

## Evaluation of four radiopharmaceuticals for imaging inflammation in a rabbit model of arthritis

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We compared the utility of four radiopharmaceuticals;  $^{111}\text{In}$ -chloride,  $^{67}\text{Ga}$ -citrate,  $^{111}\text{In}$  labeled leukocytes (WBCs) and  $^{99\text{m}}\text{Tc}$ -MDP for assessing the inflammatory response in antigen induced arthritis in a rabbit model. A total of 20 rabbits, divided into four equal groups, were included in this study. Each group was studied twice with a single radiotracer: a baseline study and a follow-up study after induction of the arthritis. Knee to knee, knee to whole body, and knee to liver (except for the group studied with  $^{99\text{m}}\text{Tc}$ -MDP) ratios were generated. Knee to knee ratios showed no significant change from baseline to arthritis studies in any of the four groups.

Significantly increased knee to total body ratios were seen in all of the groups, except for the group studied with  $^{99\text{m}}\text{Tc}$ -MDP. The greatest increase was seen in the group studied with  $^{111}\text{In}$ -chloride. Significantly increased knee to liver ratios were observed in all three groups for which these ratios were generated and again the greatest increase was observed in the group studied with  $^{111}\text{In}$ -chloride. In summary, based on the higher uptake observed in this group, of the four radiotracers evaluated,  $^{111}\text{In}$ -chloride is probably the most useful for monitoring the inflammatory response in antigen induced arthritis. The symmetry of the response suggests that it may also be useful in monitoring the response to therapy.

**Key words:** imaging joint inflammation, antigen induced arthritis in rabbits,  $^{111}\text{In}$ -chloride,  $^{67}\text{Ga}$ -citrate,  $^{111}\text{In}$ -WBCs,  $^{99\text{m}}\text{Tc}$ -MDP

### INTRODUCTION

ANTIGEN INDUCED ARTHRITIS (AIA) in rabbits, an immunologically induced inflammatory arthritis, is one of the most frequently used animal models to study rheumatic diseases because of its similarity to human rheumatoid arthritis.<sup>1</sup> This model has recently been used to evaluate the potential therapeutic utility of synovial ablation via intraarticular radionuclide administration (radiation synovectomy).<sup>2</sup>

The need for an objective, yet non-invasive, method to evaluate the degree of synovial inflammation is well

recognized and several radiopharmaceuticals have been investigated. Radiotracers such as  $^{99\text{m}}\text{Tc}$  pertechnetate and  $^{99\text{m}}\text{Tc}$  pyrophosphate have shown higher uptake in the inflamed vs. control knees but the activity was concentrated primarily in bone rather than in the inflamed synovium.<sup>3</sup> Recently,  $^{67}\text{Ga}$ -citrate<sup>4</sup> and  $^{111}\text{In}$ -chloride<sup>5</sup> have also been evaluated in the arthritic rabbit joint.

In an effort to select the optimum radiotracer(s), we prospectively compared  $^{111}\text{In}$  labeled leukocytes,  $^{111}\text{In}$ -chloride,  $^{67}\text{Ga}$ -citrate and  $^{99\text{m}}\text{Tc}$ -methylenediphosphonate in the same group of rabbits with AIA.

### MATERIALS AND METHODS

#### *Animal model*

Antigen induced arthritis (AIA) was produced by the intra-articular injection of ovalbumin (Sigma, St. Louis, MO, USA) in 20 New Zealand white rabbits (2-4 months

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old) previously immunized with this antigen in Freund's complete adjuvant (Sigma, St. Louis, MO, USA).<sup>6</sup> The immunization cycle consisted of five intradermal injections on the back of each rabbit of 1 ml of an emulsion of equal volumes of ovalbumin with Freund's complete adjuvant. Two sensitizations were performed 3 weeks apart. Three weeks after the second sensitization, a single injection of 2.5 mg of ovalbumin in 0.25 ml saline (challenge dose) was administered to each rabbit through the patellar ligament into the knee of both hind legs. By day 5 all challenged knees appeared swollen, in agreement with previously published observations on this model.<sup>7</sup>

#### Radiopharmaceutical preparation

<sup>111</sup>In-chloride: no-carrier-added <sup>111</sup>In-chloride in 0.05 N HCl (Amersham, Arlington Heights, IL, USA) was incubated at 37°C for 15 min with 1 ml of rabbit plasma to facilitate its administration in the form of a complex with plasma transferrin. After incubation, 0.3–0.4 ml containing 7–11 MBq (0.2–0.3 mCi) was injected into an ear vein.

