Successful Clinical Translation of \( ^{90}\)Y-Labeled Hydroxyapatite Particles Prepared Utilizing \( ^{90}\)Y Produced in Dhruva Research Reactor

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The prospect of using \( ^{90}\)Y produced by \((n,\gamma)\) reaction route in Dhruva research reactor, Trombay for use in the treatment of arthritis of knee joints was explored. Yttrium-90 produced by thermal neutron irradiation of Yttrium oxide \((Y_2O_3)\) target yielded \( ^{90}\)Y with specific activity and radionuclidic purity adequate for formulation of hydroxyapatite particles (HA) based radiation synovectomy agent. An optimized kit formulation strategy was developed for convenient one-step compounding of \( ^{90}\)Y labeled hydroxyapatite particles (\( ^{90}\)Y-HA) those are easily deployable at nuclear medicine hospital radiopharmacy. Subsequent to pre-clinical biological evaluation of \( ^{90}\)Y-HA particles, the clinical investigations were performed on patients suffering from chronic arthritis in knee joint by localized administration of 185 MBq \( ^{90}\)Y-HA into the diseased joints. Preliminary results demonstrated the therapeutic efficacy of the formulation.

Introduction

Intra-articular administration of biocompatible and biodegradable particulates/colloids radiolabeled with a suitable \( \beta\) emitting radionuclide is one of the most promising modalities for the treatment of acute and chronic inflammatory joint disorders \([1,2]\). Administered radiolabeled particulates are phagocytized by the macrophages of the inflamed synovial membrane and deliver selective radiation dose to the synovium leading to necrosis, fibrosis, sclerosis of the proliferating synovial tissue and ablation of the inflamed synovial membrane \([1-4]\). Three radionuclides namely, \( ^{90}\)Y (\( ^{90}\)Y-silicate/citrate colloid), \( ^{186}\)Re (\( ^{186}\)Re-sulfur colloid) and \( ^{169}\)Er (\( ^{169}\)Er-citrate colloid) are most widely used for large, medium and small joints, respectively \([1,2,5,6]\). The prospect of using \( ^{90}\)Y is unmatched for the treatment of large inflamed joints owing to its attractive nuclear decay characteristics [Half-life = 64.1 h, \( E_{\beta_{\max}} = 2.28 \text{ MeV} \) (maximum tissue range \( 11 \text{ mm} \)), no \( \gamma\) emissions].

Cost effective availability of clinically useful \( ^{90}\)Y at hospital radiopharmacy is a major impediment in tapping the huge potential of \( ^{90}\)Y-labeled particulates/colloids for the treatment of rheumatoid arthritis. While \( ^{90}\)Sr/\( ^{90}\)Y generator system is the conventional source of no carrier added (NCA) \( ^{90}\)Y on demand \([7]\), the unavailability of optimally designed \( ^{90}\)Sr/\( ^{90}\)Y generator that can provide \( ^{90}\)Y for direct use as radiopharmaceutical ingredient in commercial scale is a major impediment. Consequently, \( ^{90}\)Y suitable for \textit{in vivo} therapeutic applications is not available at an affordable cost worldwide, particularly in the developing countries. Alternatively, \( ^{90}\)Y is produced by neutron activation of natural yttrium target (yttrium is mononuclidic in \( ^{90}\)Y) in a nuclear research reactor, which yields low specific activity \( ^{90}\)Y due to the low neutron absorption cross section [(1.28 b) of \( ^{90}\)Y] \([8]\). We, at the Radiopharmaceuticals Division, BARC have successfully explored the potential therapeutic utility of low specific activity \( ^{90}\)Y produced by \((n,\gamma)\) route in the treatment of arthritis. This treatment can easily be made available to a large population of patients at an affordable cost in India.

In this article, we describe formulation of \( ^{90}\)Y-HA [HA, an inorganic polymer of \( \text{Ca}_10(\text{PO}_4)_{6}(\text{OH})_2 \) unit] particles using \( ^{90}\)Y produced in a medium flux research reactor (Dhruva), chemical and radiochemical characterization of the radiolabeled preparation, its pre-clinical evaluation in animal model and preliminary clinical investigations in human patients suffering from chronic rheumatoid arthritis of knee joints. Towards achieving successful translation of the product from radiochemistry laboratory to clinic, an effective kit formulation strategy was adapted for its expedient formulation at hospital radiopharmacy.

Production, radiochemical processing and quality control of \( ^{90}\)Y

Yttrium-90 was produced by irradiating natural \( Y_2O_3 \) (mononuclidic in \( ^{90}\)Y) target at a thermal neutron flux of \( \sim 1 \times 10^{10} \text{ n/cm}^2 \text{.s} \) for a period of 14 d. Subsequently, target was dissolved in 0.1 M suprapure HCl by gentle warming inside a lead-shielded glove box to obtain \( ^{90}\)Y-\( \text{YCl}_3 \) solution. Specific
activity of $^{90}$Y was $851 \pm 111$ MBq/mg (23 ± 3 mCi/mg) ($n = 6$) at the end of irradiation (EOI). The radionuclidic purity of $^{90}$Y produced was 99.93±0.03% ($n = 6$) at EOI, with $^{89}$Sr, $^{91}$Y, $^{160}$Tb and $^{169}$Yb being the radionuclidic impurities detected.

