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Characterization of Re-188–Sn microparticles used for synovitis treatment

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Abstract

Rhenium-188 labeled tin (Sn) microparticles were developed for pain palliation therapy in the patients suffering from synovitis with acute pain. The rhenium tin microparticles were prepared using stannous chloride and freshly eluted $^{188}ReO_4^-$ from $^{188}W/^{188}Re$ generator. The aggregated colloidal particles, packed in a spherical form after boiling for 90–120 min were analyzed using electron microscope. The size, surface morphology and stability of microparticles were analyzed by changing temperature and volume conditions. The small colloidal particles clustered and formed spherical microparticles. The 90% of microparticles were in 5–10 μm range, after 90 min and 120 min of boiling. The radiolabeling efficiency was improved to 98% after centrifugation for 10 min at 3500 rpm. The formulations were stable but the increase in volume had inverse effect on labeling efficiency. No leak was observed from knee area up to 24 h with 15–20 mCi injection of ^{188}Re–Sn microparticles. The relief in treated patients, from the pain and inflammation, was observed clinically and by ^{99m}Tc-MDP perfusion scan.

Keywords: Synovitis; Microparticles; Rhenium-188; Bone scan

1. Introduction

Synovitis is the inflammation and damage of innermost layer of the joint capsule. The inflammation leads to the development of a proliferating and infiltrating granulation tissue (pannus), resulting in progressive destruction of articular cartilage and finally of the whole joint, that causes a long suffering of pain and subsequently leads to deformities and disability. In cases of rheumatoid arthritis, the synovitis is caused by an autoimmune response. However, the mechanical stress and abrasion of cartilage or bone are the main reasons for associated synovial inflammation in severe osteoarthritis.

In many cases, conventional long-term treatment with various combinations of drugs can bring relief, but many joints need additional local therapy. In some cases, surgery is required. The surgical removal of an inflamed synovial membrane is an invasive approach and needs hospitalization. Chemical synovectomy could be performed by intra-articular application of anti-inflammatory and antiproliferative substances. Initially in 1950s, the highly toxic agents like osmic acid, alkylating substances like nitrogen mustards, methotrexate and cobra venom were used but were then abandoned because of possible joint tissue damage (Dahmen, 1971; VonReis and Swensson, 1951). The intra-articular corticosteroid injections are the widespread therapeutic approach for local treatment of synovitis (Hollander et al., 1951). However, these compounds decrease the synthesis of important extracellular matrix components (Fubini et al., 2001; Robion et al., 2001) and progressive cell death (Barrueco et al., 1993; Podbielski and Raiss, 1986). Moreover, intra-articular steroids may also exert systemic toxicity (Behrens et al., 1976).

The radionuclides are used effectively to control the inflammatory process of the synovial membrane and are indicated as an alternative therapy to early surgical synovectomy (Knapp et al., 1998; Sledge, 1979). Rheumatoid arthritis is the main indication, however, many joints having activated arthrosis with synovial inflammation can also be treated successfully. After careful evaluation and diagnosis, a small amount of radioisotope is injected into the joint in the same manner as cortisone. These radioisotopes emit beta rays which penetrate only from fraction of a millimeter to a few millimeters and destroy the inflammatory tissue, and thus, reduce swelling and pain. In many cases, if applied in relatively early stage, complete restoration of the joint is possible. Such radionuclides when attached to colloids and on reaching the joint cavity are recognized as foreign bodies by the upper most cellular layer of the synovial membrane and are taken up by type-A-synoviocytes, the phagocyte cells (Isomaeki et al.,

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1972; Webb et al., 1969). Due to the selective radiation of the synovial membrane, there is necrosis of the cells and reduction in the inflammatory cellular proliferation (Menkes, 1979). The radionuclides are injected intra-articularly as colloidal solution, bind to citrate, sulphide, ferric hydroxide and other carriers. The studies suggest that larger particles (5–15 μm) retain for longer duration of time in the joints (Noble et al., 1983; Sledge et al., 1977).

Rhenium-188 is an attractive radionuclide for radiosynovectomy because of its suitable chemistry, $t_{1/2}$ = 16.9 h and average beta energy of 776 keV (E_{max} = 2.11 MeV, 79%) (Wang et al., 1995). These properties enable knee treatment due to its maximal tissue penetration of 11 mm, and its mean range of 3.8 mm.

