

⁹⁹Tc-MDP treatment for the therapy of rheumatoid arthritis, choroidal neovascularisation and Graves' ophthalmopathy (Review)

QIAN WU¹, YANG NI², QINGRUI YANG¹ and HONGSHENG SUN¹

Departments of ¹Rheumatology and Immunology and ²Oncology, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, Shandong 250021, P.R. China

Received November 30, 2015; Accepted February 16, 2016

DOI: 10.3892/br.2016.609

Abstract. Technetium 99 conjugated with methylene diphosphonate, which is an anti-inflammatory drug, can inhibit macrophage infiltration and downregulate a number of proinflammatory cytokines, such as tumor necrosis factor- α and interleukin-1 β . Recently, numerous studies have indicated that it could improve rheumatoid arthritis (RA) activity by upregulating the frequency of peripheral $\gamma\delta$ T cells and cluster of differentiation CD4⁺CD25⁺Foxp3⁺ Tregs, affecting the serum cytokine environment, inhibiting osteoclast formation and reducing the concentrations of rheumatoid factor-immunoglobulin M (IgM)/IgG/IgA. As well, it may have a therapeutic role for choroidal neovascularisation (CNV) and Graves' ophthalmopathy (GO). Therefore, it will be a valuable choice in the treatment of RA, CNV and GO.

(patent no. ZL94113006.1), which has been used for the safe and effective treatment of rheumatoid arthritis (RA) and ankylosing spondylitis in China since 1997 (1). ⁹⁹Tc-MDP treatment can inhibit macrophage infiltration together with the downregulation of proinflammatory cytokines, including tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), in addition to intercellular adhesion molecule-1 (ICAM-1) and matrix metalloproteinases (MMPs) (1).

⁹⁹Tc-MDP is the radioactive-safe decay product of ^{99m}Tc-MDP and has been widely used in bone scintigraphy as a radioactive agent with limited radioactivity (demonstrated as harmless to the human body). The present study reviews the therapeutic use of ⁹⁹Tc-MDP in clinical diseases and the associated mechanisms of action.

Contents

1. Introduction
2. Rheumatoid arthritis
3. Choroidal neovascularization
4. Graves' ophthalmopathy
5. Conclusion

1. Introduction

Technetium 99 (⁹⁹Tc) conjugated with methylene diphosphonate (MDP) is an anti-inflammatory drug patented in China

Correspondence to: Dr Qingrui Yang or Dr Hongsheng Sun, Department of Rheumatology and Immunology, Shandong Provincial Hospital Affiliated to Shandong University, 324 Jing Wu Road, Jinan, Shandong 250021, P.R. China
E-mail: qryang720@163.com
E-mail: sunhongsheng@126.com

Key words: technetium 99 conjugated with methylene diphosphonate, rheumatoid arthritis, choroidal neovascularization, Graves' ophthalmopathy, therapy

2. Rheumatoid arthritis

RA is a chronic, progressive and inflammatory autoimmune disease (2-4), particularly affecting elderly people (5). Although it can affect numerous tissues and organs, it primarily involves flexible joints (6) resulting in synovitis, pannus formation (7) and massive bone destruction with consequent inflammation, pain and disability (8), associated with higher mortality as compared to the general population (9,10).

⁹⁹Tc-MDP was treated intravenously to active RA patients at a dose of 20 μ g/day consecutively for 10-14 days, and reported that the frequency of peripheral cluster of differentiation 3 (CD3⁺) $\gamma\delta$ T cells and CD4⁺CD25⁺Foxp3⁺ Tregs were significantly elevated, paralleled with decreased serum TNF- α and IL-6 levels and increased serum TGF- β . There was a positive correlation between the elevation of peripheral CD3⁺ $\gamma\delta$ T cells and increased serum transforming growth factor (TGF)- β and decreased disease activity, indicating that ⁹⁹Tc-MDP may improve RA activity by upregulating frequency of peripheral $\gamma\delta$ T cells and CD4⁺CD25⁺Foxp3⁺ Tregs, as well as affecting the serum cytokine environment (11).

