Upregulation of granulocyte CD11b (CR 3) after labelling with technetium-99m hexamethylpropylene amine oxime

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Abstract. Today technetium-99m hexamethylpropylene amine oxime (99mTc-HMPAO) is widely used for leucocyte scintigraphy, as 99mTc-HMPAO selectively labels granulocytes in mixed leucocyte suspensions. However, the influence of cell labelling on the expression of specific adhesion proteins has not been studied before. Therefore, we investigated five patients, four of whom had established Crohn’s disease. We found that leucocyte labelling with 99mTc-HMPAO induces increased expression of the glucoprotein receptor CD11b on granulocates, but it is not clear whether this upregulation affects the functional integrity of the granulocytes.

Key words: Granulocyte CD11b – Technetium-99m hexamethylpropylene amine oxime


Introduction

Technetium-99m hexamethylpropylene amine oxime (HMPAO) selectively labels granulocytes in mixed leucocyte suspensions [1] and is now being widely used for leucocyte scintigraphy. Extravasation of leucocytes into tissues is a central event in the inflammatory response. In order to migrate, granulocytes start to “roll” on selectins on vascular endothelial cells [2]. After the rolling speed has decreased, and the leucocytes have finally stopped, interactions between leucocyte CD11b and endothelial cell intercellular adhesion molecule-1 take part in the extravasation process [3]. Leucocyte CD11b (Mac-1, CR-3) is a β-2 integrin adhesion molecule stored in intracellular pools [4], and is readily mobilized to the cell surface upon activation with different chemotactic factors [5] or cytokines like TNF-α [4]. In this way, the surface expression of CD11b can be regarded as a marker of cellular activation in response to various stimuli.

Materials and methods

Patients

Five patients, four men and one woman aged 23–49 years, were investigated. Four patients had established Crohn’s disease. In one patient previous radiological signs of ileal Crohn’s disease had not been confirmed later. None were receiving any medication. Two patients had active and three patients quiescent disease.

Methods

Labelling protocol. Mixed leucocytes were labelled with 99mTc-HMPAO (Ceretec, Amersham AB, Sweden) in plasma according to a slight modification of a previously published method [1]. A volume of 102 ml venous blood was collected into 18 ml of acid citrate dextrose (ACD, Travenol). Twenty ml was centrifuged at 2000 g for 10 min to yield plasma, which subsequently was used to suspend cells for labelling and reinjection. Twenty ml of a 6% w/v solution of polyhydroxyethyl starch (Plasmasteril, Fresenius, Germany) was added to the remaining 100 ml of anticoagulated blood to promote red blood cell sedimentation. The leucocyte and platelet-rich plasma was centrifuged at 150 g for 5 min. A second centrifugation step for the platelet-rich plasma was omitted [6], since centrifugation might release platelet factors that in turn might influence the behaviour and the adhesiveness of granulocytes. 99mTc-HMPAO was formed by adding about 500 MBq 99mTc in 5 ml isotonic saline to a vial of Ceretec containing 0.5 mg HMPAO, 7.6 µg stannous chloride dihydrate and 4.5 mg sodium chloride with nitrogen. Five ml of the 99mTc complex was then immediately added to the mixed leucocytes in 1 ml plasma. The cells were incubated for 10 min at room temperature, washed with 10 ml autologous plasma containing polyhydroxyethyl starch, and centrifuged at 150 g for 5 min. The cell pellet was resuspended in 5 ml plasma without polyhydroxyethyl starch and reinjected.
Table 1. Expression of granulocytes CD11b during cell labelling with 99mTc-HMPAO (n=5). Values are median (inter-quartile range) values of the mean fluorescence intensity of analysed cells from each patient. Wilcoxon sign rank test

<table>
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<th>Basal</th>
<th>After sedimentation</th>
<th>After centrifugation</th>
<th>After incubation</th>
<th>Before reinjection</th>
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* P<0.05 compared to basal values and compared to values after centrifugation

The median labelling efficiency of the leucocytes, i.e. the quotient between cell-bound activity and total activity after the last centrifugation step, was 49.2% (interquartile range, 38.4%-55.4%) and the median amount of activity injected was 200 MBq (interquartile range, 153–226 MBq).