2. Materials and methods

Dihydrated $SnCl_2$ and Ascorbic acid was purchased from Sigma–Aldrich, ^{188}Re was obtained from an alumina-based ^{188}W–^{188}Re generator from Oak Ridge National Laboratory. Other reagents were of analytical grade.

2.1. Preparation and radiolabeling

The ^{188}Re–Sn microparticles were prepared by the method described by Lee et al. (2003) with some modifications. HCl (0.1N, 2.0 ml) was added in 25 mg $SnCl_2{\cdot}2H_2O$ and 10 mg ascorbic acid was added in it. The solution was divided into five equal parts in separate vials and the vials were sealed properly. Freshly eluted $^{188}ReO_4^-$ (perrhenate) with saline, from an alumina-based ^{188}W/^{188}Re-generator, was added in each of the $SnCl_2{\cdot}2H_2O$ containing vials. Solutions were boiled for 0 min, 30 min, 60 min, 90 min and 120 min, respectively. The formulations were prepared by changing reaction volumes, amount of radioactivity and heating time. ^{188}Re–Sn microparticles were neutralized by adding a 0.2 M sodium phosphate buffer (pH 8.0). The microparticles were centrifuged for 10 min at 3500 rpm. Supernatant was removed to get rid of free perrhenate. The pellets of ^{188}Re–Sn microparticles were resuspended in physiological saline (0.9% saline). All the experiments were performed in triplets.

2.2. Characterization

The radioactivity, in supernatant and precipitate, was measured with the help of well counter (Berthold Well Type Counter, LB-2040). Stability of ^{188}Re–Sn microparticles was assessed in normal saline solution. Radiolabeling efficiency was determined by chromatography (ITLC-SG/normal saline plates) and radioactivity was monitored using a TLC scanner (AR 2000, Bioscan Imaging Scanner System). The batches of ^{188}Re–Sn microparticles were kept at room temperature for 24 h and 48 h after labeling and stability was evaluated by Chromatography, ITLC-SG/normal saline. All the samples of microparticles, without heating, 30 min, 60 min, 90 min and 120 min after heating, were visualized under scanning microscope and the sizes were determined using Lieca Q-win software programme after decay.

2.3. Patient studies

Patients were selected on the basis of elevated blood-pool pattern in a pre-therapeutic three-phase ^{99m}Tc-MDP-bone scan and having clinical history of knee joint pain. Proper ethical clearance from the institutional review board for using ^{188}Re–Sn microparticles and written consent from the patients were obtained. The ^{99m}Tc-MDP images were taken using duel head BG Millennium SPECT gamma camera. A passive movement of the joint was performed after injecting 15–20 mCi, as prescribed by Lee et al. (2003), of ^{188}Re–Sn microparticles (size 5–10 μm) to achieve a homogenous intra-articular distribution of the radionuclide and the joint was strictly immobilized for 48 h to prevent any significant leakage of radionuclide into venous or lymphatic vessels. In joints treated with ^{188}Re–Sn microparticles (γ = 155 keV) a distribution scan was acquired with a gamma-camera system, after injection and after 24 h after injection, to verify successful intra-articular injection and to observe the leakage.

3. Results

3.1. Morphology and size distribution

It was observed that as soon as the HCl was added in $SnCl_2$, the small spherical colloidal particles of ≤1 μm were formed (Fig. 1a). As the heating was progressed at 100 °C, the colloidal particles clustered and formed spherical microparticles. At 30 min, the microparticles prepared were of ≤3.0 μm in size (Fig. 1b). After 60 min of boiling, the spherical microparticles were within 3–5 μm range. However, after 90 min and 120 min of heating, most of the microparticles were of 5–10 μm size (Fig. 1c). After centrifugation at 3500 rpm for 10 min of the formulation (after 90 min heating), the 98–99% of the microparticles were within 5–10 μm range (Fig. 1c).

3.2. Labeling efficiency and stability

Labeling efficiencies of all batches were determined. The labeling efficiency of microparticles formed at room temperature was approximately 30%. After boiling 30 min, the labeling efficiency was less than 50%; rest of the perrhenate (ReO_4^-) was present in the supernatant. After 60 min, the labeling efficiency was more than 90%. After 90 min and 120 min of boiling, labeling efficiencies were more than 95% and reached up to 98–99% after centrifugation (Fig. 2). The free ^{188}Re was moved to the solvent-front and the colloidal and ^{188}Re–Sn microparticles remained at the origin in this condition. There was no change in labeling efficiencies of microparticles just after preparation and after 48 h at room temperature and at 37 °C.