Ji *et al* (12) isolated peripheral blood mononuclear cells from RA patients and cultured them in medium with 25 μ g/l of receptor activator of nuclear factor- κ B ligand (RANKL), 25 μ g/l macrophage-colony stimulating factor and various concentrations of ⁹⁹Tc-MDP (5, 10, 20 and 50 mg/l), and found that after 12 or 16 days of culture, large multinuclear

cells were observed, and that ^{99}Tc -MDP markedly inhibited the changes. Those inhibitory effects were positively associated with the increased ^{99}Tc -MDP concentration, indicating protective effect of ^{99}Tc -MDP on RA via inhibition of osteoclast formation.

Similarly, Gong *et al* (13) reported that $0.01\ \mu\text{g/ml}$ ^{99}Tc -MDP significantly inhibited RANKL-induced osteoclastogenesis with no cytotoxicity, and significantly abolished the appearance of multinucleated osteoclasts, inhibited the expression of the transcription factors c-Fos, nuclear factor of activated T cells and inflammatory factors, such as IL-6, TNF- α and IL-1 β . Thus, ^{99}Tc -MDP has anti-osteoclastogenic activity on RANKL-induced osteoclast formation.

After $0.2\ \text{g}$ of ^{99}Tc -MDP was administered daily by intravenous drip for 5 days to RA patients and patients with joint pain/arthritis, the injection A and B models of ^{99}Tc -MDP were used with one set daily for 10 days, which was one course of treatment. The next course started after 10 days. Each case used it for 2-4 courses of treatment. In the RA patients, the concentrations of rheumatoid factor-immunoglobulin M (RF-IgM) were 296.2 ± 108.4 , 189.5 ± 92.3 and $107.8\pm72.5\ \text{IU/ml}$; the concentrations of RF-IgG were 325.6 ± 126.2 , 209.7 ± 98.2 and $160.2\pm80.8\ \text{IU/ml}$; and the concentrations of RF-IgA were 330.4 ± 136.3 , 210.7 ± 89.2 and $148.8\pm72.2\ \text{IU/ml}$ prior and subsequent to 2 and 4 courses of treatment, respectively. The concentrations of the aforementioned RFs were significantly lower following 2 and 4 courses compared to those prior to treatment ($P<0.05$ and $P<0.01$, respectively). Consequently, ^{99}Tc -MDP appeared to reduce the abnormally high concentrations of RFs, implicating that ^{99}Tc -MDP could have an important role in controlling the activities of RA (14).

Wu *et al* (15) also demonstrated that ^{99}Tc -MDP could suppress the secretion of IL-1 and soluble IL-2R in RA patients.

3. Choroidal neovascularization

Choroidal neovascularisation (CNV), a common vision-threatening complication (16), accounts for 90% of cases of severe vision loss in patients with advanced age-related macular degeneration (17-19). Its pathogenesis involves a disruption of the homeostasis between the retinal pigment epithelium and Bruch's membrane (20). The proliferating choroidal blood vessels extend into the macula region of the subretinal space, and leak fluid, leading to serous retinal detachment and scarring (20) and central vision loss.

Lai *et al* (1) used C57BL/6J mice to induce CNV by laser photocoagulation, and following this they intraperitoneally injected ^{99}Tc -MDP at the doses of 0.05 , 0.1 and $0.2\ \mu\text{g/kg}$ or the same volume of phosphate-buffered saline (PBS) daily. After 7 days, areas of CNV were significantly suppressed by ^{99}Tc -MDP treatment without toxicity to the retina, compared with PBS treatment, in a dose-dependent manner. ^{99}Tc -MDP treatment of 0.05 , 0.1 and $0.2\ \mu\text{g/kg}$ suppressed the development of CNV by 36.12 , 58.76 and 73.48% , respectively. Furthermore, ^{99}Tc -MDP treatment inhibited the infiltration of macrophages to CNV and the downregulation of protein expressions of vascular endothelial growth factor (VEGF), ICAM-1, TNF- α , and MMPs. At the same time, ^{99}Tc -MDP also inhibited VEGF-induced migration and capillary-like tube formation of endothelial cells. In consequence, anti-inflammatory treatment

with ^{99}Tc -MDP has therapeutic potential for CNV-related diseases.

