significantly from the CUR group in terms of the inhibition of oxidative stress or anti-scorching effects. To further clarify that the inhibitory effect of CUR on pyroptosis was mediated by its antioxidant effect, $COCL_2$ was added after CUR treatment. The results showed that the inhibitory effect of CUR on pyroptosis was reversed and that IL-18 and IL-1β levels increased ($P < 0.05$) (Fig. 1H, I). Through these experiments, we demonstrated that CUR could inhibit the oxidative stress state induced by pyroptosis and reduce the level of pyroptosis.

An EM mouse model was successfully constructed as described in our previous study.[5] According to previous research by our team, the daily dose of curcumin was 200 mg/kg for each mouse. Adhesion scores were significantly higher in the EM model group than in the control group ($P < 0.05$). Adhesions were reduced in the CUR and dienogest groups compared with the EM group ($P < 0.05$), and there was no significant difference in the degree of adhesions between the CUR and dienogest groups. Compared with that in the EM model group, the number of lesions and lesion volume were significantly different between the CUR and dienogest groups ($P < 0.05$), and there was no significant difference between the dienogest and CUR groups, which indicated that CUR had a clear therapeutic effect on EM (Fig. 1; Fig. S7). To determine the therapeutic effect due to the inhibition of pyroptosis mediated by the antioxidant effect of CUR, SOD, and MDA assays were performed on ectopic lesions from EM mice to determine the oxidative stress levels of the lesions. Compared with those in the control group, SOD levels in the EM model group decreased significantly ($P < 0.05$), and MDA levels increased significantly ($P < 0.05$), indicating significant oxidative stress and a significant decrease in antioxidant capacity in ectopic lesions. Compared with the EM model group, the CUR group showed a significant increase in SOD levels ($P < 0.05$) and a decrease in MDA levels ($P < 0.05$), indicating that CUR could reduce the level of oxidative stress and enhance the antioxidant capacity of the lesions. There was a significant increase in SOD levels ($P < 0.05$) and a decrease in MDA levels ($P < 0.05$) in the dienogest group compared with the EM model group, indicating that the antioxidant capacity of the lesions was increased in the dienogest group (Fig. 1K, L). The WB results showed that compared with those in normal endometrial tissue from control mice, the protein levels of *NLRP3*, *GSDMD*, *GSDMD-N*, and *CASPASE-1* in the lesions of EM model mice were significantly increased, indicating the presence of pyroptosis in EM mice. Compared with that in the EM model group, CUR significantly inhibited the protein expression of *NLRP3*, *GSDMD*, *GSDMD-N*, and *CASPASE-1* in the ectopic lesions of mice and reduced the occurrence of pyroptosis. The levels of *NLRP3* and *caspase-1* in the dienogest group were not significantly different from those in the other groups, but the two key pyroptosis indicators *GSDMD* and *GSDMD-N* were significantly decreased, which may be due to effects on the *GSDMD* protein through other pyroptosis pathways (Fig. 1M; Fig. S8). The PCR results were consistent with the WB results (Fig. S9). The results indicated that compared with those in the control group, the levels of IL-1β and IL-18 in abdominal fluid in the EM model group were significantly increased ($P < 0.05$). Compared with those in the EM model group, the levels of IL-1β and IL-18 in peritoneal fluid in the CUR group and the dienogest group were significantly decreased ($P < 0.05$), and there was no significant difference between the CUR group and the dienogest group (Fig. 1N, O).

In this study, we found that pyroptosis was involved in EM disease progression and was closely related to EM-

Figure 1 Curcumin (CUR) modulates oxidative stress to inhibit pyroptosis and improve the inflammatory microenvironment to treat endometriosis. **(A)** Immunohistochemical analysis of *NLRP3*, *caspase-1*, *GSDMD*, IL-1β, and IL-18 expression in lesions. **(B, C)** ELISA analysis of IL-1β and IL-18 levels in human peritoneal fluid (***$P < 0.001$). **(D–F)** ROS, SOD, and MDA levels in endometrial stromal cells (***$P < 0.001$). **(G)** Differences in the levels of the pyroptosis proteins *NLRP3*, *CASPASE-1*, *ASC*, and *GSDMD* in endometrial stromal cells. **(H, I)** Effects of CUR on IL-1β and IL-18 (***$P < 0.001$, *$P < 0.05$). **(J)** Endometriosis lesions in model mice, CUR-group mice, and denogestrel mice. The red clipping head points to the lesion. **(K, L)** Analysis of SOD and MDA levels in mouse lesions (***$P < 0.001$, **$P < 0.01$). **(M)** Differences in the levels of the pyroptosis proteins *NLRP3*, *CASPASE-1*, *GSDMD*, and *GSDMD-N* in model mice, CUR-group mice, and denogestrel mice. **(N, O)** ELISA analysis of IL-1β and IL-18 in mouse peritoneal fluid (***$P < 0.001$).

associated oxidative stress. CUR can treat EM by inhibiting oxidative stress and reducing pyroptosis, thus regulating the level of inflammation in the disease state. These findings warrant further evaluation in clinical studies, and the interaction between pyroptosis and oxidative stress needs further evaluation.

Author contributions

DJ, MSS, and WKL made equal contributions. DJ, MSS, and WKL designed and completed the experiments, completed the data analysis, and wrote the manuscript. CW and SS participated in the collection and analysis of clinical specimens. NZX participated in the supplementary experiments. WXQ and YCQ provided ideas for the experimental design and modified the manuscript to ensure the integrity of the entire experimental design. All authors participated in discussions of the experimental results and the final manuscript. We confirmed that all authors meet the ICMJE and agreed to submit the manuscript.

Conflict of interests

The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.06.022.

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