3.3. Effect of volume

It was observed that increase in volume of perrhenate (eluted in saline solution) with the amount of $SnCl_2$ had inverse effect on labeling efficiency (Fig. 3). For single patient preparation (10 mg $SnCl_2$), the volume studied was 2.0–7.0 ml, 98% label-

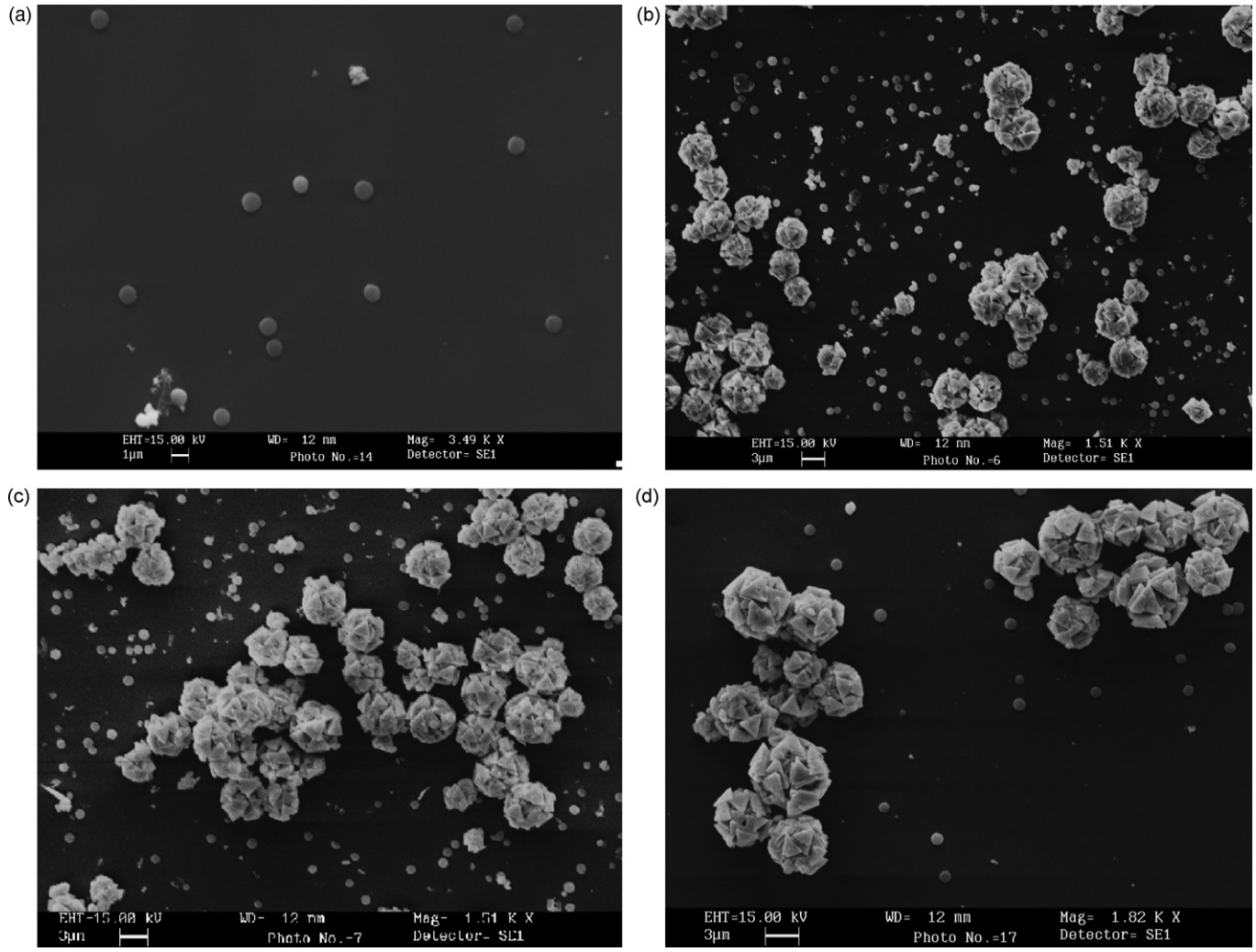

Fig. 1. (a) ^{188}Re–Sn microparticles (without heating), (b) ^{188}Re–Sn microparticles (after 60 min heating), (c) ^{188}Re–Sn microparticles (after 90 min heating) and (d) ^{188}Re–Sn microparticles (after 90 min of heating and centrifugation).

ing efficiency was achieved with 2–3 ml volume. For four to five patients dose (40–50 mg $SnCl_2$) the volume range studied was 4.0–10 ml, 98% labeling efficiency was achieved by 4.5–5.0 ml and for eight patients dose (70–80 mg $SnCl_2$), the volume range studied was 6.0–16 ml but 7–8 ml was observed to be optimal with good labeling efficiency. Labeling efficiencies were not altered even 48 h after the preparation of formulations.

