Berberine Induces Dendritic Cell Apoptosis and Has Therapeutic Potential for Rheumatoid Arthritis

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Objective. To investigate the effects of berberine on dendritic cell (DC) apoptosis and its potential as a therapeutic agent in rheumatoid arthritis (RA).

Methods. Bone marrow (BM)–derived myeloid DCs (MDCs) and plasmacytoid DCs (PDCs) were generated by culturing BM cells with granulocyte–macrophage colony-stimulating factor/interleukin-4 or flt3L, respectively. Splenic DCs, T cells, and B cells were purified using a magnetic-activated cell sorting system. In vitro apoptosis was assessed by annexin V/propidium iodide or Hoechst 33258 staining. The in vivo effects of berberine were examined in mice with collagen-induced arthritis (CIA). Immune responses against type II collagen (CII) were determined by assaying serum antibody levels, lymphocyte proliferation, and cytokine production. The proportions of DCs and apoptosis of different immune cell subsets in spleens and lymph nodes were analyzed by flow cytometry and immunohistochemistry after subset-specific surface marker labeling and TUNEL staining.

Results. Exposure of MDCs to berberine during BM cell differentiation reduced cell recovery by inducing apoptosis. Sensitivity to berberine-induced apoptosis was acquired starting on day 3 of DC differentiation, and mature DCs were more sensitive to berberine than immature DCs. Murine peritoneal macrophages, RAW 264.7 cells, and Jurkat cells were insensitive to berberine-induced apoptosis. Splenic DCs were more sensitive to berberine than T and B cells. Susceptibility of PDCs to berberine-induced apoptosis was similar to that of MDCs. In mice with CIA, berberine treatment ameliorated arthritis, suppressed CII-specific immune responses, and selectively increased the incidence of apoptosis in DCs within spleens and lymph nodes.

Conclusion. These findings show that berberine selectively induces apoptosis in DCs. Berberine may thus represent a novel therapeutic agent for RA.
agents such as dexamethasone (DEX), cyclosporin A, rapamycin, and 1,25(OH)₂D₃, whose immunosuppressive effects on T cell activation have been well documented, have been found to suppress key functions of DCs or to evoke DC apoptosis (17–20). Therefore, DCs might represent a novel and important target for immunosuppressive therapy in RA.

Although immunosuppressive drugs have greatly improved the treatment of RA, their clinical use is limited due to potentially serious toxicity. Thus, there is still a need for alternative therapeutic agents. Chinese herbal medicine has been practiced for thousands of years and provides a vast source of pharmaceutical materials. Natural compounds purified from herbal medicines that have known indications for inflammatory conditions often have a low toxicity profile (21,22). Thus, exploring new herbal medicine–based immunosuppressive agents that selectively target DCs may contribute to the development of novel pharmaceutical agents for RA.

Berberine is an isoquinoline alkaloid that is present in numerous plants of the genera Berberis and Coptis (23). These herbs are commonly used in Chinese traditional medicine for the treatment of “damp-heat” syndromes. Several traditional remedies that have been used in the treatment of rheumatic diseases are based on extracts of plants that belong to the genus Berberis (24). As one of the main active ingredients of these extracts, berberine has been demonstrated to exert significant antiinflammatory and immunosuppressive properties (24–27). However, there have been few reports concerning its effect on DCs. In the present study, we first conducted a series of in vitro experiments to investigate the direct effects of berberine on DC maturation and survival. Upon observing that berberine selectively induces apoptosis in DCs, we subsequently used the collagen-induced arthritis (CIA) mouse model to determine whether berberine could also induce DC apoptosis in vivo and whether it has antiarthritic potential.

MATERIALS AND METHODS

Reagents. Berberine, lipopolysaccharide (LPS), and DEX were obtained from Sigma. Annexin V–fluorescein isothiocyanate (FITC), propidium iodide (PI), and 7-aminoactinomycin D (7-AAD) were purchased from BD PharMingen. 7-aminoactinomycin D (7-AAD) were purchased from BD PharMingen. 7-aminoactinomycin D (7-AAD) were purchased from BD PharMingen.