4. Graves' ophthalmopathy

Graves' ophthalmopathy (GO), also known as thyroid-associated ophthalmopathy, is an organ-specific autoimmune disease (21,22).

In the general population, the estimated incidence of GO is 16 women and 3 men per 100,000 population per year (23,24), and it is clinically present in 20-25% of patients at the time of their diagnosis of hyperthyroidism (23,25). The exact etiology and underlying mechanism of GO remain to be elucidated. The natural history of GO varies from spontaneous remission to persistent or progressive disease (23,26), and glucocorticoid therapy is the primary treatment (23,27-29).

Yan *et al* (30) incubated retroocular fibroblasts of GO patients for 72 h with $100\ \text{U/ml}$ interferon- γ , $100\ \text{U/ml}$ IL-1 or $100\ \text{U/ml}$ TNF- α in the presence of ^{99}Tc -MDP, recognizing that at base conditions, the percentage of positive cells of human leucocyte antigen-DR (HLA-DR) and ICAM-1 on retroocular fibroblasts was 6.70 ± 3.06 and $5.29\pm3.02\%$, respectively, and the synthesis of hyaluronic acid (HA) was $337.8\pm42.7\ \text{ng/ml}$. Compared to the basal values, 72 h incubation with the aforementioned cytokines clearly enhanced the expression of HLA-DR and ICAM-1 together with the synthesis of HA. In addition, ^{99}Tc -MDP inhibited cytokine-induced retroocular fibroblasts activation and proliferation in a dose-dependent manner.

Pan *et al* (31) evaluated the efficacy of immunosuppressive agents, ^{99}Tc -MDP and the two together in treating patients with GO, finding that in 22 patients treated with immunosuppressive agents, the general efficacy rate was 19/22, and the incidence rate of a serious side effect was 8/22. In 20 patients treated with ^{99}Tc -MDP, the general efficacy rate was 17/20, and the incidence rate of a serious side effect was 2/20. In 24 patients treated with immunosuppressive agents and ^{99}Tc -MDP, the general efficacy rate was 22/24, and the incidence rate of a serious side effect was 2/24. The results suggested that using immunosuppressive agents in combination with ^{99}Tc -MDP could obtain satisfactory efficacy, and avoid the serious side effect and 'rebound' of symptoms in the treatment of GO (31).

5. Conclusion

^{99}Tc -MDP may have numerous important roles in the therapy of clinical disease, as it can improve the activity of RA, treat CNV-related diseases, and avoid the serious side effect in the treatment of GO in combination with immunosuppressive agents, thus, it will be a valuable choice for the treatment of RA, CNV and GO.

Acknowledgements

The study was supported by the Natural Science Foundation of China Grants (nos. 81172861/H1008 and 81501399/H1008), the Foundation of Scientific and Technological Project of Shandong Province (grant no. 2011GG21829), Projects of Medical and Health Technology Development Program in Shandong province (grant no. 2014WS0344), and the

Foundation for Outstanding Young Scientist in Shandong Province (grant no. BS2014YY012).

