A prospective study of macrophage migration inhibitory factor as a marker of inflammatory detection

Chao Zhang a, b, Guihua Hou b, #, Ting Liang b, Jing Song b, Lili Qu b, Fuqiang Chen b, Jia Xu b, Dan Wang b, Jiankui Han a, #, *

a Department of Nuclear Medicine, Qilu Hospital of Shandong University, Jinan, Shandong, P. R. China
b Institute of Experimental Nuclear Medicine, Shandong University, School of Medicine, Jinan, Shandong, P. R. China

Received: July 27, 2008; Accepted: September 23, 2008

Abstract

This study was to evaluate whether macrophage migration inhibitory factor (MIF) can be used as a better marker of inflammatory detection through the biodistribution and inflammatory imaging study with 131I-labelled anti-MIF McAb and control antibody in inflammatory model mice. The mRNA and protein expression of MIF in inflammatory lesions were proved by RT-PCR and immunohistochemistry. The model mice were injected with 3.7 MBq of each agent and killed at 24, 48 and 72 hrs after injection. Whole-body images were obtained with storage phosphor screen. The organs, blood, abscesses muscles were removed, weighed and counted with a γ counter. The percentage of uptake by organs and per gram tissues and abscesses normal tissue (%ID/g) concentration ratios were calculated. The abscesses in mice were well visualized from 24 hrs. The target-to-non-target (T/NT) ratios were 6.71 ± 1.09 (24 hrs), 8.57 ± 0.81 (48 hrs) and 11.41 ± 0.37 (72 hrs) for 131I-labelled anti-MIF McAb group; while in control group of 131I-IgG, T/NT ratios were 4.65 ± 0.63 (24 hrs), 6.44 ± 0.60 (48 hrs) and 8.23 ± 0.35 (72 hrs) (P < 0.05). MIF mRNA expression was threefold increased in inflammatory tissues at 24 hrs compared with normal tissues, and twofold increased at 48 hrs. MIF protein expression was stronger in the inflammatory tissues at 48 hrs after focal inflammation occurred. Our findings suggest that the 131I-labelled anti-MIF McAb appears to be more specific and suitable than 131I-labelled IgG for targeting focal inflammation, which means MIF can be used as a better marker of inflammatory detection.

Keywords: anti-MIF McAb • IgG • radioiodine • inflammation • storage phosphor screen • RT-PCR • immunohistochemistry

Introduction

The diagnosis of inflammatory processes is an important goal in medicine, especially for deep and insidious infection. It has been well proved that using radiopharmaceuticals to detect inflammation has apparent advantages than other methods [1, 2]. The radiopharmaceuticals routinely used for inflammation scintigraphic detection include 67Ga-citrate [3, 4], 99mTc or 111In-labelled leucocytes [5], 99mTc-nanocolloid [6] and 99mTc or 111In-labelled human polyclonal immunoglobulin [7, 8]. However these agents have different disadvantages such as non-specific, side effect, high dangerous using in human. Therefore, we need to urgently find a reasonable imaging agent for inflammatory detection. Recently, the use of radio-labelled receptor ligands that specifically bind to different leucocyte subsets is under study and it is hoped that they will significantly improve the diagnosis and management of patients with inflammatory diseases.

Macrophage migration inhibitory factor (MIF) was originally described as a product derived from activated T lymphocytes, which could prevent the random migration of macrophage. Recent studies have identified a key role for MIF as a pro-inflammatory cytokine [9–13], and may be an important mediator of various inflammatory diseases including septic shock [14, 15], rheumatoid arthritis [16, 17], delayed-type hypersensitivity [18, 19] and inflammatory lung diseases [20, 21]. MIF acts as an initiator of inflammatory and immunological reactions to regulate the production of a number of cytokines including TNF-α, IL-1β and IL-6 [15–17], and to activate T cells and macrophage, and to be implicated in the activation of T cells and macrophage. The over-expression of MIF stimulates Th1 [19, 20] and thereby prolongs an
exaggerated inflammatory response [21]. Moreover, anti-MIF antibody has shown a potent therapeutic action in severe inflammatory conditions in experimental animals [22, 23].