Mice. C57BL/6 and DBA/1 mice (6–8 weeks old) were purchased from Shanghai Laboratory Animal Company. All animal procedures were approved by the animal care committee of the Second Military Medical University in accordance with institutional and Chinese government guidelines for animal experiments.

Cell preparation and culture. All cells were cultured in RPMI 1640 medium (Sigma) supplemented with 10% fetal bovine serum (Gibco), 2 mM l-glutamine, 100 units/ml penicillin, and 100 µg/ml streptomycin, at 37°C in a 5% CO₂ atmosphere. RAW 264.7 and Jurkat cells were obtained from Shanghai Institute of Cell Biology and Biochemistry. BM cells were prepared by flushing the femurs of C57BL/6 mice, filtering through a 70-µm nylon cell strainer (BD Labware), and depleting red blood cells by 5-minute incubation with ammonium chloride (0.8% [weight/volume]). BM-derived MDCs were generated by culturing BM cells with 10 ng/ml of recombinant mouse granulocyte–macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4) (both from R&D Systems) for 6 days as previously described (28). BM-derived PDCs were generated by culturing BM cells with 100 ng/ml of recombinant human flt3L (PeproTech) for 10 days as previously described (29,30). Peritoneal macrophages were obtained by pelting cells lavaged from the mouse peritoneal cavity in 24-well flat-bottomed plates and removing nonadherent cells after 3 hours of culture (31).

Spleen and lymph node (LN) single-cell suspensions were prepared by digesting disrupted spleen or inguinal LN tissue with collagenase D (1 mg/ml) and DNase I (0.1 mg/ml) (both from Roche Diagnostics) for 30 minutes at 37°C, and further dissociating the cells in Ca²⁺-free Hanks' balanced salt solution in the presence of 5 mM EDTA for 5 minutes at 37°C. Single-cell suspensions were passed through a cell strainer (70 µm), and red blood cells were lysed using ammonium chloride (0.8% [w/v]). Splenic DC, B cell, and T cell subsets were purified by sequential positive selection of CD11c⁺ cells, followed by CD19⁺ cell selection from the CD11c-depleted fraction and CD3⁺ cell selection from the CD11c- and CD19-depleted cells, using magnetic-activated cell sorting (MACS) beads and MS columns according to the instructions of the manufacturer (Milteny Biotech). Flow cytometric analysis showed that the DC fraction contained >90% CD11c⁺MHCIId⁺ (class II major histocompatibility complex) cells, and the B cell and T cell fractions contained >95% CD19⁺CD3⁻ and CD19⁻CD3⁺ cells, respectively.

Analysis of cell surface marker expression. Fluorescence-conjugated monoclonal antibodies (mAb) recognizing CD11c, I-A/I-E, CD40, CD80, CD86, CD11b, B220, CD3, CD19, and the respective isotype controls were purchased from BD PharMingen. The F4/80 (C:E/A3-1) mAb was obtained from Caltag. Cells were blocked with FcBlock (BD PharMingen) and stained with specific antibodies or isotype controls as described previously (32). PI or 7-AAD staining was used to detect dead cells. Stained cells were analyzed using a FACSCalibur, and data were analyzed with the CellQuest software package (Becton Dickinson). During analysis, debris and dead cells (PI⁺ or 7-AAD⁺) were excluded through gating, except during the apoptosis analysis of annexin V–stained samples, when only debris was excluded.

