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MIF mediates bladder pain, not inflammation, in cyclophosphamide cystitis

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ABSTRACT

Macrophage migration inhibitory factor (MIF), a proinflammatory mediator, is recognized as a player in inflammatory and neuropathic pain. Cyclophosphamide (CYP) results in bladder inflammation and pain and it's a frequently used animal model of interstitial cystitis/bladder pain syndrome (IC/BPS). Because pretreatment with a MIF inhibitor (ISO-1) prevented both CYP-induced bladder pain and inflammation we used genetic MIF knockout (KO) mice to further investigate MIF's role in CYP-induced bladder pain and inflammation. Abdominal mechanical threshold measured bladder pain induced by CYP in wild type (WT) and MIF KO mice at several time points (0–48 h). End-point (48 h) changes in micturition parameters and histological signs of bladder inflammation were also evaluated. Abdominal mechanical hypersensitivity developed within 4 h after CYP injection (and lasted for the entire observation period: 48 h) in WT mice. MIF KO mice, on the other hand, did not develop abdominal mechanical hypersensitivity suggesting that MIF is a pivotal molecule in mediating CYP-induced bladder pain. Both WT and MIF KO mice treated with CYP showed histological signs of marked bladder inflammation and showed a significant decrease in micturition volume and increase in frequency. Since both changes were blocked in MIF KO mice by pretreatment with a MIF inhibitor (ISO-1) it is likely these are non-specific effects of ISO-1. MIF mediates CYP-induced bladder pain but not CYP-induced bladder inflammation. The locus of effect (bladder) or central (spinal) for MIF mediation of bladder pain remains to be determined.

1. Introduction

Macrophage migration inhibitory factor (MIF), a pro-inflammatory molecule, is increasingly recognized as a fundamental constituent of many pathologic conditions, including sepsis, traumatic inflammation and neural degenerative diseases [1]. It is expressed both peripherally and centrally in a variety of cells such as macrophages, colonic and urinary epithelia, spinal cord and brain neurons and glia [2–5]. MIF is released from pre-formed pools upon immune responses and/or nervous system trauma [6]. MIF concentration in cerebrospinal fluid and expression in spinal cord were elevated after foot plantar injection of formalin or sciatic nerve injury [7,8]. In addition, formalin inflammation insult or sciatic nerve injury induced hypersensitivity was blocked by either MIF inhibition or MIF gene deletion [7,8]. Meanwhile, MIF knockout (KO) mice showed lower pain responses to either thermal or mechanical stimulation in inflammation and nerve injury models [8,9]. Therefore, the literature supports a role for MIF in the mediation of pain.

Chemically-induced bladder inflammation is commonly used in animal models of interstitial cystitis/bladder pain syndrome (IC/BPS) where bladder pain is secondary to inflammation [10–12]. Cyclophosphamide (CYP), one of those chemicals, is known to cause hemorrhagic cystitis through its metabolite acrolein [13]. CYP caused dramatic bladder inflammation, increased micturition frequency, decreased micturition volume and most importantly, induced abdominal mechanical hypersensitivity, an indicator of bladder pain [14–16].

Abbreviations: MIF, Macrophage migration inhibitory factor; CYP, cyclophosphamide; KO, knockout; WT, wild type; ERK, extracellular signal regulated kinases 1/2; IC/BPS, interstitial cystitis/bladder pain syndrome

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A MIF inhibitor, ISO-1 ((S, R)-3-(4-hydroxyphenyl)-,5-dihydro-5-isoxazole acetic acid, methyl ester) prevented bladder inflammation, micturition changes and bladder pain after CYP treatment, providing evidence of MIF mediating bladder pain elicited by inflammation [14].

It remains undetermined whether genetic MIF deletion will block CYP-induced bladder inflammation, micturition changes and accompanying bladder pain. The present study measured CYP effects on bladder inflammation, micturition changes, and abdominal mechanical sensitivity in MIF knockout mice compared to wild type (WT) control mice. In addition, since extracellular signal regulated kinases 1/2 (ERK) phosphorylation are activated by MIF signaling [17] and CYP treatment increased pERK activation in the bladder [18], we also examined the effect of MIF deletion on CYP-induced ERK phosphorylation changes in the bladder.

2. Materials and methods

All animal experiments were approved by the Lexington Veterans Affairs Medical Center Institutional Animal Care and Use Committee (VER-11-016-HAF) and performed according to the guidelines of the National Institutes of Health. MIF knockout mice were obtained from Yale University, bred in our animal facility and genotyped to confirm MIF-deletion [9]. Wild type controls (C57/BL6) were purchased from Jackson Laboratory (Jackson Laboratory, Bar Harbor, ME). Experiments were performed on mice from both sexes.