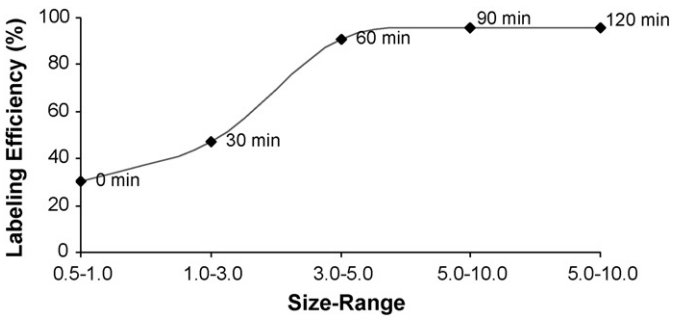

Fig. 2. Effect of heating on size-distribution and labeling efficiencies (%) of ^{188}Re–Sn microparticles.

3.4. Effect of boiling time

All batches of ^{188}Re–Sn microparticles, prepared by boiling for different times, were analyzed for stability and size- range. It was observed, in our set of experiments, that boiling time had

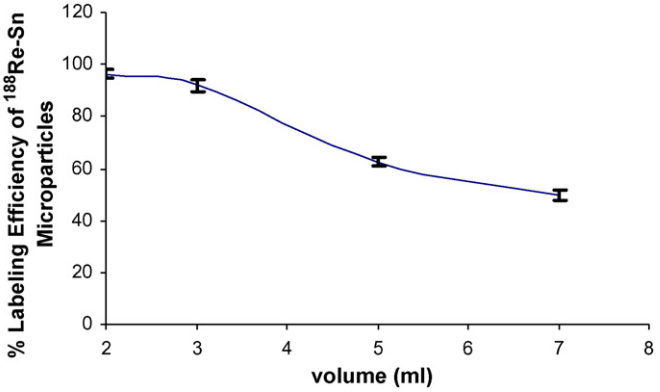

Fig. 3. Effect of volume of ^{188}Re-perrhenate on %yields of ^{188}Re–Sn microparticles.

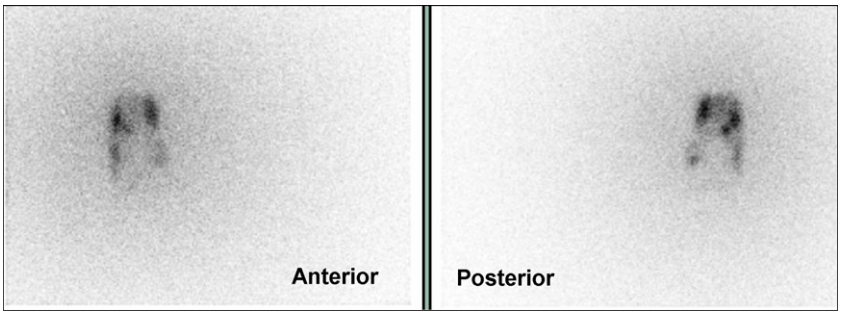

Fig. 4. ^{188}Re–Sn-microparticle distribution scan of knee joint after 24 h.

influence on both, radioactivity tagged to microparticles and the size of microparticles. The ^{188}Re–Sn particles-size was determined by scanning electron microscope for each batch and the distribution of the activity was plotted as a function of particle size grouped according to boiling time for microparticles preparation (<1.0 μm, 1.0–3.0 μm, 3.0–5.0 μm and 5.0–10.0 μm), as shown in Fig. 2. It had been observed that more than 90% of the radioactivity was found in particles bigger than 5 μm. The particle size distribution and labeling efficiencies were altered by increasing the heating time (60 min and 90 min), however, these properties remained unaltered even after heating for 120 min (Fig. 2).