Apoptosis assay. Cells were stained with annexin V–FITC and PI to detect early apoptotic cells (annexin V positive, PI negative) and late apoptotic cells (annexin V positive, PI positive) by flow cytometry. For analysis of apoptosis among different immune cell subsets, cells were initially labeled with various combinations of mAb recognizing subset-
specific surface markers, and then were stained with annexin V–FITC or FITC-conjugated TUNEL reaction mixture provided with the ApopTag Plus Fluorescein In Situ Apoptosis Detection kit, according to the instructions of the manufacturer (Chemicon International). The apoptotic cells (annexin V or TUNEL positive) in the different gated subsets were analyzed on annexin V or TUNEL histogram plots. To determine the morphologic nuclear changes, cells were harvested and fixed with 4% paraformaldehyde on ice. Cytospin preparations were made and stained with Hoechst 33258 (Molecular Probes) for 10 minutes at room temperature. Cells were washed 3 times in distilled water, and then mounted with glycerol. Cell morphology was then determined by fluorescence microscopy (Axiovert 200; Zeiss).

**Induction, assessment, and treatment of CIA.** As described previously (33), DBA/1 mice were injected intradermally at the base of the tail with 200 μg of bovine type II collagen (CII; Sigma) emulsified in Freund’s complete adjuvant (Difco). A booster injection of 200 μg CII in phosphate buffered saline (PBS) was administered intraperitoneally 21 days later. Arthritis symptoms were assessed using a previously described scoring system (34). Three groups of mice were used in each experiment: nonarthritic mice (normal control), arthritic mice injected intraperitoneally with 1 mg/kg of berberine every day from day 24 to day 38 postimmunization (CIA–berberine treatment), and arthritic mice injected with PBS instead of berberine (CIA control).

**Spleen and LN cell proliferation assay.** Mice were killed on day 38 postimmunization. Single-cell suspensions made from the spleens or inguinal LNs were plated in 96-well plates at 5 × 10^5 cells/well and stimulated with CII (25 μg/ml) for 3 days. In control wells, ovalbumin was added instead of collagen. The proliferation of cells was examined using a Cell Proliferation bromodeoxyuridine enzyme-linked immunosorbent assay (ELISA) kit (Roche Diagnostics) (35). Proliferation was quantified based on the stimulation index, calculated by dividing the absorbance value in collagen-stimulated cultures by the absorbance value in ovalbumin-stimulated cultures.

**Cytokine assay.** Levels of IL-12p70, interferon-γ (IFN-γ), IL-17, IL-2, and IL-10 in culture supernatants were measured by ELISA, according to the instructions of the manufacturer (R&D Systems).

**Immunohistochemistry and TUNEL staining.** Inguinal LNs and spleens isolated from mice with CIA were quickly...
frozen in OCT medium, cryosectioned in 10-μm sections, and then immediately fixed in ice-cold acetone for 4 minutes. The cryosections were stained with phycoerythrin-conjugated anti-CD11c mAb, after which the TUNEL method was applied in order to identify apoptotic cells using an ApopTag Plus Fluorescein In Situ Apoptosis Detection kit. Fluorescence-labeled LN and spleen tissue were visualized by fluorescence microscopy (Axiovert 200).

**Statistical analysis.** Data are expressed as the mean ± SEM. The statistical significance of differences between groups was determined by Student’s t-test. P values less than 0.05 were considered significant.

**RESULTS**

Addition of berberine to murine BM cultures reduces the viable cell yield by inducing apoptosis and arrests DCs in an immature state. The effects of berberine on DCs were initially investigated in BM cells cultured for 6 days with GM-CSF/IL-4, which generates mainly BM-derived MDCs. Berberine (0–50 μM) was added on day 3, and LPS (100 ng/ml) was added on day 5 to stimulate DC maturation. Addition of berberine markedly reduced the number of viable cells on day 6, as assessed by trypan blue dye exclusion (Figure 1A). Phenotypic analysis demonstrated that >90% of the cells on day 6 expressed CD11c, and LPS stimulation markedly up-regulated the expression of DC maturity markers (class II MHC, CD40, CD80, and CD86) (Figure 1B). Berberine did not influence the proportion of CD11c+ cells, but down-regulated the expression levels of CD80 and CD86 on viable CD11c+ cells and inhibited the release of IL-12p70 in response to stimulation with LPS (Figures 1A and C). To examine whether berberine reduced cell recovery by inducing apoptotic cell death, the extent of apoptosis in the day-6 DCs was further assessed using annexin V/PI staining. A significant degree of apoptosis was detected in the berberine-treated DCs (Figures 1A and D).