2.1. Drug treatments

Cyclophosphamide (ISOPAC C7397; Sigma; St. Louis, MO) was prepared by dissolving in saline to 20 mg/ml. CYP (300 mg/kg) was intraperitoneally injected once following baseline von Frey testing. ISO-1 (EMD Millpore, Billerica, MA) was dissolved in saline with 20% DMSO to 10 mg/ml. 20 mg/kg ISO-1 was intraperitoneally injected twice, 10 min before and 24 h after CYP injection.

2.2. Abdominal mechanical sensitivity test

Abdominal mechanical sensitivity was tested by two different methods in separate experiments:

1. Percentage of responses to von Frey stimulation [19]. Briefly, von Frey filaments of ascending bending forces (0.008, 0.02, 0.04, 0.07 g; Stoelting, Wood Dale, IL) were pressed to the lower abdominal region in trials of 10. A positive response was defined as any one of three behaviors: (1) licking the abdomen, (2) flinching/jumping, or (3) abdomen withdrawal. Numbers of positive responses were recorded to calculate percentage of responses.
2. 50% mechanical threshold [20–22]. Briefly, mechanical threshold was measured with eight von Frey fibers (0.008, 0.02, 0.07, 0.16, 0.4, 1.0, 2.0 and 6.0 g). Whenever a positive response to a stimulus occurred, the next smaller von Frey filament was applied. Otherwise, the next higher filament was applied.

Abdominal mechanical sensitivity was tested at baseline (prior to treatment) and 4, 24, 48 h after intraperitoneal injection of CYP (300 mg/kg in saline). Control mice were treated as above and received an intraperitoneal injection of saline (vehicle; 200 μl).

2.3. pERK/ERK measurement

A pilot experiment examined the time-course of bladder ERK activation after CYP treatment. The following groups were studied: 2-, 4-, 6-, 8-, 24-, 48-h post-CYP injection. CYP-induced changes in bladder ERK phosphorylation in WT and MIF KO mice at the maximal time point (4 h) were compared to naïve wild type and naïve MIF knockout mice. Mice were anesthetized and bladder tissue was harvested for ERK measurement according to Bio-Plex assay kit instructions (Bio-Rad, Hercules, CA). Briefly, a quarter of bladder tissue was homogenized in cell lysis buffer with PMSF and QG (Bio-Rad, Hercules, CA), centrifuged and lysates were adjusted protein concentration to 200 μg/ml. Samples were loaded onto a Bio-Plex plate and analyzed (Bio-Plex 200 systems, Bio-Rad, Hercules, CA). Concentrations of pERK and ERK were measured and the ratio of pERK/ERK was calculated.

2.4. Voided stain on paper (VSOP)

Micturition volume and frequency were measured in using the Voided Stain on Paper (VSOP) method [23] at the end of the experiment, 48 h after CYP injection, and following the abdominal mechanical behavioral test. Briefly, mice were gavaged with water (50 μl/g body weight) to induce diuresis, and then placed in a plastic enclosure. Mice were free to move and filter paper was placed under the animal to collect urine during a 2-h observation period. Micturition volumes were determined by linear regression using a set of known volumes. Micturition frequency was defined as the number of micturition per hour.

2.5. Histological measurements

Bladders were excised, under isoflurane anesthesia, 48 h after CYP injection immediately following micturition observation. Bladder paraffin sections (5 μm) were processed for routine hematoxylin and eosin (H&E) staining. H&E stained sections were evaluated by a pathologist blinded to the experimental treatment and scored separately for edema and inflammation as described earlier [24]. Briefly, the following scale was used to assess edema or inflammation: 0, No edema/no infiltrating cells; 1, Mild submucosal edema/few inflammatory cells; 2, Moderate edema/moderate number of inflammatory cells; 3, Frank edema, vascular congestion/many inflammatory cells.

2.6. Statistical analyses

All statistical analyses were performed using R [25]. Changes in positive response frequency (%) to von Frey stimulation baseline were evaluated using within subject 2-way (Time × Filament Strength) ANOVA. When the Time factor (baseline, 4, 24, 48 h post CYP injection) was significant, differences at each filament strength were compared at each time point using t-tests with a multiple comparison adjustment (Holm-Sidak). Changes in micturition parameters and bladder histology scores were analyzed using ANOVA followed by Tukey tests.