In a previous study [24], we reported that Na$^{125}$I radioiodinated anti-MIF McAb could localize in three inflammatory models (Staphylococcus aureus [S. aureus] group, Escherichia coli group and turpentine oil group) specifically. But the relation of local MIF expression with inflammatory visualization and the advantages of this agent compared to non-specific IgG in inflammatory detection are not clear. As an extension of our previous studies, we now report that the $^{131}$I-labelled anti-MIF McAb appears to be more specific and suitable than $^{131}$I-labelled IgG for targeting focal inflammation, which means MIF can be used as a better marker of inflammatory detection.

**Materials and methods**

**Radioiodination of anti-MIF McAb**

All commercially available chemicals were of analytic grade and anti-MIF McAb (R&D Systems, Minneapolis, MN, USA), IgG (ZSGB-BIO, Beijing, China) were pharmaceutical grade. Anti-MIF McAb and control IgG were iodinated with Na$^{131}$I (specific activity 37 MBq/mg, China Institute of Atomic Energy, Beijing, China) using the iodogen technique. Radioiodinated anti-MIF McAb and control IgG were separated from free iodine using size exclusion columns (Sephadex G-25, Pharmacia Biotech, Uppsala, Sweden). The specific activity of radioiodinated anti-MIF McAb is 29.56 GBq/μmol and that of radioiodinated IgG is 30.12 GBq/μmol. The radiochemical purity of radioiodinated anti-MIF McAb is >95% (paper chromatography) and that of radioiodinated IgG is >95% (paper chromatography). The immunological activities of $^{131}$I-labelled anti-MIF McAb was proved through ELISA (data not shown).

**Preparation of inflammatory animal model**

The animal experiments were carried out in accordance with institutional, national and international guidelines for humane use of animals for research. BALB/c mice (18–22 g, Animal Center of Shandong University, Jinan, Shandong, China) were induced inflammation by intramuscularly injecting $2 \times 10^8$ colony forming units of S. aureus in 0.2 ml, into the left thigh muscle. Twenty-four hours after inoculation, focal inflammation occurred. Those inflammatory models were proved by histological studies (data not shown). Forty-eight mice of inflammatory models were divided into two groups for biodistribution study, each group consisting of 24 mice. Eight mice of inflammatory models were used for whole-body autoradiography. Twelve mice of inflammatory models were used for histology and immunohistochemical analysis and RT-PCR.

**Biodistribution of $^{131}$I-anti-MIF McAb**

Mice with the left thigh inflammation were injected intravenously via the tail vein by 3.7 MBq $^{131}$I-anti-MIF McAb or $^{131}$I-IgG (0.2 ml in PBS), respectively. Eight mice of each group were killed by cervical dislocation at 24, 48 and 72 hrs after injection, respectively. A sample of 1 ml blood was collected at the time of decapitation. Samples of two thigh muscles (left as target, right as control), lung, heart, liver, spleen and kidney were excised, rinsed with saline, weighted and counted on a γ counter. Organ uptake was calculated as a percentage of the injected dose per gram of wet tissue mass (%ID/g).

**Whole-body autoradiography**

Eight mice of inflammatory models were divided into two groups; each group consisted of four mice, respectively. $^{131}$I-anti-MIF McAb (3.7 MBq in 0.2 ml PBS) or $^{131}$I-IgG (3.7 MBq in 0.2 ml PBS) were injected intravenously via the tail vein. Serial images were performed at 24, 48 and 72 hrs after injection. The anaesthetized mice were placed on the storage phosphor screen plate with the ventral side facing the plate, in subdued light. The plate was exposed to a mouse for 40 min. At cessation of exposure, the plate was immediately covered with an opaque plastic sheet, then transferred to the scanner, and scanned by typhoon trio (+ laser red 633 nm, pixel size 200 microns, phosphor mode: best sensitivity).