Figure 2. Occurrence of berberine-induced apoptosis during differentiation of BMDCs, and difference between mature DCs and immature DCs in sensitivity to berberine. A, Freshly isolated murine BM cells or BMDCs generated from 6-day cultures of BM cells were exposed to 10 μM berberine for 48 hours, and apoptosis was analyzed by annexin V/PI staining. Numbers in the dot plots represent the percentages of annexin V–negative, PI-negative cells, annexin V–positive, PI-positive cells, and annexin V–positive, PI-negative cells. B, Cell morphology was assessed by Hoechst 33258 staining. Viable cells display diffuse fluorescence in the nuclei. Apoptotic cells show concentrated dense granular fluorescence (arrows). Original magnification × 400. C, Berberine (10 μM) was added after culture of BM cells with granulocyte–macrophage colony-stimulating factor and IL-4 for various lengths of time. After 48 hours of incubation with or without berberine, cells were harvested, and apoptosis was analyzed by annexin V/PI staining. D, Mature DCs after LPS stimulation and immature DCs without LPS stimulation were treated with berberine (2–50 μM) for 48 hours or with 50 μM berberine for various lengths of time (3–48 hours), and apoptosis was analyzed by annexin V/PI staining. Values in C and D are the mean ± SEM from triplicate cultures; results are representative of 3 independent experiments. * = P < 0.05 versus untreated controls, by Student’s t-test. See Figure 1 for definitions.
apoptosis, as assessed by annexin V/PI staining (Figure 2A) or Hoechst 33258 staining (Figure 2B). This suggests that the sensitivity of DCs to berberine-induced apoptosis is acquired during differentiation. To examine this possibility, we next performed a daily analysis by adding 10 μM berberine on different days of DC culture, harvesting cells after 48 hours of incubation, and analyzing apoptosis using annexin V/PI staining. Daily analysis demonstrated that berberine sensitivity became apparent between days 3 and 4 of DC differentiation, and that mature DCs stimulated with LPS showed the highest sensitivity to berberine-induced apoptosis (Figure 2C).

We further compared berberine-induced apoptosis in mature DCs and immature DCs by treating day-6 BMDCs stimulated with LPS (mature DCs) and day-6 BMDCs without LPS stimulation (immature DCs) with berberine (0–50 μM) for 48 hours or with 50 μM berberine for different periods of time (3–48 hours), and subsequently assessing apoptosis. The results demonstrated that berberine induced apoptosis in both mature and immature DCs in a dose- and time-dependent manner, and mature DCs were more sensitive to berberine than immature DCs (Figure 2D).

**Berberine selectively induces apoptosis in DCs.** To determine whether berberine selectively targeted DCs, its proapoptotic effects were also tested on other types of immune cells. Incubation with 50 μM berberine for 48 hours did not induce any increase of apoptosis in freshly isolated murine peritoneal macrophages, RAW 264.7 cells, or Jurkat cells, whereas it resulted in a >30% and >50% increase of apoptosis in day-6 immature BMDCs without LPS stimulation and mature BMDCs after LPS stimulation, respectively (Figure 3A). This indicates that peritoneal macrophages, RAW 264.7 cells, and Jurkat cells are not sensitive to berberine-induced apoptosis.