3. Results

3.1. MIF knockout mice did not develop abdominal mechanical hypersensitivity after CYP

We measured abdominal mechanical hypersensitivity before treatment with CYP (baseline) and at several time points after treatment. Saline injection (vehicle control; i.p.) did not result in abdominal hypersensitivity in wild type mice (Fig. 1A) or MIF KO mice (Fig. 1C). As expected, CYP treatment significantly increased number of responses to von Frey filaments on abdominal/perianal area in wild type mice. At all three time-points (4; 24; 48 h), wild type mice showed significant increases in percentage of responses to all strength of filaments (0.008, 0.02, 0.04 and 0.07 g) compared with baseline ($p < 0.05$, 0.01, 0.001 on all comparison between baseline and after CYP injection by all four filaments) (Fig. 1B).

MIF KO mice treated with an intraperitoneal injection of CYP, however, showed no evidence of abdominal mechanical hypersensitivity at any of the time points tested. For each filament, there was no increase in percent response to abdominal mechanical stimuli at 4, 24 and 48 h after CYP injection compared with baseline ($p > 0.05$ on each filament along each time point) (Fig. 1D).

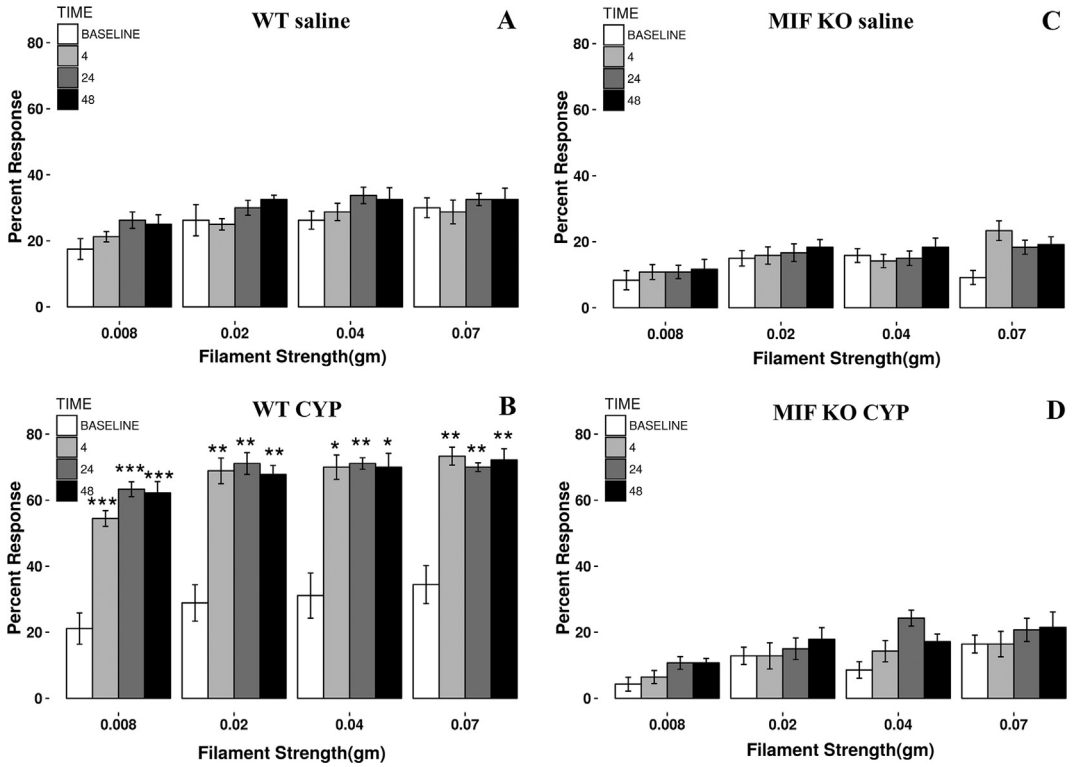

Fig. 1. CYP induced abdominal mechanical hypersensitivity. CYP caused abdominal mechanical hypersensitivity in wild type but not MIF knockout mice on 0.008, 0.02, 0.04 and 0.07 filaments at 4, 24 and 48 h after CYP injection. (A) Intraperitoneal injection of saline did not cause behavioral change in wild type mice (male n = 4, female n = 4). (B) Intraperitoneal injection of CYP significantly increased percent response to von Frey filament in wild type mice (male n = 4, female n = 5). (C) Intraperitoneal injection of saline did not induce percent response change in MIF knockout mice (male n = 7, female n = 5). (D) Intraperitoneal injection of CYP did not cause abdominal mechanical hypersensitivity in MIF knockout mice (male n = 8, female n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ *vs.* baseline.