**Preparation of $^{90}$Y labeled HA particles**

A kit based approach was evaluated for convenient one-step compounding of $^{90}$Y-HA that is easily deployable at nuclear medicine hospital radiopharmacy. HA particles of 1-10 µm size range (Fig. 1) were synthesized and characterized in as per procedure reported earlier [9,10]. Kits for radiolabeling with $^{90}$Y were prepared based on the optimized parameters from systematic preformulation experiments. HA particles (5.0±0.2 mg) were weighed into each of the several sterile glass vials inside a laminar flow hood. Subsequently, 8.4±0.3 mg sodium bicarbonate were weighed and added into each of the glass vials, mixed with HA particles and sealed. Sterile water for injection (1 mL) was added to kit vial followed by addition of 200 ± 10 MBq of $^{90}$Y activity as YCl$_3$ solution. The contents of the kit vials are mixed thoroughly for 5 min using vortex mixture and set aside for 60 min at room temperature without any further agitation. Subsequently, the supernatant is carefully separated from the precipitated $^{90}$Y-HA particulates. The radiolabeled HA particles obtained as precipitate are washed using 1 mL sterile saline to ensure the removal of unlabeled (or loosely held) $^{90}$Y activity. Finally, the radiolabeled particulates were suspended in sterile normal saline, autoclaved, used for animal studies and human clinical applications after measurement of $^{90}$Y activity content.

The yield of $^{90}$Y-HA prepared using kits was found to be 98.5 ± 1.1% ($n = 25$), while the radiochemical purity of labeled particles subsequent to washing with normal saline was 99.5 ± 0.2% ($n = 25$). $^{90}$Y-HA particles showed excellent in vitro stability upto a period of 10 d (>3 half-lives of $^{90}$Y) in normal saline at 37 °C. Radiochemical purity of the preparation was found to be retained to the extent of >99% during the entire study period. The yields, radiochemical purity, stability of $^{90}$Y-HA formulation prepared utilizing reactor produced $^{90}$Y-YCl$_3$ compares well with the formulation prepared using NCA $^{90}$Y from Nordion and HA from Bio-Rad as reported by Renata et al. [11].

**Pre-clinical biological studies**

The pre-clinical biological evaluation of $^{90}$Y-HA particles was studied by carrying out biodistribution and bioluminescence imaging studies in Wistar rats artificially induced with arthritis in one of the knee joints. The results showed retention of > 98% of the injected activity within the joint cavity even after 168 h post-administration (Fig 2). Activity
detected in blood and other major organ/tissue was insignificantly low. The results of biological evaluations obtained by indigenously developed $^{90}$Y-HA formulation are comparable to those obtained from NCA $^{90}$Y as reported by Renata et al. by Bremsstrahlung scintigraphy of $^{90}$Y-HA in Wistar rats [11].

**Clinical study**

Radiation synovectomy (RSV) using $^{90}$Y-HA was performed on five patients with clinically proven rheumatoid arthritis (RA) of knee joints who are suffering from persistent joint pain and lack of mobility despite ongoing pharmacotherapy with anti-inflammatory and analgesic drugs. Dose of $^{90}$Y-HA particles (185 MBq) dispersed in 1 mL of sterile, pyrogen free normal saline was administered intra-articularly into each affected joint. The clinical study was approved by the local institutional ethics committee (Reference No. E C/ A P/ 2 4 4 / 0 7 / 2 0 1 3 , R e g i s t r a t i o n N o E C R/113/INST/TN/2013), and the patients provided written informed consent. Results of the preliminary clinical investigations showed that the administered $^{90}$Y-HA activity was retained completely within the knee joint cavity as no leakage of $^{90}$Y activity into any other non-target organs were visible in the serial whole body scans recorded upto 7 d post-administration of $^{90}$Y-HA. The representative scan of a typical treated knee joint recorded 24 h post-administration of $^{90}$Y-HA recorded using Bremsstrahlung radiation from $^{90}$Y depicts excellent localization of the radiolabeled particulates in the joint cavity with almost no extra-articular leakage. Assessment of treatment efficacy carried out over a period of six months based on the information from the patients showed substantial improvement in the disease conditions such as, reduction in joint effusion, local pain and improvement in the range of motion. Analysis of RSV treatment outcome at 6 months follow-up was based on the detailed information received from patients, clinical examinations and three phases bone scintigraphy (BS). Treatment outcome was examined in terms of joint pain during exercise, improvement measured with a 100–point visual analog scale (VAS) pain score, before and at 6 months after treatment. Six months after RSV, the VAS improvement from baseline values of the knee pain for the patient was 75 ±15%. A comparison of pre-therapy, 3 months post-therapy and 6 months post-therapy $^{99m}$Tc-MDP scans of the knee joint region of a patient (Fig. 3) clearly demonstrates significant reduction of synovial inflammation as a direct evidence of therapeutic efficacy of $^{90}$Y-HA. More the improvement in VAS score, more decrease in blood pooling compared to pretherapy scintigraphic changes were noted. Long-term treatment efficacy based on quantitative data obtained from clinical and pathological examinations are encouraging.

**Conclusion**

The utility of $^{90}$Y obtained from neutron activation production route for the formulation of clinical doses of $^{90}$Y-HA particles using ready-to-use single vial kits of HA particles at the hospital radiopharmacy set up is successfully developed and demonstrated. The single-vial kit, provided a convenient and reproducible method for facile preparation of $^{90}$Y-HA particles (200 ± 10 MBq) with high yield (>98%) and radiochemical purity (>99%) in a clinical setting. Preliminary clinical studies demonstrate the effectiveness of $^{90}$Y-HA in terms of pain control, functional improvement and prevention of disease progression in rheumatoid arthritis patients. Although radiation synovectomy (RSV) with $^{90}$Y based radiopharmaceuticals have been used extensively in Europe for the past 25 years to treat rheumatoid arthritis in the knee joint [12], it has generated only modest clinical interest in India till date. The comprehensive and systematic study from radiochemistry laboratory to nuclear medicine clinic demonstrates a potential therapeutic utility of indigenous (n,$\gamma$) produced $^{90}$Y in India.
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References