We also investigated the proapoptotic effects of berberine on MACS-purified splenic DCs and T and B cells, by incubating these cells with berberine (0–50 μM) for 18 hours and subsequently analyzing apoptosis. In this set of experiments LPS was not added, and DEX, a well-known inducer of apoptosis in DCs and T and B cells (19,20,36,37), was used as a positive control. We observed that ~18% of splenic DCs, ~23% of T cells, and ~32% of B cells underwent spontaneous apoptosis after 18 hours of culture without any stimulation, which is consistent with previous reports (36–38). Berberine treatment increased the apoptosis of splenic DCs in a dose-dependent manner, while it only slightly increased the apoptosis of splenic T cells and B cells (Figure 3B).

In contrast to berberine, DEX induced a greater increase of apoptosis in splenic T and B cells than in DCs.
These results clearly demonstrate that berberine induces apoptosis of splenic DCs with high selectivity, and splenic T and B cells appear to be more sensitive to DEX than to berberine in terms of apoptosis. Similar susceptibility of different DC subsets to berberine-induced apoptosis. To determine whether the proapoptotic effect of berberine is DC subset dependent, we compared the sensitivities of PDCs and MDCs to berberine-induced apoptosis. After culture with flt3L for 10 days without LPS stimulation, ~85% of the differentiated BM cells were CD11c+, and 20% exhibited a PDC phenotype (CD11c+B220+) (Figure 4A), consistent with findings in a previous study (30). The addition of berberine (2–50 μM) on day 8 of BM cell culture resulted in a dose-dependent increase in annexin V–positive cells in both the MDC (CD11c+B220−) and PDC (CD11c+B220+) subsets on day 10 (Figure 4B), demonstrating that berberine could induce apoptosis in both MDCs and PDCs. The addition of DEX (10 nM or 100 nM) on day 8 of BM cell culture resulted in a significantly higher percentage of apoptosis in PDCs than in MDCs on day 10 (Figure 4B), confirming that DEX preferentially induces apoptosis in PDCs, consistent with previous results (39).

We also compared the sensitivities of splenic PDCs and MDCs to berberine- and DEX-induced apoptosis. Incubation of MACS-purified splenic DCs (CD11c+) with berberine (2–50 μM) and DEX (10 nM or 100 nM) for 18 hours resulted in a marked increase in the apoptosis of both PDCs (CD11b−B220+) and MDCs (CD11b+B220−). Berberine showed similar proapoptotic effects on PDCs and MDCs, but DEX had a stronger proapoptotic effect on PDCs (Figures 4C and D).

Attenuation of CIA and suppression of the CIA-specific immune responses in mice with CIA. The finding that berberine selectively induces DC apoptosis prompted us to further investigate its antiinflammatory and immunosuppressive properties in mice with CIA. Daily intraperitoneal injection of berberine (1 mg/kg) beginning on day 24 postimmunization decreased the
incidence and severity of CIA through day 38 (Figure 5A). Histologic analysis of joints from mice with CIA showed severe proliferation of the synovium, with significant infiltration of inflammatory cells, cartilage damage, and bone erosion. Treatment with berberine suppressed these pathologic changes (Figure 5B).

Moreover, anti-CII antibodies were barely detectable in the sera of normal healthy mice, but were markedly induced in mice with CIA. Berberine treatment resulted in a significant decrease in the level of anti-CII IgG. The level of anti-CII IgG2a was more significantly reduced by berberine than was that of IgG1, as demonstrated by the 38% reduction in IgG2a versus the 19% reduction in IgG1 (Figure 5C). Spleen and LN cells obtained from berberine-treated arthritic mice showed a significant reduction in collagen-stimulated proliferation compared with cells obtained from mice with CIA that was not treated. In addition, the production of IFNγ, IL-17, IL-2, and IL-10 by collagen-stimulated splenocytes was reduced by berberine treatment (Figure 5D).
These results demonstrate that both the humoral and cell-mediated immune responses against CII were suppressed by berberine treatment. 