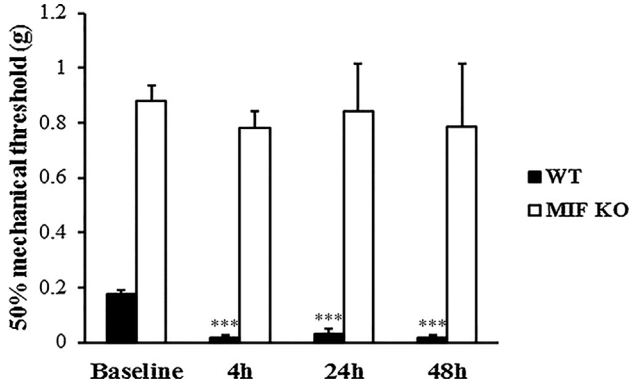

Fig. 2. CYP effects shown by 50% mechanical threshold test. 50% mechanical threshold result showed that CYP significantly decreased abdominal mechanical threshold in wild type (n = 6) but not MIF knockout (n = 6) female mice at 4, 24 and 48 h after CYP injection. $^{***}p < 0.001$ *vs.* baseline.

Similar results were obtained when using 50% mechanical threshold to test abdominal mechanical hypersensitivity. Significant decreases in mechanical threshold were shown in wild type mice at 4, 24 and 48 h after CYP compared with baseline ($p < 0.001$) while MIF knockout mice did not show any behavior changes on any of the time points after CYP (Fig. 2).

It should be noted that the baseline abdominal mechanical hypersensitivity prior to any treatment, measured either as percent response (Fig. 1A) or 50% threshold (Fig. 2) was significantly lower in MIF KO mice compared to WT ($p < 0.001$).

3.2. pERK/ERK changes after CYP injection

A pilot experiment determined the time-course of ERK activation in the bladder of WT male mice. Activation of ERK in the bladder was analyzed at 0, 2, 4, 8, 24 and 48 h after CYP injection by measuring total ERK and phosphorylated ERK (pERK) and displayed as a ratio. pERK/ERK ratio reached peak at 4 h after CYP injection and the pERK/ERK at 4 h was used for comparison between groups (Table 1). Four hours after CYP injection, pERK/ERK ratio was significantly increased in wild type mice compared with naïve group (pERK/ERK = 1.51 ± 0.32; $p < 0.001$). In MIF KO mice, CYP treatment caused an elevation in pERK/ERK (0.60 ± 0.11) in the bladder compared to naïve MIF KO mice but this difference did not reach the level of statistical significance (Fig. 3).

3.3. Micturition changes after CYP injection

Micturition volume and frequency were measured 48 h after saline

Table 1
Bladder pERK/ERK in WT and MIF KO mice (values reported are mean ± SE).

Time (h)	0	2	4	8	24	48
pERK/ERK	0.4 ± 0.07	1.06 ± 0.1***	1.63 ± 0.27***	1.05 ± 0.14**	0.51 ± 0.07	0.31 ± 0.04

Compared to 0 h: $^{**}p < 0.01$, $^{***}p < 0.001$.

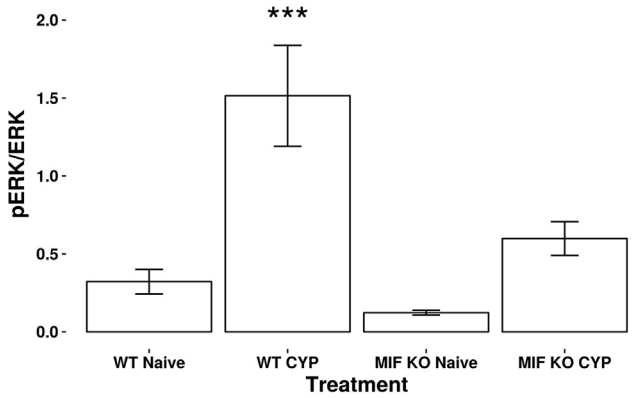

Fig. 3. CYP induced pERK/ERK increase in wild type mice. CYP significantly increased pERK/ERK in wild type but not MIF knockout mice 4 hours after CYP injection. $^{***}p < 0.001$ *vs.* naïve wild type mice.