<sup>67</sup>Ga-citrate: approximately 18.5 MBq (0.5 mCi) of Ga-67 was intravenously injected as <sup>67</sup>Ga-citrate (Du Pont, Billerica, MA, USA) to facilitate solubilization.

<sup>111</sup>In-labeled WBC: cells were labeled with 22–29 MBq (0.6–0.8 mCi) of <sup>111</sup>In-oxine (Amersham, Arlington Heights, IL, USA) as described elsewhere<sup>8</sup> with 35 ml of human venous blood, from a single human volunteer. Three to four MBq (0.08–0.12 mCi) of labeled cells from this preparation were administered to each rabbit.

<sup>99m</sup>Tc-MDP: a kit of medronic acid (Du Pont, Billerica, MA, USA) was labeled with generator produced Tc-99m according to the routine procedure. An aliquot was further diluted 1 to 10 with normal saline in order to obtain 30–37 MBq (0.8–1.0 mCi) of Tc-99m activity in 0.3–0.4 ml.

#### Imaging studies

**Experimental design:** The 20 rabbits were divided into four groups of 5 animals each. Each group underwent two separate imaging studies with a single radiotracer: the first study (baseline) prior to the intra-articular injection of the antigen and the second (arthritis) 7 to 10 days after antigen administration. Images were acquired 48 hours after injection of <sup>111</sup>In-chloride and <sup>67</sup>Ga-citrate, 24 hours after injection of <sup>111</sup>In-WBCs and 4 hours after injection of <sup>99m</sup>Tc-MDP.

**Scanning techniques:** Anterior total body images were acquired for 10 minutes with a large field of view gamma camera (GE 500 Starport, Milwaukee, WI, USA) equipped with either a high resolution low energy, or medium energy parallel hole collimator, depending on the radiotracer. Knee images were acquired with a second

**Table 1** Ratios right/left knee (mean  $\pm$  SD) for baseline and arthritis studies

	Baseline	Arthritis	Significance
<sup>111</sup> In-chloride	0.98 $\pm$ 0.08	1.03 $\pm$ 0.05	p > 0.06
<sup>67</sup> Ga-citrate	1.12 $\pm$ 0.09	1.18 $\pm$ 0.14	p > 0.06
<sup>111</sup> In-WBCs	1.05 $\pm$ 0.07	1.25 $\pm$ 0.22	p > 0.06
<sup>99m</sup> Tc-MDP	1.06 $\pm$ 0.11	1.14 $\pm$ 0.17	p > 0.06

**Table 2** Percent knee to total body ratios ( $\pm$  SD) for baseline and arthritis studies

	Baseline	Arthritis	Significance
<sup>111</sup> In-chloride	3.68 $\pm$ 0.46	11.85 $\pm$ 1.74	p < 0.06
<sup>67</sup> Ga-citrate	5.60 $\pm$ 0.85	10.14 $\pm$ 2.26	p < 0.06
<sup>111</sup> In-WBCs	2.89 $\pm$ 0.37	4.51 $\pm$ 0.66	p < 0.06
<sup>99m</sup> Tc-MDP	13.38 $\pm$ 0.55	12.04 $\pm$ 4.62	p > 0.06

**Table 3** Percent knee to liver ratios ( $\pm$  SD) for baseline and arthritis studies

	Baseline	Arthritis	Significance
<sup>111</sup> In-chloride	16.90 $\pm$ 2.93	60.56 $\pm$ 9.60	p < 0.06
<sup>67</sup> Ga-citrate	27.37 $\pm$ 1.29	48.60 $\pm$ 5.36	p < 0.06
<sup>111</sup> In-WBCs	7.19 $\pm$ 1.20	11.15 $\pm$ 2.48	p < 0.06

gamma camera (Picker Dyna 4/15, Cleveland, OH, USA) equipped with a pin-hole collimator. Imaging was performed with the knees in 30° flexion.