**Adhesion molecules.** The first sample for analysis of leucocyte CD11b was taken from each patient in ethylene diamine tetra-acetic acid (EDTA) tubes before the blood sample for 99mTc labelling was drawn. The expression of leucocyte CD11b was analysed by adding 10 μl phycoerythrin-conjugated monoclonal anti-CD11b antibody (Beckton-Dickinson, Mountain View, Calif., USA) to 100 μl of the EDTA blood. The mixture of cells and monoclonal antibodies was held at room temperature for 10 min. The red blood cells were then lysed and the leucocytes fixed using the Coulter Q-PREP EPICS system [7] (Coulter Inc., Hialeah, FL, USA).

Samples of the cell suspension were taken at four different steps of the labelling procedure: (1) after whole blood sedimentation for approximately 60 min, (2) after centrifugation (150 g, 5 min) of plasma rich in leucocytes and platelets, (3) after incubation for 10 min with 5 ml 99mTc-HMPAO and (4) after final centrifugation (150 g, 5 min) and resuspension with autologous plasma just before reinjection.

At each of the four steps, 100-μl samples of the cell suspension were taken. The expression of leucocyte CD11b was analysed by adding 20 μl phycoerythrin-conjugated monoclonal anti-CD11b antibody (Beckton-Dickinson, Mountain View, Calif., USA) to 100 μl of the cell suspension. The mixture of cells and monoclonal antibodies was held on ice for 30 min. The leucocytes were then fixed using the Coulter Q-PREP EPICS system [7] (Coulter Inc., Hialeah, FL, USA).

In all analyses, phycoerythrin-labelled isotype-specific irrelevant monoclonal antibodies were incubated in parallel as negative controls.

All samples were analysed using a EPICS Profile flow cytometer (Coulter Inc., Hialeah, FL, USA) with an air-cooled 15 mW argon-ion laser with an excitation wavelength of 488 nm. The leucocytes were detected and separated by their different light scattering properties. Combinations of granulocytes, monocytes and lymphocytes were represented by well-separated clusters in two-parameter scatter plot histograms. A discrimination gate was placed around the granulocyte cluster, and the instrument gave the total number of cells and the mean fluorescence intensity of the cells within the gate.

The instrument was calibrated daily with Immunocheck (Coulter Corporation, Hialeah, FL, USA) fluorospheres for optical alignment, and before each experiment with Standard Brite (Coulter Corporation, Hialeah, FL, USA) standardised fluorescent particles.

Differences were analysed with the Wilcoxon sign rank test. A probability of less than 5% was accepted as statistically significant.

**Results and discussion**

The expression of CD11b on granulocytes during the different steps of the labelling procedure is shown in Table 1. No significant changes occurred during non-specific cell handling. After incubation with 99mTc-HMPAO the CD11b expression increased.

Any change in behaviour or morphology of labelled cells can result from: (a) physical damage during the labelling procedure, (b) chemical damage due to the employed radiopharmaceutical compound, and (c) intracellular radiation [8]. The mobilization of CD11b was not of an artefactual nature since it did not occur during non-specific cell handling such as sedimentation or centrifugation. Furthermore, the cells were continuously maintained in (20%) plasma during the labelling procedure [1], a condition which for indium-111 labelled granulocytes has been shown to preserve the functional integrity of the cells compared to when labelling is performed in saline [9]. We cannot, however, separate the relative effects of the lipophilic HMPAO, its secondary hydrophilic complex and 99mTc.

In conclusion, leucocyte labelling with 99mTc-HMPAO induces an increased expression of the glucoprotein receptor CD11b on granulocytes. At present we do not know whether this upregulation affects the functional integrity of the granulocytes, or possibly influences the uptake in inflammatory foci and parenchymatous organs. Further studies are in progress.

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**References**