(vehicle control) or CYP injection. CYP treatment significantly decreased micturition volume (in μl) and increased micturition frequency (volume: 205 ± 29.9, $p < 0.01$; frequency: 2.9 ± 0.5, $p < 0.05$) in wild type mice, compared with saline injected mice (volume: 341 ± 25.3 μl; frequency: 1.6 ± 0.2) (Table 1). Similarly, in MIF knockout mice CYP also significantly decreased micturition volume and (volume: 303 ± 24.1 *vs* 654 ± 42.3 μl, $p < 0.001$) when compared with saline injected mice. Frequency showed a trend towards increasing in CYP-treated MIF knockout mice when compared to saline treatment (1.2 *vs* 0.6 micturition/hour; Table 1) but this difference was not statistically significant. When expressed as percent change from saline treatment, CYP treatment resulted in similar decreases in micturition volume (40% in wild type *vs.* 54% in MIF KO) and percentage of increase in micturition frequency (84% in wild type *vs.* 110% in MIF KO) in both strains.

There were significant differences in micturition parameters between the two strains in the control groups (saline treated). MIF knockout mice with saline injection had significantly higher micturition volume (2-fold larger volumes) and lower micturition frequency when compared to wild type mice ($p < 0.001$) (Table 2).

3.4. Bladder histological changes after CYP injection

H&E stained bladder sections from wild type and MIF knockout mice were examined by a pathologist blinded to the treatment and scored for inflammation as well as edema changes. Intraperitoneal saline injections did not cause any inflammation or edema in either wild type or MIF knockout mice (Fig. 4A). Forty-eight hours after CYP injections, bladders showed increased inflammation (1.6 ± 0.3, $p < 0.001$) and edema (1.8 ± 0.2, $p < 0.001$) in wild type mice (Fig. 4B, Table 3). Genetic MIF deletion did not block bladder damage caused by CYP (Fig. 4C) and there was inflammatory cell infiltration (2.5 ± 0. 2, $p < 0.001$) and tissue edema (2.4 ± 0.2, $p < 0.001$) in the bladders of CYP treated MIF knockout mice (Fig. 4D, Table 2). These changes were significantly different from those seen in MIF knockout mice receiving saline injections.

3.5. MIF inhibitor (ISO-1) pretreatment on MIF knockout mice: effects on CYP-induced micturition and bladder changes

Because we previously reported that ISO-1 (20 mg/kg; MIF inhibitor) prevented CYP-induced bladder inflammation in mice, we tested its effects on MIF KO mice [14].

Pretreatment with ISO-1 prevented CYP-induced changes in micturition volume and frequency in MIF KO mice. Micturition volume (590 ± 85.8 μl) and frequency (1.1 ± 0.2) were similar to that of saline treatment in MIF KO mice (Table 2). There was a significant increase in micturition volume ($p < 0.001$) when compared with CYP treated MIF knockout group. Micturition frequency trended downward but not significantly when compared with wild type mice. ISO-1 alone did not change micturition volume and frequency (volume: 582 ± 49.4; Frequency: 1.1 ± 0.1) (Table 2).

ISO-1 alone did not cause any bladder histological changes (inflammation: 0.3 ± 0.2, edema: 0.2 ± 0.1, Fig. 5A) in MIF KO mice. Pretreatment with ISO-1 blocked bladder inflammation or edema induced by CYP (inflammation: 0.5 ± 0.2, edema: 0.6 ± 0.2, $p < 0.001$, Fig. 5B) (Table 3). No changes in abdominal mechanical sensitivity were seen after ISO-1 only (Fig. 5C) or pretreatment with ISO-1 followed by CYP when compared to baseline (Fig. 5D).

4. Discussion

The present study showed that CYP caused abdominal mechanical hypersensitivity in wild type mice but not in MIF knockout mice, suggesting that MIF is a pivotal molecule in CYP induced bladder pain. The finding that MIF gene deletion blocked CYP induced bladder pain was confirmed using two different methods of assessing abdominal mechanical hypersensitivity, percent response method and 50% mechanical threshold method. This finding agrees well with our recent observations that MIF mediates bladder pain in a model that does not rely on overt bladder inflammation [19,26] and thus suggests that MIF mediates bladder pain in general and not secondary to bladder inflammation.

Bladder nerve grow factor (NGF) and substance P are also reported to be involved in bladder pain [27,28]. The role (if any) of MIF in modulating NGF or Substance P is not known and was not investigated in the present study. In addition, whether MIF modulates bladder hyperalgesia by acting at local (bladder) or central (spinal cord; brain) levels was not investigated in this study and remains an important question. Intrathecal administration of a MIF inhibitor decreased number of paw flinch in formalin test and increased mechanical threshold and thermal paw withdrawal latency [7,8]. Furthermore, spinal MIF mRNA and protein were significantly increased after LPS induced bladder pain, suggesting spinal MIF is playing a role in bladder pain [29]. Therefore, it is possible that MIF may be acting centrally (at the spinal cord level), as well as peripherally, to mediate bladder hyperalgesia.