3.5. Patient studies

All the patients showed good intra-articular injection in distribution scan of ^{188}Re–Sn microparticles with no visual leakage out side of the knee joint (Fig. 4). Same distribution pattern was observed even after 24 h. Patients were clinically evaluated after 3 months, 6 months and 12 months after treatment by comparing pre and post treatment ^{99m}Tc-MDP bone scans. The clinical outcome was assessed on the basis of pain relief, improvement in joint swelling, use of analgesic and improvement in mobility. Improvement was observed clinically after 1 month follow up and also by perfusion scans.

4. Discussion

Jeong et al. (2000) demonstrated that 10 mg/ml stannous chloride was necessary to radiolabel ^{188}Re–tin-colloid. In our studies, 10 mg was sufficient for one patient dose with good labeling efficiency (>95%), however, volume could vary up to 3 ml. The radiopharmaceutical particle size must be smaller enough to be engulfed by the superficial cells of the synovium but should be large enough to impede the fast biological clearance from articulation (Eduardo et al., 2004; Lee et al., 2003). The optimum size-range for the therapeutic application was reported between 2 and 10 μm (Johnson and Christian, 1967). The biggest activity loss from the particles of less than 2 μm was possible due to leakage from the joints which could cause extra radiation burden to the body organs especially to the liver and the bigger particles (>15 μm) would not be taken by superficial cells of the synovium for required therapeutic effect. But the sizes 5–15 μm were suggested for longer retention in the knee joints (Noble et al., 1983, Sledge et al., 1977). In our studies, more than 80% of the microparticles were of 5–10 μm. Moreover, 90 min of heating at 100 °C gave the good labeling efficiency (more than 90%). The yield of radiolabeled microparticles was further increased to 95–98% after centrifugation of formulations at 3500 rpm for 10 min. The unlabeled $^{188}ReO_4^-$, being water-soluble, was removed with supernatant. Our studies demonstrated comparative quick, efficient and in-house method of preparing ^{188}Re–Sn microparticles. In contrary to earlier findings (Ures et al., 2002), our modified aseptic synthesis method demonstrated that particle size and activity distribution in the microparticles were affected by heating time. The labeling efficiency was increased from 50% to 98% by increasing the heating time. The small colloidal particles clustered to form bigger spherical microparticles as the heating progressed up to 90 min and led to the more activity deposition onto the big microparticles (Fig. 1a–d). The treatment was by intra-articular injections and the distribution scan of ^{188}Re–Sn microparticles showed no visual leakage out side of the knee joint. The treatment was safe for the patients.

The safety and feasibility of ^{188}Re-microspheres had been confirmed in rabbit knee joints (Wang et al., 1998) and the effectiveness of ^{188}Re–sulphide was also demonstrated in knee joints with hemophilic arthritis (Li et al., 2004). But ^{188}Re–Sn colloid was demonstrated as well tolerated with significant improvement in patients with rheumatoid arthritis (Lee et al., 2003). Moreover, ^{188}Re–Sn colloid had higher labeling efficiency, control of particle size and greater retention in the knee joint as compared to other radiopharmaceuticals (Jeong et al., 2000). The kinetic energy of the β-particles (Re-188) impart within 11 mm (mean = 3.8 mm) of the synovial tissue. The bio-physical results of this interaction between radiation and tissue is comprised of excitation and ionization of the different atoms and molecules inside the tissue. Additional reactive particles are produced by the generation of secondary electrons, the so-called shower effect (Johnson and Yanch, 1991) and lead to higher effectiveness of radiosynovectomy as compared to anti-inflammatory or antiproliferative drugs. The biological effects inside the irradiated tissues are due to direct interactions and arising from highly reactive secondary oxygen radicals resulting in the destruction of cellular membranes by lipid peroxidation and reducing the inflammatory pannus tissue (Pavelka et al., 1975). The development of fibrous tissue, with decreased secretory activity, by superficial capillaries of the synovial membrane significantly reduces the pain (Kerschbaumer et al., 1998). Our

studies showed greater retention of injected ^{188}Re–Sn microparticles in the knee joints and good therapeutic outcome in the treated patients.

5. Conclusion

The particles of 5–10 μm are optimum for phagocytosis and retention in the joint area for longer duration. Moreover, small amount of activity is sufficient for the desired outcome. The ^{188}Re–Sn microparticles are useful for the pain relief in patients suffering from synovitis with low absorbed dose in the patient's whole body.

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