Induction of DC apoptosis in the LNs and spleens of mice with CIA by berberine. Significantly lower frequencies of DCs (identified as CD11c+/MHCI+ cells) were observed in both the spleens and LNs of berberine-treated mice with CIA compared with untreated controls. The lower frequencies of DCs corresponded to significant losses in the total number of DCs detected in the spleens and LNs of berberine-treated mice with CIA (Figure 6A). The incidence of apoptosis in DCs was also significantly higher in both LNs and spleens from berberine-treated arthritic mice than in those from untreated controls, while the incidence of apoptosis in macrophages was not significantly altered by berberine treatment. In addition, a slight but significant increase in the apoptosis of B and T cells was observed in berberine-treated mice, but this was not as pronounced as that in DCs (only a <5% increase in T and B cells, versus a <20% increase in DCs) (Figure 6B). These results suggest that the selective apoptosis of DCs is a major factor underlying the loss of DCs in berberine-treated mice. 

Furthermore, berberine treatment resulted in a significant decrease in the ratio of mature DCs (MHCI(high)CD80(high)) to immature DCs (MHCI(low)CD80(low)) in the spleen (Figure 6C). This result indicates that berberine may preferentially induce apoptosis in mature DCs, which is consistent with our in vitro findings. Finally, immunohistochemical analysis further showed that the loss of DCs correlated with the appearance of apoptotic, CD11c+ cells in both LNs and spleens (Figure 6D).

DISCUSSION

This is the first study to demonstrate that berberine can induce apoptosis in DCs. Murine BM-derived MDCs underwent strong apoptosis in the presence of berberine, in a time- and dose-dependent manner. Interestingly, freshly isolated BM cells were insensitive to berberine, and the sensitivity to berberine-induced apoptosis was acquired during DC differentiation. Further-
more, mature DCs after LPS stimulation were found to be more sensitive to berberine than immature DCs. This observation may explain why the DCs generated from BM cells in the presence of berberine expressed relatively low levels of CD80/CD86 and produced less IL-12 in response to LPS stimulation. These results indicate that berberine can limit the maturation of DCs and shorten their lifespan by selective induction of apoptosis in mature DCs.

The present study also provides evidence that the proapoptotic effect of berberine is specific to DCs. Berberine showed no proapoptotic effects on murine peritoneal macrophages, RAW 264.7 cells, or Jurkat cells even at concentrations up to 50 μM, at which it exerted strong proapoptotic effects on BMDCs. Splenic DCs have higher susceptibility to berberine-induced apoptosis than splenic T and B cells, whereas DEX, a well-known inducer of apoptosis in various types of immune cells (19,20,36,37,39), exhibited stronger proapoptotic effects on splenic T and B cells than on DCs in the same experimental system. However, different DC subsets, namely MDCs and PDCs, in both BM-derived DCs and splenic DCs, showed similar susceptibility to berberine-induced apoptosis, whereas PDCs appeared more sensitive than MDCs to DEX, in accordance with a previous report (39). These results suggest that the proapoptotic effect of berberine is DC subset independent.

Studies have shown that both DC subsets have functional plasticity that allows them to guide appropriate T cell responses depending on the microenvironment of cytokines and/or inflammation mediators (40,41). MDCs produce a large amount of IL-12, thus preferentially inducing Th1 development, and exhibit a primarily proinflammatory effector phenotype during the inflammation process (16). PDCs drive the development of protective antiviral inflammation through their ability to produce type I IFN in response to viral stimulation (42). However, long-term activation of PDCs and secretion of type I IFN in the absence of infection may result in autoimmune diseases such as RA and systemic lupus erythematosus (16,43–45). Due to the crucial roles of MDCs and PDCs in the development of the immune response and the pathogenesis of inflammation, the berberine-induced apoptosis in both MDCs and PDCs is likely to be closely associated with its immunomodulatory and antiinflammatory effects.