MIF signaling is strongly dependent on activation of ERK phosphorylation through binding to MIF's receptor CD74 [30]. We observed

Table 2
Micturition in WT and MIF KO mice (values reported are mean ± SE).

	WT saline (N = 10)	WT CYP (N = 10)	MIF KO saline (N = 10)	MIF KO CYP (N = 10)	MIF KO CYP + ISO-1 (N = 6)	MIF KO ISO-1 (N = 6)
Volume (μl)	341 ± 25.3	**205 ± 29.9***	**654 ± 42.3*****	**303 ± 24.1**$^{\#\#\#}$	**590 ± 85.8**†††	582 ± 49.4
Frequency (micturition/hour)	1.6 ± 0.2	**2.9 ± 0.5****	0.6 ± 0.1	1.2 ± 0.2	0.6 ± 0.1	0.6 ± 0.1

Compared to WT saline: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.
Compared to MIF KO saline: $^{\#\#\#}p < 0.001$.
Compared to MIF KO CYP: $^{\dagger\dagger\dagger}p < 0.001$.

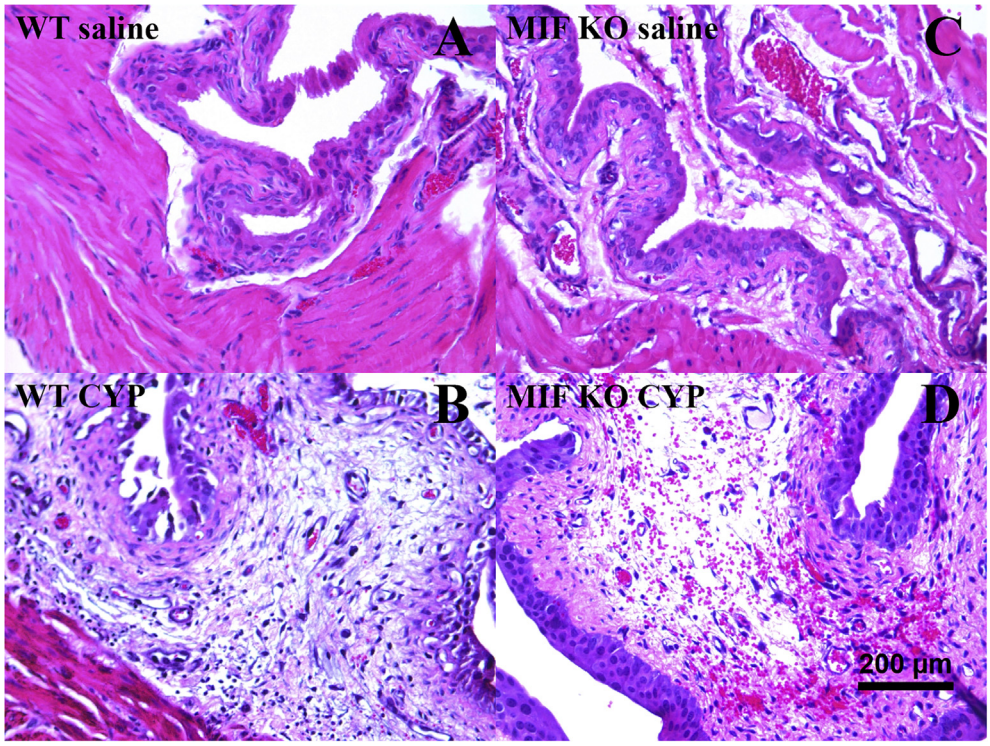

Fig. 4. CYP induced bladder inflammation in wild type and MIF knockout mice. CYP caused dramatic edema and inflammation in bladder urothelium of both wild type and MIF knockout mice. Inflamed bladders showed increased urothelial thickness, inflammatory cell infiltration and blood cell presentation. (A) Wild type mice with saline injection showed normal bladder histology. (B) CYP induced significant edema and inflammation in bladder urothelium. (C) MIF knockout mice with saline injection showed normal bladder histology. (D) CYP caused dramatic urothelial edema and inflammation in bladder of MIF knockout mice.