**RT-PCR**

The expression of MIF mRNA was evaluated by RT-PCR. Total RNA was isolated from inflammatory tissues with TRizol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA), and contaminant DNA was removed by DNase I (Invitrogen Life Technologies) according to the manufacturer’s instructions. Total RNA (5 μg) (Fermentas Inc., Glen Burnie, MD, USA) was reverse transcribed by Revert Aid™ First Strand cDNA Synthesis Kits (Fermentas), cDNAs were amplified with murine MIF specific primer using TaKaRa PCR Amplification Kit (TaKaRa Biomedical Technology (Beijing) Co. Ltd., Beijing, China). The primers were designed as follows: MIF (368 bp), sense primer, 5′-CCATGCCTATGTTCATCGTG-3′; and antisense primer, 5′-GAACAGCGGTGCAGGTAAGTG-3′.

Cycling conditions for amplification as follows: 4 min. denaturation step at 94°C, followed by 35 cycles of 30 sec. at 94°C, 1 min. at 55°C and 1 sec. at 72°C. PCR products were analysed on 1.5% agarose gels and stained with ethidium bromide. All the experiments were performed in duplicate and they were repeated at least three times. GAPDH mRNA expression was used as a loading control.

**Histology and immunohistochemical analysis**

Inflammatory tissues were fixed in 10% PBS-buffered formalin, and paraffin sections were stained with haematoxylin and eosin and examined by light microscopy to assess the histological changes. Immunohistochemical analysis was done using a SP-9002 Histostain™-Plus kit (ZSGB-BIO) according to the manufacturer’s protocol. The sections were microscopically examined and the positively stained fields were observed. Ten fields per each section were observed.

**Statistics analysis**

The data were analysed using SPSS11.0 software. Statistical analysis was performed using the unpaired Student’s t-test. Difference were considered significant when $P < 0.05$, and two-sided. All data were expressed as the means ± S.D.
Result

Accumulation in the inflammatory tissue

The concentration of $^{131}$I-anti-MIF McAb or $^{131}$I-IgG in the inflammatory tissue was expressed as percentage of the initial dose (%ID/g) (Fig. 1A). It is apparent that both radiopharmaceuticals localized at sites of inflammation to a significant extent at 24 hrs after injection. $^{131}$I-anti-MIF McAb has a high initial uptake ($0.352 \pm 0.021\%$ID/g at 24 hrs after injection) and retained in muscle inflammation more than 72 hrs ($0.0925 \pm 0.0013\%$ID/g at 72 hrs after injection). The uptake of $^{131}$I-anti-MIF McAb was significantly higher compared to that of $^{131}$I-IgG ($P < 0.05$). Target-to-non-target (T/NT) ratio was given in Fig. 1B. It shows that the $^{131}$I-anti-MIF McAb group T/NT ratio was $>6$ at 24 hrs, and increased continually, T/NT ratio was $>9$ until 72 hrs, but the $^{131}$I-IgG group T/NT ratio was $>6$ at 48h and $>8$ at 72 hrs. The highest uptake happened in the $^{131}$I-anti-MIF McAb group ($P < 0.05$). Target-to-blood ratios (T/B,%ID/g) are given in Fig. 1C. It shows that the T/B ratios for the $^{131}$I-anti-MIF McAb group were $1.41 \pm 0.031$, $1.53 \pm 0.018$, $2.58 \pm 0.025$ at 24, 48 and 72 hrs after injection,
respectively. These ratios were significantly higher than that of $^{131}$I-IgG group ($P < 0.05$).

All these data showed that the $^{131}$I-labelled anti-MIF McAb appears to be more specific and suitable than $^{131}$I-labelled IgG for targeting focal inflammation.

**The Imaging of the inflammatory foci**

The whole-body autoradiography images of the two radiotracers at 24, 48 and 72 hrs after injection are shown in Fig. 2. Both radiotracers showed focally increased uptake in the inflammatory muscles beginning at 24 hrs. Compared analysis of the scintigrams of three time-points showed that the $^{131}$I-anti-MIF McAb group had much more clear images than the $^{131}$I-IgG groups, which is in accordance with the high T/NT ratio ($P < 0.05$). These also demonstrate that the $^{131}$I-labelled anti-MIF McAb appears to be more specific than $^{131}$I-labelled IgG for targeting focal inflammation.