References

- Lai K, Xu L, Jin C, Wu K, Tian Z, Huang C, Zhong X and Ye H: Technetium-99 conjugated with methylene diphosphonate (99Tc-MDP) inhibits experimental choroidal neovascularization in vivo and VEGF-induced cell migration and tube formation in vitro. *Invest Ophthalmol Vis Sci* 52: 5702-5712, 2011.
- Choi JK, Kim SW, Kim DS, Lee JY, Lee S, Oh HM, Ha YS, Yoo J, Park PH, Shin TY, *et al*: Oleonic acid acetate inhibits rheumatoid arthritis by modulating T cell immune responses and matrix-degrading enzymes. *Toxicol Appl Pharmacol* 290: 1-9, 2016.
- Favalli EG, Becciolini A and Biggioggero M: Structural integrity versus radiographic progression in rheumatoid arthritis. *RMD Open* 1 (Suppl 1): e000064, 2015.
- Meednu N, Zhang H, Owen T, Sun W, Wang V, Cistrone C, Rangel-Moreno J, Xing L, Med B and Anolik JH: A link between B cells and bone erosion in rheumatoid arthritis: Receptor activator of nuclear factor kappa-B ligand production by memory B cells. *Arthritis Rheumatol*: Nov 10, 2015 (Epub ahead of print).
- Owens GM: Optimizing rheumatoid arthritis therapy: Using objective measures of disease activity to guide treatment. *Am Health Drug Benefits* 8: 354-360, 2015.
- Magyari L, Varszegi D, Kovacs E, Sarlos P, Farago B, Javorhazy A, Sumegi K, Banfai Z and Melegh B: Interleukins and interleukin receptors in rheumatoid arthritis: Research, diagnostics and clinical implications. *World J Orthop* 5: 516-536, 2014.
- Rosado-de-Castro PH, Lopes de Souza SA, Alexandre D, Barbosa da Fonseca LM and Gutfilen B: Rheumatoid arthritis: Nuclear Medicine state-of-the-art imaging. *World J Orthop* 5: 312-318, 2014.
- Al-Nahain A, Jahan R and Rahmatullah M: Zingiber officinale: A potential plant against rheumatoid arthritis. *Arthritis* 2014: 159089, 2014.
- Scarno A, Perrotta FM, Cardini F, Carboni A, Annibali G, Lubrano E and Spadaro A: Beyond the joint: Subclinical atherosclerosis in rheumatoid arthritis. *World J Orthop* 5: 328-335, 2014.
- Sonomoto K, Yamaoka K and Tanaka Y: An approach to bone and cartilage repair of rheumatoid arthritis by mesenchymal stem cells. *J UOEH* 36: 141-146, 2014 (In Japanese).
- Su D, Shen M, Gu B, Wang X, Wang D, Li X and Sun L: 99 Tc-methylene diphosphonate improves rheumatoid arthritis disease activity by increasing the frequency of peripheral $\gamma\delta$ T cells and CD4⁺ CD25⁺ Foxp3⁺ Tregs. *Int J Rheum Dis*: Jan 28, 2014 (Epub ahead of print).
- Ji Y, Huo X and Zhang H: Technetium 99Tc methylenediphosphonate inhibits osteoclast formation from PBMCs in patients with rheumatoid arthritis. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* 34: 684-688, 2009 (In Chinese).
- Gong W, Dou H, Liu X, Sun L and Hou Y: Technetium-99 conjugated with methylene diphosphonate inhibits receptor activator of nuclear factor- κ B ligand-induced osteoclastogenesis. *Clin Exp Pharmacol Physiol* 39: 886-893, 2012.
- Huang A, Yu L and Shen L: Effect of technetium-99 conjugated with methylene diphosphonate on IgM-RF, IgG-RF and IgA-RF. *J Huazhong Univ Sci Technolog Med Sci* 23: 266-268, 2003.
- Wu YG, Ma QL and Liu GF: Effect of 99Tc-MDP on cytokine production by peripheral blood mononuclear cells of patients with rheumatoid arthritis. *Hunan Yi Ke Da Xue Xue Bao* 27: 173-175, 2002 (In Chinese).