that CYP increased ERK1/2 activation in the bladder of WT mice that peaked early (4 h after CYP) during 48 h of observation, in agreement with earlier findings by other investigators [18]. However, although there was an increase in ERK activation in MIF KO mice treated with CYP, the ratio was still considerably smaller than the change observed in WT mice and was not statistically different from naive MIF KO mice. ERK activation has been shown to play a role in mediating pain both at peripheral and central sites [12,31]. Therefore, it is tempting to infer that decreased ERK signaling in the bladder after CYP treatment is due to impaired MIF-CD74 signaling in these animals and that decreased MIF-activated ERK signaling in MIF KO mice may be also be responsible for decreased sensation. Decreased sensation in MIF KO may explain the baseline differences on abdominal mechanical sensitivity observed between the strains. Furthermore, the much larger (3-fold increase) micturition volume of MIF KO mice compared to WT at baseline, strongly suggests that MIF plays a role in mediating normal bladder sensation thus affecting micturition sensation, typically carried by A-delta fibers [32].

CYP still induced bladder inflammation, edema and micturition changes in MIF KO mice and these findings run counter to our previous observations that MIF antagonism blocked these CYP-induced changes in mice [14]. Interestingly, pretreatment with a MIF inhibitor (ISO-1), blocked bladder inflammatory and micturition changes in MIF KO mice. Therefore, we consider it likely that ISO-1 is blocking the deleterious effects of cyclophosphamide (or its metabolite, acrolein) in the bladder and this possibility may account for our earlier findings with CYP-treated WT mice [14]. Mesna is a commonly used uroprotective agent to prevent hemorrhagic cystitis in patients treated with cyclophosphamide [33]. It appears that ISO-1, through MIF-independent mechanisms has a similar effect and this is, to our knowledge, is a significant novel finding.

CYP is a severe stimulus to the bladder causing hemorrhagic cystitis and bladder inflammation through a variety of inflammatory mediators [34]. It is likely that these mechanisms are still active in MIF KO mice although this possibility was not tested in the current study. The current study measured histological signs of bladder inflammation induced by CYP. It is possible that MIF may be involved in milder forms of inflammation or mediating non-histological signs of inflammation (such as cytokine changes). Regardless, the histological differences between WT and MIF KO mice treated with CYP were very similar and suggested the overall effect was similar between the two strains.

CYP still induced micturition changes in volume and frequency in WT and MIF KO mice and it is likely that those changes reflect mechanical changes in the inflamed bladder. However, since MIF KO mice did not show increased abdominal sensitivity after CYP treatment, it is likely that micturition changes after CYP do not reflect bladder pain sensation. The present results indicate that MIF mediates bladder pain directly and not secondary to bladder inflammatory changes.

Based on our current and previous results we propose the following model for MIF-mediated bladder pain (Fig. 6): Bladder injury or insult

Table 3
Histological changes in WT and MIF KO mice (values reported are mean ± SE).

	WT saline (N = 10)	WT CYP (N = 10)	MIF KO saline (N = 13)	MIF KO CYP (N = 14)	MIF KO CYP + ISO-1 (N = 6)	MIF KO ISO-1 (N = 6)
Inflammation	0.0 ± 0.0	**1.6 ± 0.3*****	0.0 ± 0.0	**2.5 ± 0.2**$^{\#\#\#}$	**0.5 ± 0.2**†††	0.3 ± 0.2
Edema	0.0 ± 0.0	**1.8 ± 0.2*****	0.2 ± 0.1	**2.4 ± 0.2**$^{\#\#\#}$	**0.6 ± 0.2**†††	0.2 ± 0.1

Compared to WT saline: $^{***}p < 0.001$.
Compared to MIF KO saline: $^{\#\#\#}p < 0.001$.
Compared to MIF KO CYP: $^{\dagger\dagger\dagger}p < 0.001$.