**MIF mRNA expression in inflammatory and normal tissues**

In order to make sure that the high intake of $^{131}$I-labelled anti-MIF McAb in inflammatory tissue is caused by high expression of MIF in locus, we analysed the expression of MIF in inflammatory tissue. Result of RT-PCR showed the expression of MIF mRNA in inflammatory and normal tissues in Figure 3. There were little changes in MIF gene expression in normal tissues at the three time-points. However, there was a threefold increase in MIF mRNA expression in inflammatory tissues at 24 hrs compared with normal tissues ($P < 0.05$). There was a twofold increase in MIF mRNA levels in inflammatory tissues at 48 hrs compared with normal tissues ($P < 0.05$). Semi-quantitative RT-PCR was performed in duplicate to minimize experimental error on the value calculated. All columnar values were expressed as means and standard deviations. A pattern of results were analysed by repeating at least three times. $P < 0.05$ compared with the normal group.
MIF protein expression

Immunohistochemistry staining showed that high MIF was expressed at the inflammatory tissues (Fig. 4). It shows the high localization of MIF protein in tissues specimens from inflammatory mouse at 24, 48, 72 hrs after focal inflammation occurred. MIF was negative expressed in normal tissues (Fig. 4A). However, there was a significant increase in MIF expression in inflammatory specimens according to the time after focal inflammation occurred in the first 48 hrs (Fig. 4B and C); and then decreased at 72 hrs (Fig. 4D). MIF expression was localized among the inflammatory cells strongly after focal inflammation occurred. Strikingly, up-regulation of MIF in the inflammatory tissues was associated with numerous macrophage accumulations. This was particularly profound in the inflammatory tissues, it also demonstrated that high intake of $^{131}$I-labelled anti-MIF in the locus corresponds with the high expression of MIF in inflammatory tissue.

Discussion

Inflammatory diseases remain prominent in clinical medicine up date. Definitive diagnosis of inflammation depends primarily on positive culture of the offending pathogens. However, the anatomical delineation of the site and the extent of focal inflammation are critical to the clinical management of inflammatory processes, both for diagnosis and for monitoring the response to therapy. All inflammatory processes develop along a known sequence leakage of fluid, followed by consequent leakage of small molecules, proteins, then by transudation and local accumulation of cells. From this process we can predict which radiopharmaceuticals combined with inflammatory cells and related products might have a role in visualization of the various inflammatory processes in clinic.

Scintigraphic imaging detection of inflammation allows determination of both the location and the number of inflammation foci throughout the body. Since scintigraphic images are based on
functional tissue changes, these foci can be localized in their early-phases, when anatomic changes are not yet apparent [25]. The search for an ideal radiopharmaceutical for the rapid, accurate and unequivocal identification of inflammation is necessary. Over the past decades, research efforts have been directed towards the development of monoclonal antibodies as radiopharmaceuticals for application in fields of radioimmunodiagnosis. Antibodies can be made to specifically target the immunizing antigen. As such, their potential as specific targeting agents for both diagnosis and therapy has been well recognized.

In this study, we compared $^{131}$I-anti-MIF McAb with $^{131}$I-IgG in extensive studies of animals and found that this radiopharmaceutical rapidly specifically localizes at the inflammation sites; $^{131}$I-anti-MIF McAb group T/NT ratio was >6 at 24 hrs, and increased continually, until 72 hrs T/NT ratio was >11, but the $^{131}$I-IgG group T/NT ratio was >6 at 48 hrs and >8 at 72 hrs, so the highest uptake happened in the $^{131}$I-anti-MIF McAb group ($P < 0.05$). The T/B ratios for the $^{131}$I-anti-MIF McAb group were $1.41 = 0.031$, $1.53 = 0.018$, $2.58 = 0.025$ at 24, 48 and 72 hrs after injection, respectively. These data showed that $^{131}$I-anti-MIF McAb group significantly higher than that of $^{131}$I-IgG group ($P < 0.05$). Furthermore, serial images of whole-body autoradiography also showed that the $^{131}$I-anti-MIF McAb group had much more clear images than the $^{131}$I-IgG groups in accordance with the high T/NT ratio. RT-PCR also showed that there was a threefold increase in MIF mRNA expression in inflammatory tissues at 24 hrs compared with normal tissues and a twofold increase at 48 hrs. Immunohistochemistry confirmed an increase in MIF protein expression, strong expression in the inflammatory tissues at 48 hrs after focal inflammation occurred. This maybe because MIF is a kind of cytokine produced by T lymphocytes and macrophages in response to inflammatory stimuli, resulting in antigen-specific immune responses between $^{131}$I-anti-MIF McAb and MIF while uptake of $^{131}$I-IgG in inflammatory loci is caused by nonspecific binding to Fe fragments of normal IgG. Though radiolabeled human polyclonal IgG had received considerable attention as a radiopharmaceutical for inflammation imaging [30–32], it is far from the ideal inflammation imaging reagent since it is not specific for inflammation.