- Wong TY, Ohno-Matsui K, Leveziel N, Holz FG, Lai TY, Yu HG, Lanzetta P, Chen Y and Tufail A: Myopic choroidal neovascularisation: Current concepts and update on clinical management. *Br J Ophthalmol* 99: 289-296, 2015.
- Li T, Aredo B, Zhang K, Zhong X, Pulido JS, Wang S, He YG, Huang X, Brekken RA and Ufret-Vincenty RL: Phosphatidylserine (PS) is exposed in choroidal neovascular endothelium: PS-Targeting antibodies inhibit choroidal angiogenesis in Vivo and ex Vivo. *Invest Ophthalmol Vis Sci* 56: 7137-7145, 2015.
- Weber ML and Heier JS: Choroidal Neovascularization Secondary to Myopia, Infection and Inflammation. *Dev Ophthalmol* 55: 167-175, 2016.
- Nagai N, Ju M, Izumi-Nagai K, Robbie SJ, Bainbridge JW, Gale DC, Pierre E, Krauss AH, Adamson P, Shima DT, *et al*: Novel CCR3 Antagonists Are Effective Mono- and Combination Inhibitors of Choroidal Neovascular Growth and Vascular Permeability. *Am J Pathol* 185: 2534-2549, 2015.
- Baxter SL, Pistilli M, Pujari SS, Liesegang TL, Suhler EB, Thorne JE, Foster CS, Jabs DA, Levy-Clarke GA, Nussenblatt RB *et al*: Risk of choroidal neovascularization among the uveitides. *Am J Ophthalmol* 156: 468-477.e2, 2013.
- Bahn RS: Clinical review 157: Pathophysiology of Graves' ophthalmopathy: the cycle of disease. *J Clin Endocrinol Metab* 88: 1939-1946, 2003.
- Wei H, Guan M, Qin Y, Xie C, Fu X, Gao F and Xue Y: Circulating levels of miR-146a and IL-17 are significantly correlated with the clinical activity of Graves' ophthalmopathy. *Endocr J* 61: 1087-1092, 2014.
- Hahn E, Laperriere N, Millar BA, Oestreicher J, McGowan H, Krema H, Gill H, DeAngelis D, Hurwitz J, Tucker N, *et al*: Orbital radiation therapy for Graves' ophthalmopathy: Measuring clinical efficacy and impact. *Pract Radiat Oncol* 4: 233-239, 2014.
- Bartley GB, Fatourehchi V, Kadrmas EF, Jacobsen SJ, Ilstrup DM, Garrity JA and Gorman CA: The incidence of Graves' ophthalmopathy in Olmsted County, Minnesota. *Am J Ophthalmol* 120: 511-517, 1995.
- Burch HB and Wartofsky L: Graves' ophthalmopathy: Current concepts regarding pathogenesis and management. *Endocr Rev* 14: 747-793, 1993.
- Perros P, Crombie AL and Kendall-Taylor P: Natural history of thyroid associated ophthalmopathy. *Clin Endocrinol (Oxf)* 42: 45-50, 1995.
- Zang S, Ponto KA and Kahaly GJ: Clinical review: Intravenous glucocorticoids for Graves' orbitopathy: efficacy and morbidity. *J Clin Endocrinol Metab* 96: 320-332, 2011.
- Prummel MF and Wiersinga WM: Immunomodulatory treatment of Graves' ophthalmopathy. *Thyroid* 8: 545-548, 1998.
- Mou P, Jiang LH, Zhang Y, Li YZ, Lou H, Zeng CC, Wang QH, Cheng JW and Wei RL: Common Immunosuppressive Monotherapy for Graves' Ophthalmopathy: A Meta-Analysis. *PLoS One* 10: e0139544, 2015.
- Yan SX, Wang Y, Peng GJ, Lu XP and Fu Y: Effects of technetium-99 methylenediphosphonate on cytokine-induced activation of retro-ocular fibroblasts from patients with Graves' ophthalmopathy. *Nucl Med Commun* 32: 142-146, 2011.
- Pan W, Tan T, Wang Q and Zheng J: Treatment of patients with Graves' ophthalmopathy by immunosuppressive agent and 99Tc-MDP. *Sheng Wu Yi Xue Gong Cheng Xue Za Zhi* 19: 300-301, 323, 2002 (In Chinese).