In vivo evaluation of $^{111}$In-DTPA-$N$-TIMP-2 in Kaposi sarcoma associated with HIV infection

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Abstract. Matrix metalloproteinases are the major agents responsible for the degradation of extracellular matrix and are produced at high levels by transformed and tumour cells, where they participate in the metastatic process by allowing local invasion. They are also more active at sites of new normal growth and angiogenesis. In the early stages of Kaposi sarcoma (KS), in vitro studies have demonstrated that vascular invasion can be inhibited by inhibitors of matrix metalloproteinases. Imaging of visceral and cutaneous KS presents a problem and therefore the potential use of a labelled inhibitor of metalloproteinases, $N$-TIMP-2, with indium-111 was thought to present a possible imaging tool. The biokinetics, dosimetry and potential for imaging with $^{111}$In-DTPA-$N$-TIMP-2 were assessed in five patients with HIV infection and KS. Between 103.1 and 108.0 MBq of this agent was injected into each patient, and the dynamic uptake over the kidneys was assessed, whole body scans were performed and blood samples were obtained. The clearance from the blood was rapid, with a first component half-time of 16.6±3.4 min and a second component half-time of 9.68±2.68 h. Two out of five patients experienced minor shivering but one of these patients was generally unwell before the study. The last three patients had no such problems. The tracer distributed predominantly to the kidneys and did not localise in other tissues. No KS lesions were clearly identified. $^{111}$In-DTPA-$N$-TIMP-2 can be successfully prepared and administered to patients safely, with a biodistribution and dosimetry which would allow its use as an imaging tracer. It is unlikely to be of use for imaging KS, but may have a role in other tumours that produce matrix metalloproteinases.

Keywords: TIMP-2 – Matrix metalloproteinases – Kaposi sarcoma – Indium-111 – Tumour

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Introduction

Kaposi sarcoma (KS) is a rare multicentric, multi-organ tumour which occurs more frequently in homosexual or bisexual men than in other patient groups with human immunodeficiency virus (HIV) infection. Human herpes virus type 8 (HHV-8), also known as KS-associated herpes virus, is now understood to be a major aetiological factor in the development of KS of all types. It is transmitted not only sexually but also by non-sexual routes. Unlike classical KS, this disease in the immunosuppressed patient can vary markedly both in the extent of disease at presentation and in the rate of progression. The clinical features can vary depending on the site and size of the sarcomatous lesions.

KS represents a major challenge to the nuclear medicine clinician. The treatment and prognosis will undoubtedly be related to the bulk of disease and its site. The localisation of the extent of disease is difficult. Gallium-67 is not taken up [1, 2, 3]. Thallium-201 has been shown to identify disease in the skin, lymph nodes and lung [4, 5] but has a variable uptake in KS and cannot be used to identify abdominal disease as it is excreted through the bowel. Positron emission tomography has also been used with little success. Both fluorodeoxyglucose and methionine have low or no uptake in pulmonary KS [6]. The problem with all of these agents is their uptake in other conditions associated with HIV, such as infections and lymphoma. A positive imaging agent is required for KS imaging.

We decided to investigate matrix metalloproteinases (MMPs) as a possible target for radionuclide imaging of
KS. MMPs are the major mediators of extracellular matrix degradation and are produced at high levels by transformed and tumour cells, where they participate in the metastatic process by allowing local invasion. Their activity is regulated by endogenous tissue inhibitors of metalloproteinases (TIMPs). There is considerable evidence that increased MMP expression is implicated in KS progression. Benelli et al. [7] showed that host cells are recruited into the KS lesions by the release of cytokines, growth factors and angiogenic factors, and that supernatants from KS cell culture induce vascular cell invasion in vitro accompanied by the release of MMPs. Addition of the MMP inhibitors TIMP-2 and peptide 74, a peptide from the MMP propeptide region, inhibited this endothelial cell invasion. Albini et al. [8] demonstrated that such supernatants also induce KS lesion characteristics in vivo when injected into mice. Co-injection of TIMP-2 with the supernatant inhibited this angiogenic response by 65% [8, 9]. Meade-Tollin et al. [10] demonstrated that KS-derived cells express a variety of MMPs not produced by normal endothelial cells, and Blankaert et al. [11] showed that KS-derived cells release MMP-2 and MMP-9 (gelatinase A and B, respectively).