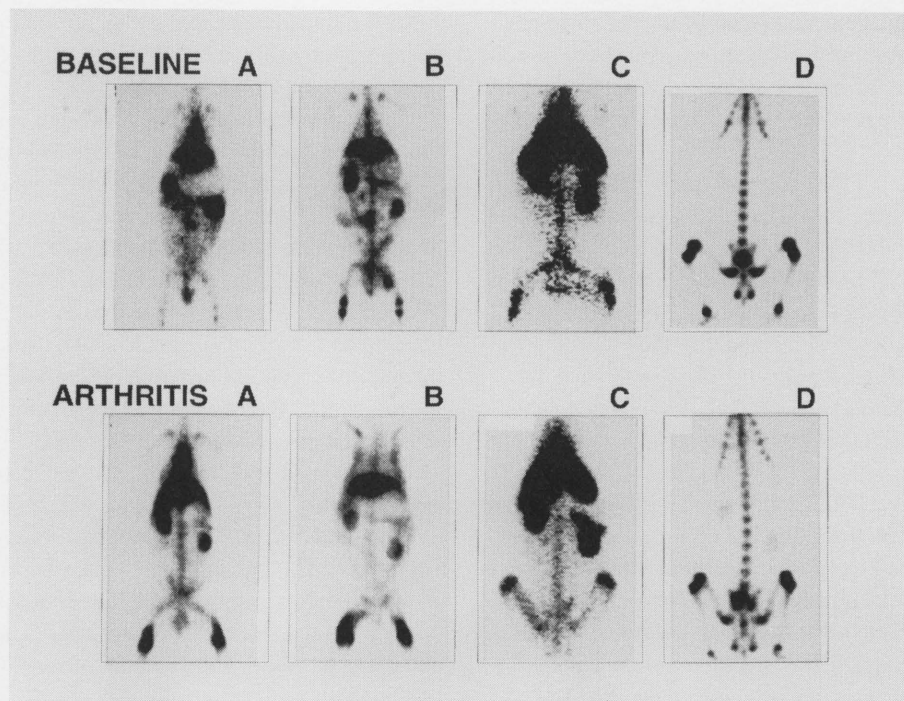
**Image processing:** To quantitate radiopharmaceutical accumulation in the joints under study, regions of interest (ROI) were drawn over each knee, the liver and the entire body. Knee to knee and knee to total body ratios (%) were generated for all 20 rabbits. Knee to liver ratios (%) were determined for all animals except those studied with <sup>99m</sup>Tc-MDP.

**Statistical Analysis:** Because the number of measurements in each group was small (< 12), the nonparametric Mann-Whitney test was selected for assessing the significance of the differences between radiotracer uptakes in baseline and arthritis studies. The level of significance with this method is p < 0.06.

## RESULTS

There was no statistically significant (p > 0.06) difference between the mean knee/knee ratios in the baseline and arthritis studies in any of the four groups (Table 1).