MIF has been identified as a ubiquitous protein performing an important role in the pathogenesis of various inflammatory disease conditions in different organs [33]. MIF was originally discovered as a lymphokine involved in delayed hypersensitivity and various macrophage functions, including production of pro-inflammatory cytokines, glucocorticoid-induced immunomodulator, and natural killer cell inhibitory factor, regulation of toll-like receptor expression, adherence and phagocytosis of macrophages, as well as induction of metalloproteinase. MIF activates macrophages to enhance the production of TNF-$\alpha$ and nitric oxides. Accordingly, macrophage-derived MIF has the capacity to act in an autocrine fashion to promote the secretion of additional pro-inflammatory mediators, thereby sustaining a local inflammatory reaction. The potential of an anti-MIF therapeutic strategy was highlighted by the ability of a neutralizing anti-MIF monoclonal antibody to attenuate pro-inflammatory cytokine production. Therefore MIF is considered as a potential target protein in many pathophysiological states.

This study demonstrated that the $^{131}$I-anti-MIF McAb achieves very high target-to-background ratio, has relatively low level of accumulation in non-target tissues, it possesses a high relations with local expression of MIF and it is much better than non-specific IgG in inflammatory radioimmunoimaging. So radioiodinated anti-MIF McAb could be used as a prospective inflammation-imaging agent, and MIF is preferred as a good marker of inflammatory detection.