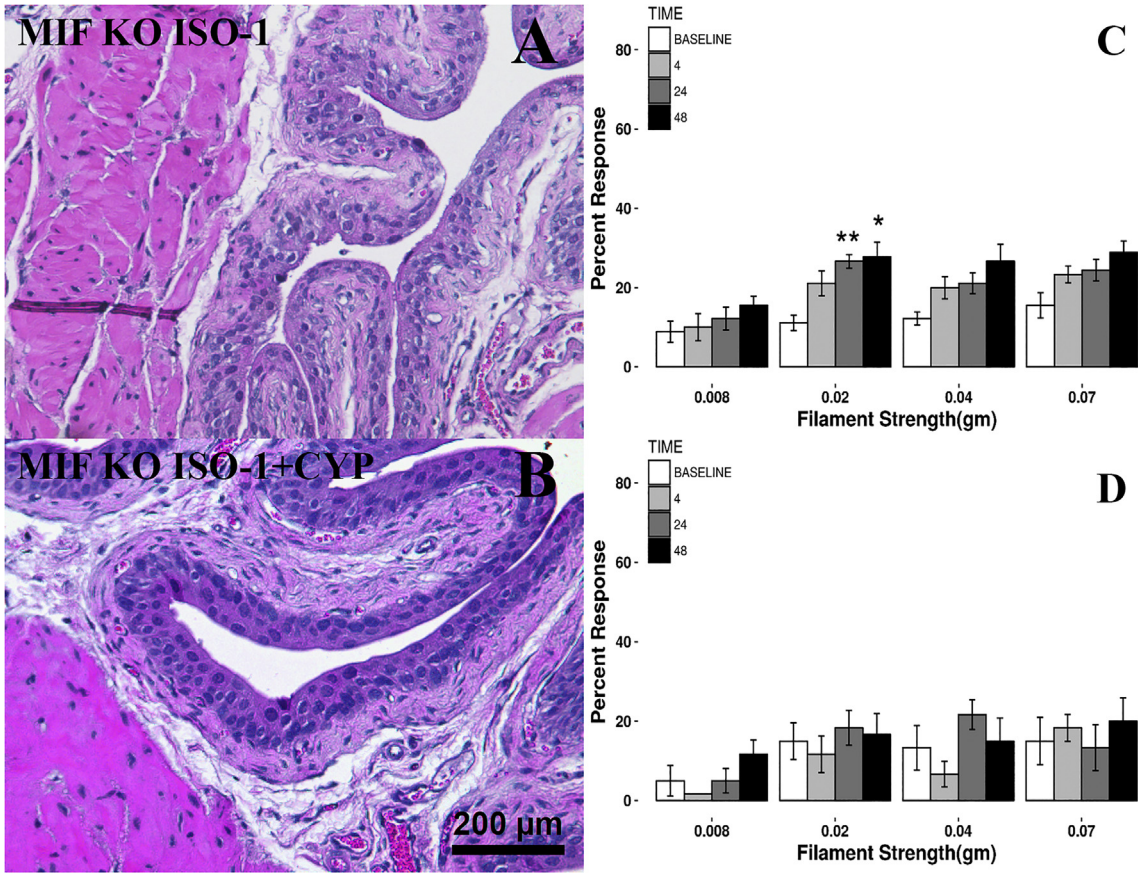

Fig. 5. ISO-1 blocked CYP induced bladder inflammation in MIF knockout mice. ISO-1 blocked CYP induced bladder inflammation and did not affect percent response to von Frey filament in MIF knockout mice treated with CYP. (A) ISO-1 alone did not induce bladder histological change. (B) MIF knockout mice with ISO-1 pretreatment showed normal bladder histology after CYP injection. (C) ISO-1 only did not affect percent response to von Frey filament in MIF knockout mice. (D) ISO-1 did not cause any change in percent response to von Frey filament after CYP treatment in MIF knockout mice. $^{*}p < 0.05$, $^{**}p < 0.01$ *vs.* baseline.

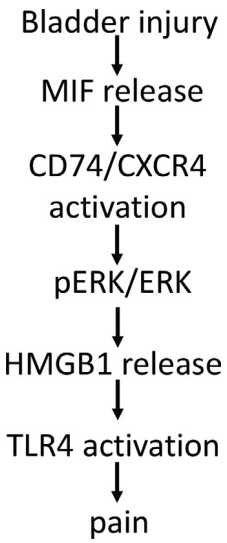

Fig. 6. Diagram shows MIF mediating bladder pain. Urothelial MIF is released upon bladder injury or insult that activates its receptor CD74/CXCR4. MIF receptor activation promotes HMGB1 release possibly through ERK phosphorylation and then induces bladder pain through TLR4.

(such as activation of PAR receptors) leads to release of urothelial MIF [19]. MIF binds to MIF receptors [19] or CD74 [30] resulting in EKR phosphorylation (current result) which promotes HMGB1 release from bladder urothelia [35,36]. A specific redox form of HMGB1 (disulfide) then acts on toll like receptor 4 (TLR4) either on bladder urothelial or nerve ending to induce bladder pain [24].

5. Conclusions

MIF mediates bladder pain but not bladder inflammation or micturition changes caused by CYP cystitis. This effect may be mediated through ERK phosphorylation at the bladder level. ISO-1, a well-known MIF inhibitor, blocks CYP induced bladder inflammation and micturition changes through pathways other than MIF.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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