

## Direct Labelling of Bioactive Molecules with Technetium to evaluate their Biodistribution in Arthritic Joints by Imaging.

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### Introduction

Core peptide (CP; GLRILLKLV) is a hydrophobic peptide derived from the TCR- $\alpha$  transmembrane region. Previous studies have shown that CP can inhibit T cell-mediated immune responses both *in vitro* and *in vivo*. CP given subcutaneously or intraperitoneally was found to significantly reduce the induction of T cell-mediated inflammation in adjuvant-induced arthritis, allergic encephalomyelitis and delayed-type contact hypersensitivity in animal model [1,2]. In humans, Gollner *et al.* [3] had found its effectiveness in the treatment of autoimmune skin conditions. However, the actual mechanism of how CP acts remains unknown. Here we report the use of technetium for evaluating biodistribution of arthritic drugs *in vivo*.

The labelling of biologically active molecules with <sup>99m</sup>Tc for radiopharmaceutical purposes is a field of intense research. Naturally occurring bidentate ligands such as N-terminal histidines in peptide chains could be effectively labelled with <sup>99m</sup>Tc-pertechnetate through tricarbonyl linkers [4]. They have further demonstrated that histidine could be introduced at the N-terminal end of any peptide, which would allow labelling with <sup>99m</sup>TcO<sub>4</sub><sup>-</sup> at low ligand concentration [5]. Based on this established method, <sup>99m</sup>Tc-CP was prepared with histidine attached at the N-terminal end of CP and its utility was explored in detecting inflammatory arthritis in adjuvant induced arthritis in animals.

We also here report technetium-99m labelled 2-deoxyglucoseamine (ECDG) as described by Yang *et al.* [6,7] which is a glucose analogue radiolabelled with technetium-99m via a N<sub>2</sub>S<sub>2</sub> chelator. <sup>99m</sup>Tc-ECDG was prepared and its utility was explored in detecting inflammatory arthritis in adjuvant induced arthritis in animals.

### Results and Discussion

When either <sup>99m</sup>Tc-His-CP or <sup>99m</sup>Tc-His-Control peptides (5-10 MBq each) were injected in the adjuvant induced arthritic mice, there was intense focal uptake of the agents at the arthritic joints and at the arthritic lesions in the tail bones (Figure 1). There was no qualitative difference in the biodistribution between these agents although the intensity of <sup>99m</sup>Tc-His-CP uptake was significantly higher than <sup>99m</sup>Tc-His-Control peptide. When <sup>99m</sup>Tc-labelled isolink was injected in these mice, as a control experiment, there was an intense uptake of the agent at the kidneys, liver, heart and the bladder. There was no uptake at the arthritic joints or at the arthritic lesions as was observed with peptide compounds (eg. <sup>99m</sup>Tc-His-CP).

Studies were performed to demonstrate the potential imaging capability of <sup>99m</sup>Tc-ECDG in detecting similar inflammation conditions. The radiochemical purity (RCP) of <sup>99m</sup>Tc-ECDG preparation was >98% by ITLC-SG/acetone chromatography, which was stable for more than 24 hrs post-reconstitution (RCP >95%). The adjuvant induced arthritis mice were intravenously injected with approximately 5 to 10 MBq of <sup>99m</sup>Tc-ECDG and imaged at 1, 2 and 24 hrs post-injection. SPECT imaging studies showed focal uptake of <sup>99m</sup>Tc-ECDG at the arthritic inflammatory lesions. On the other hand, when <sup>99m</sup>Tc-EC was injected in these mice, there was an intense physiological uptake of the agent in the kidneys, liver and bladder but not at the inflammatory lesions. These experimental evidence strongly suggest that <sup>99m</sup>Tc-ECDG has specifically accumulated at the arthritic inflammatory lesions.

### Acknowledgments

We would like to express our sincere appreciation to Ms Valentina Valova from the Mass Spectrometry Facility, Children's Medical Research Institute for obtaining the mass spectrometry data. Financial support from the NSW Arthritis Foundation is also acknowledged. M Ali acknowledges the University of Sydney and the Faculty of Medicine for the Henry Langly Fellowship.

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**Figure 1.** Imaging inflammatory arthritis with  $^{99m}\text{Tc}$ -His-Core-Peptide ( $^{99m}\text{Tc}$ -His-CP)