References

1. Okarvi SM. Peptide-based radiopharmaceuticals: future tools for diagnostic imaging of cancers and other diseases. Med Res Rev. 2004; 24: 357–97.
2. Richard JC, Chen DL, Ferkol T, et al. Molecular imaging for pediatric lung diseases. Pediatr Pulmonol. 2004; 37: 286–96.
3. Staab EV, McCartney WH. Role of 67Ga in inflammation disease. Semin Nucl Med. 1978; 8: 219–34.
4. Seabold JE, Palestro CJ, Brown ML, et al. Procedure guideline for gallium scintigraphy in inflammation. Society of Nuclear Medicine. J Nucl Med. 1997; 38: 994–7.
5. Seabold JE, Forstrom LA, Schauwecker DS, et al. Procedure guideline for indium-111-leukocyte scintigraphy for suspected infection/inflammation. Society of Nuclear Medicine. J Nucl Med. 1997; 38: 997–1001.
6. De Schrijver M, Streule K, Senekowitsch R, et al. Scintigraphy of inflammation with nanometer-sized colloidal tracers. Nucl Med Commun. 1987; 8: 895–908.
7. Buscombe JR, Miller RF, Lui D, et al. Combined 67Ga citrate and 99Tcm-human immunoglobulin imaging in human immunodeficiency virus-positive patients with fever of undetermined origin. Nucl Med Commun. 1991; 12: 583–92.
8. McAfee JG, Gagne G, Subramanian G, et al. The localization of indium-111-leukocytes, gallium-67-polyclonal IgG and other radioactive agents in acute focal inflammatory lesions. J Nucl Med. 1991; 32: 2126–31.
9. Donnelly SC, Haslett C, Reid PT, et al. Regulatory role for macrophage migration inhibitory factor in acute respiratory distress syndrome. Nat Med. 1997; 3: 320–3.
10. Bernhagen J, Calandra T, Mitchell RA, et al. MIF is a pituitary-derived cytokine that potentiates lethal endotoxaemia. Nature. 1993; 365: 756–9.
11. Bernhagen J, Mitchell RA, Calandra T, et al. Purification, bioactivity, and secondary structure analysis of mouse and human macrophage migration inhibitory factor (MIF). Biochemistry. 1994; 33: 1414–55.
12. Kleemann R, Hauser A, Geiger G, et al. Intracellular action of the cytokine MIF to modulate AP-1 activity and the cell cycle through Jab1. Nature. 2000; 408: 211–6.
13. Bucala R. Signal transduction. A most interesting factor. Nature. 2000; 408: 146–7.
14. Calandra T, Spiegel LA, Metz CN, et al. Macrophage migration inhibitory factor is a critical mediator of the activation of immune cells by exotoxins of Gram-positive bacteria. Proc Natl Acad Sci USA. 1998; 95: 11383–8.
15. Calandra T, Echtenacher B, Roy DL, et al. Protection from septic shock by neutralization of macrophage migration inhibitory factor. Nat Med. 2000; 6: 164–70.
16. Mikulowska A, Metz CN, Bucala R, et al. Macrophage migration inhibitory factor is involved in the pathogenesis of collagen type II-induced arthritis in mice. J Immunol. 1997; 158: 5514–7.
17. Leech M, Metz C, Santos L, et al. Involvement of macrophage migration inhibitory factor in the evolution of rat adjuvant arthritis. Arthritis Rheum. 1998; 41: 910–7.
18. Bernhagen J, Bacher M, Calandra T, et al. An essential role for macrophage migration inhibitory factor in the tuberculin delayed-type hypersensitivity reaction. J Exp Med. 1996; 183: 277–82.
19. Lan HY, Mu W, Yang N, et al. De novo renal expression of macrophage migration inhibitory factor during the development of rat crescentic glomerulonephritis. Am J Pathol. 1996; 149: 1119–27.
20. Rossi AG, Haslett C, Hirani N, et al. Human circulating eosinophils secrete macrophage migration inhibitory factor (MIF). Potential role in asthma. J Clin Invest. 1998; 101:2869–74.
21. Makita H, Nishimura M, Miyamoto K, et al. Effect of anti-macrophage migration inhibitory factor antibody on lipopolysaccharide-induced pulmonary neutrophil accumulation. Am J Respir Crit Care Med. 1998; 158: 573–9.
22. Hoi AY, Morand EF, Leech M. Is macrophage migration inhibitory factor a therapeutic target in systemic lupus erythematosus? Immunol Cell Biol. 2003; 81: 367–73.
23. Kobayashi S, Nishihiro J, Watanabe S, et al. Prevention of lethal acute hepatic failure by antimacrophage migration inhibitory factor antibody in mice treated with bacille Calmette-Guerin and lipopolysaccharide. Hepatology. 1999; 29:1752–9.
24. Zhang C, Hou GH, Han JK, et al. Radiiodine labeled anti-MIF McAb: a potential agent for inflammation imaging. Mediators Inflamm. 2007; 2007: 50180.
25. Rennen HJ, Boerman OC, Oyen WJ, et al. Optimal quality (131)I-monoclonal anti-bodies on high-dose labeling in a large reaction volume and temporarily coating the antibody with IODO-GEN. J Nucl Med. 2001; 42: 509–19.
26. Jayson GC, Zweit J, Jackson A, et al. Molecular imaging and biological evaluation of HuMV833 anti-VEGF antibody: implications for trial design of antiangiogenic antibodies. J Natl Cancer Inst. 2002; 94: 1484–93.
27. Verel I, Visser GW, Boellaard R, et al. Quantitative 89Zr immuno-PET for in vivo scouting of 90Y-labeled monoclonal antibodies in xenograft-bearing nude mice. J Nucl Med. 2003; 44: 1663–70.
28. Abrams MJ, Juweid M, tenKate CI, et al. Technetium-99m-human polyclonal IgG radiolabeled via the hydrazino nicotinamide derivative for imaging focal sites of infection in rats. J Nucl Med. 1990; 31: 2022–8.
29. Bucala R, Donnelly SC. Macrophage migration inhibitory factor: a probable link between inflammation and cancer. Immunity. 2007; 20: 281–5.