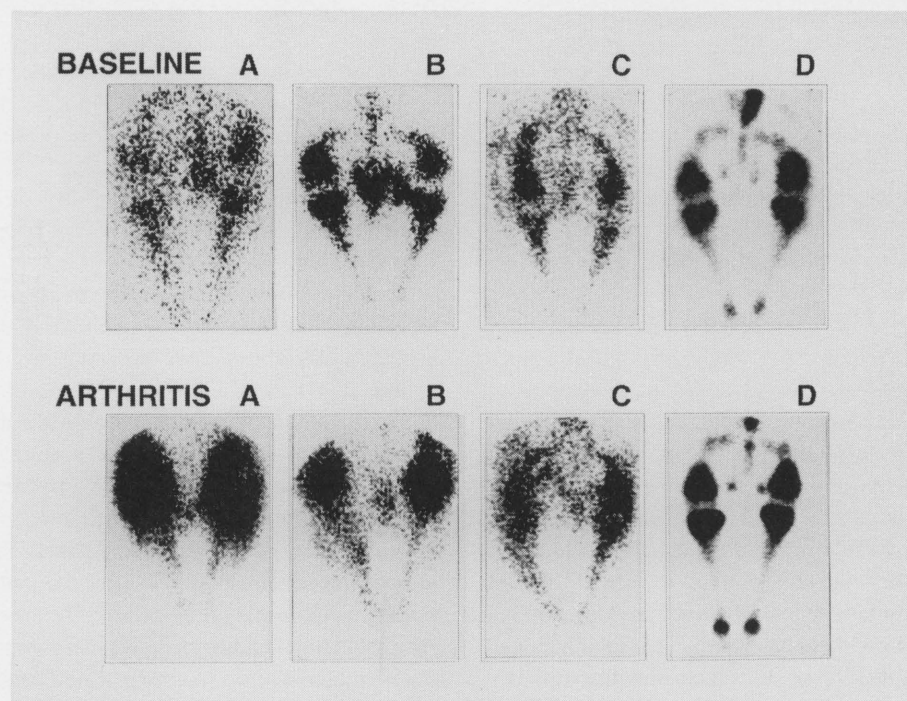
A significant increase in the mean knee to total body ratios, from baseline to arthritis studies was observed in the groups studied with <sup>111</sup>In-chloride, <sup>67</sup>Ga-citrate, and <sup>111</sup>In-WBCs. The magnitude of the increase was greatest with <sup>111</sup>In-chloride. No significant change between baseline and arthritis results was identified in the <sup>99m</sup>Tc-MDP groups (Table 2).



**Fig. 1** Representative anterior total body images of rabbits before (baseline) and after (arthritis) bilateral induction of articular inflammation.

$^{111}\text{In}$ -chloride (A);  $^{67}\text{Ga}$ -citrate (B);  $^{111}\text{In}$ -WBCs (C);  $^{99\text{m}}\text{Tc}$ -MDP (D).

Increased knee uptake on the arthritis studies is most obvious in the rabbits studied with  $^{111}\text{In}$ -chloride and  $^{67}\text{Ga}$ -citrate. Virtually no change from baseline to arthritis is noted in the  $^{99\text{m}}\text{Tc}$ -MDP images.



**Fig. 2** Representative pin-hole images of rabbit knees before (baseline) and after (arthritis) bilateral induction of articular inflammation.

$^{111}\text{In}$ -chloride (A);  $^{67}\text{Ga}$ -citrate (B);  $^{111}\text{In}$ -WBCs (C);  $^{99\text{m}}\text{Tc}$ -MDP (D).

Again, the most dramatic changes are seen with  $^{111}\text{In}$ -chloride and  $^{67}\text{Ga}$ -citrate. Note also the symmetry of the inflammatory response.

Similarly, a significant increase in the mean knee to liver ratios was observed in all three groups for which these ratios were computed. Again, the magnitude of change was greatest in the group studied with  $^{111}\text{In}$ -chloride (Table 3).

Visual inspection of the images correlated well with the quantitative data: the most drastic changes observed between baseline and arthritis studies were found in the group studied with  $^{111}\text{In}$ -chloride followed by  $^{67}\text{Ga}$ -citrate and  $^{111}\text{In}$ -WBCs. It was virtually impossible to distinguish the baseline from the arthritis images in the group studied with  $^{99\text{m}}\text{Tc}$ -MDP (Figs. 1 and 2).

## DISCUSSION

The arthritis induced in this rabbit model is histologically well described in the literature.<sup>9</sup> The initial cellular immune response consists of an intraarticular polymorphonuclear leukocyte accumulation, which peaks at about 1 day after injection of the antigen. By 4 days the cellular response consists primarily of a mononuclear infiltrate made up of lymphocytes, plasma cells, macrophages, and fibroblasts. The arthritis itself is chronic and may last several months following a single injection of antigen.

The pathologic changes that occur in this animal model can explain the different results obtained with the various radiotracers under evaluation. The failure of  $^{99\text{m}}\text{Tc}$ -MDP to show differences between the baseline and arthritis may be attributed to several factors. The animals were studied early in the course of their disease, prior to the development of any significant bony abnormalities, and therefore the lack of changes between the baseline and following bone scans is not surprising. Furthermore while some increased uptake of radiotracer secondary to local hyperemia might be expected, the rabbits used were immature; the (normally) intense uptake of the bone agent in active epiphyseal growth plates could have easily obscured subtle changes.