We have identified N-TIMP-2 as a potential carrier of radionuclides for imaging MMP expression. N-TIMP-2 is the N-terminal domain of human TIMP-2. It should be rapidly cleared from circulation owing to its modest molecular weight (14,084 Da). It is a human protein and thus should not elicit an immune response. Recombinant N-TIMP-2 is readily synthesised in pure form [12]. We have synthesised a DTPA conjugate of N-TIMP-2 for labelling with indium-111 and shown that it retains full MMP-inhibitory activity and is stable in serum [13].

Here we report the results of a pilot study to evaluate the biodistribution, vascular clearance and possible lesion uptake of 111In-DTPA-N-TIMP-2 in HIV-positive patients with KS.

**Materials and methods**

This study was approved by the St Thomas’ Hospital Research Ethics committee and the Administration of Radioactive Substances Advisory Committee (ARSAC). Patients who were HIV antibody positive and had either widespread clinically evident KS or a histological diagnosis of KS (four patients had biopsy-proven KS and one, clinically evident KS) were recruited for this study. Patient characteristics are defined in Table 1. All patients had new KS lesions developing on their skin or oral pharynx and were known to have visceral KS. All had received previous chemotherapy and/or highly active antiretroviral therapy (HAART). Patient 5 had extensive skin KS and an oropharyngeal lesion as well as extensive pulmonary disease, as shown on the chest X-ray (Fig. 1). Other patients had a mixture of old and new lesions at the time of the study.

Each patient was injected with a nominal 100 MBq (mean 104.6 MBq, range 103.1–108.0 MBq) of 111In-DTPA-N-TIMP-2 prepared as previously described. [13] All image data were acquired on a rectangular dual-headed Toshiba GCA-7200A/DI camera system (full field of view 50 cm×40 cm) using a pair of medium-energy general-purpose collimators. With the camera positioned in the anterior/posterior projection over the abdominal re-

**Table 1. The demographic data for the subjects**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>CD4</th>
<th>Viral load</th>
<th>Skin KS</th>
<th>Visceral KS</th>
<th>Chemotherapy given + time before study</th>
<th>HAART</th>
<th>Risk factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45 yrs</td>
<td>&lt;50</td>
<td>7,132</td>
<td>Widespread</td>
<td>Not confirmed</td>
<td>2 courses of bleomycin and vincristine – last 2 months before study</td>
<td>Yes/poor compliance</td>
<td>Homosexual</td>
</tr>
<tr>
<td>2</td>
<td>38 yrs</td>
<td>&lt;50</td>
<td>100,000</td>
<td>Limbs and eyelid</td>
<td>Pulmonary</td>
<td>2 courses of bleomycin and vincristine – last 1 day before study</td>
<td>Started within 3 months</td>
<td>Heterosexual</td>
</tr>
<tr>
<td>3</td>
<td>36 yrs</td>
<td>109</td>
<td>&lt;50</td>
<td>Face and legs</td>
<td>Nil</td>
<td>Pt. on 6-weekly lipodaunorubicin – new KS appearing between therapies</td>
<td>Yes</td>
<td>Homosexual</td>
</tr>
<tr>
<td>4</td>
<td>33 yrs</td>
<td>70</td>
<td>417,900</td>
<td>Limbs</td>
<td>Not confirmed</td>
<td>2 courses of bleomycin and vincristine – last 2 weeks before study</td>
<td>Started within 3 months</td>
<td>Heterosexual</td>
</tr>
<tr>
<td>5</td>
<td>22 yrs</td>
<td>&lt;50</td>
<td>100,000</td>
<td>Oral cavity and pulmonary</td>
<td>Limbs</td>
<td>2 courses of bleomycin and vincristine – last 2 weeks before study</td>
<td>No</td>
<td>Haemophiliac</td>
</tr>
</tbody>
</table>