Although the present study demonstrates that berberine selectively induces apoptosis in DCs, the precise intracellular mechanisms remain to be elucidated. In a preliminary study, we found that berberine treatment resulted in the generation of reactive oxygen species (ROS) and the loss of mitochondrial membrane potential in DCs (results not shown), suggesting involvement of ROS and mitochondrial depolarization in the berberine-induced apoptosis in DCs. We also observed that berberine treatment resulted in the activation of caspase 3 in DCs. However, we did not detect any effect of a pan-caspase inhibitor on berberine-induced DC apoptosis (results not shown). Further research is needed to explore the precise molecular mechanism(s) underlying berberine-induced apoptosis in DCs.

It has been shown that overexpression of apoptotic inhibitors or conditional deletion of proapoptotic genes in DCs caused defects in DC apoptosis, resulting in DC accumulation in transgenic mice, and through subsequent chronic lymphocyte activation, the development of autoimmunity (8–10). The present study demonstrated that pharmaceutical enhancement of DC apoptosis was therapeutically beneficial in a model of RA. Berberine ameliorated CIA in mice, and simultaneously resulted in a significant loss of DCs and an increase in the apoptosis of DCs within the LNs and spleens of these mice. Furthermore, berberine treatment resulted in a significant decrease in the ratio of mature DCs to immature DCs in the spleen, indicating that it may preferentially induce apoptosis of mature DCs in vivo, which is in accordance with the results of our in vitro experiments. Moreover, berberine treatment inhibited both humoral and cellular immune responses against CII in mice with CIA, further supporting the notion that it may interfere with immune responses at an early stage by specifically targeting DCs. Because mature DCs are major contributors to the immunopathogenic responses in RA, berberine-mediated elimination of mature DCs represents an important mechanism of immunomodulation that may account, at least in part, for the antiarthritic and immunosuppressive effects observed in the mice with CIA. Berberine is currently used in China for treating gastrointestinal diseases, including acute gastroenteritis and bacillary dysentery (46). In most clinical situations, berberine is safe when administered orally at dosages of 200 mg 2–4 times daily. One clinical study demonstrated that daily oral administration of 1.2 gm of berberine to patients for 2 weeks yielded plasma concentrations of berberine of 0.2–0.5 μM (47), which is below the concentration range (2–50 μM) used for induction of DC apoptosis in this study.

However, changing the route of administration can increase the plasma level of berberine, because of its poor intestinal absorption. Indeed, studies have indi-
icated that plasma levels of berberine were in the range of 2–25 μM after intramuscular or intravenous injection in rabbits (48) and beagle dogs (49). We can reasonably infer from these data that the berberine concentrations of 2–50 μM used in this study are attainable in humans following administration via an optimized route. In our mouse CIA experiments, we found that intraperitoneal injection of berberine at 1 mg/kg led to a much better antiarthritic effect than oral administration at 50 mg/kg (results not shown), further suggesting that a change in the route of administration can result in a better systemic antiinflammatory effect. The intraperitoneal 50% lethal dose of berberine, as determined by our group as well as by other investigators (50), was ~50 mg/kg in mice, which is 50 times the effective dose used in our mouse CIA experiments, and we observed no deaths, alterations in body weight, or any other obvious adverse effects in the berberine-treated mice. This indicates that berberine is safe in mice at a dose capable of exerting an in vivo antiinflammatory effect sufficient to bring about amelioration of arthritis in these animals. Of course, further investigations of the safety and efficacy of berberine for RA will be needed before it can be used in clinical trials in humans.

Taken together, the findings of the present study demonstrate that berberine selectively induces apoptosis in DCs, and that it significantly suppresses the immune response against CIA and effectively ameliorates disease in mice with CIA. These results not only elucidate a novel cellular mechanism for the antiinflammatory and immunosuppressive effects of berberine, but also suggest that it has therapeutic potential for RA. Further studies in animals and humans are needed to recognize the full potential of this important component of herbal medicine.

**AUTHOR CONTRIBUTIONS**

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. W. Zhang had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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