Labeled leukocyte imaging is useful for identifying sites of infection. When using a mixed population of leukocytes, the majority of cells labeled are neutrophils: consequently the study is most useful for conditions that incite a neutrophilic response. We labeled human leukocytes because neutrophils constitute 60–70% of circulating leukocytes unlike the rabbit in which the lymphocyte is the predominant circulating leukocyte and neutrophils comprise only 30%.<sup>10</sup> Since lymphocytes are uniquely sensitive to self-irradiation,<sup>11</sup> the radioactivity doses necessary for camera imaging may severely alter cell kinetics and may render invalid all but short-term imaging studies. Indium-111-oxine lymphocytes, reinjected into rats, have shown uptake in the lymph nodes as well as liver and spleen.<sup>11</sup> For this reason, in the present study, human leukocytes, rich in neutrophils, which are known to localize in foci of inflammation,<sup>12</sup> were used. By the time these animals were imaged the neutrophil response had sub-

sided, having been replaced by a mononuclear cell infiltrate making this radiotracer less sensitive in detecting arthritis in this animal model.

The most striking changes between baseline and arthritis studies were seen in the rabbits studied with  $^{67}\text{Ga}$  and  $^{111}\text{In}$ . Both of these radiocations are bound tightly to transferrin and albumin, and are transported by these protein complexes into the interstitial space.<sup>13</sup> The experimental observations, demonstrating that tracer ionic indium is bound entirely both *in vitro* and *in vivo* to transferrin,<sup>14</sup> prompted us to incubate the low pH  $^{111}\text{In}$ -chloride solution with 1 ml of rabbit plasma in order to facilitate its administration. The increased capillary membrane permeability that is present in inflammation is probably responsible, at least in part, for local uptake of these two radiotracers. Despite these similarities, however, a greater magnitude of change between baseline and arthritis was found in the uptake of  $^{111}\text{In}$  than in that of  $^{67}\text{Ga}$ . Part of the explanation for this discrepancy may lie in the fact that iron binding proteins and cells accumulate at sites of active synovitis.<sup>15</sup> While both gallium and indium are bound to these structures, indium competes more specifically than gallium for the iron-binding sites of transferrin.

The utility of these two agents in imaging joint inflammation has previously been reported in separate studies. In the acute arthritis model, induced in rabbit knees by the intra-articular injection of Zymosan,<sup>4</sup>  $^{67}\text{Ga}$ -citrate showed increased uptake in the inflamed knee compared to the control contralateral knee in the first five days following arthritis induction. Increased knee-to-soft tissue ratios in the involved knees compared to the control was also observed after injection of  $^{111}\text{In}$ -chloride, in a rabbit model of AIA, similar to that used in the present communication.<sup>5</sup>

Another potentially useful radiotracer which has been shown to localize in a variety of infection/inflammation sites is the human polyclonal IgG labeled with  $^{111}\text{In}$  or  $^{99\text{m}}\text{Tc}$ .<sup>16</sup> Until now, however, the exact mechanism of its localization has not quite been elucidated. Although significant correlations have been observed between joint uptake of  $^{99\text{m}}\text{Tc}$  IgG and clinical scores for joint pain and/or swelling,<sup>17</sup> other authors have shown that the index of uptake of  $^{99\text{m}}\text{Tc}$ -IgG in 18 patients with rheumatoid arthritis was always lower than that of  $^{99\text{m}}\text{Tc}$ -MDP regardless of the degree of inflammation in the joints.<sup>18</sup>

In contrast to other reports,<sup>5</sup> we have induced arthritis in both knees. The reason is that this animal model has as one of its major applications the assessment of the efficacy of treatments by histological comparison of treated vs. untreated joints. In order to noninvasively monitor response to local therapy, the method to be used must be sensitive to the inflammatory changes induced.

Both  $^{67}\text{Ga}$ -citrate and  $^{111}\text{In}$ -chloride are acceptable in this regard. Moreover, the symmetry of the inflammatory response elicited suggests that these two radiotracers may be, by both qualitative and quantitative comparison with

the untreated knee, useful for monitoring response to therapy.

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