IN SILICO, *IN VITRO* AND *IN VIVO* EVALUATION OF TECHNETIUM-99m AND RHENIUM THYMIDINE COMPLEXES FOR POTENTIAL USE IN SUICIDE GENE THERAPY AND CANCER DIAGNOSIS

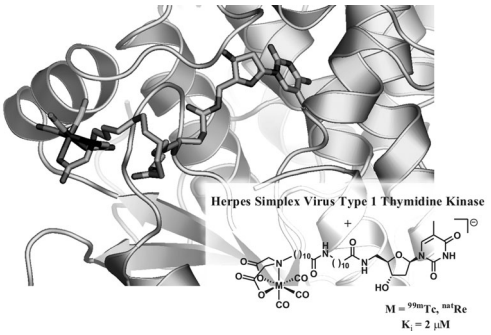
D. Desbouis,[2] M. Stichelberger,[2] V. Spiwok,[2] L. Scapozza,[3] R. Schibli.[1,2]
[1]Chemistry and Applied Biosciences, ETH Zurich, Zurich, Switzerland; [2]Center for Radiopharmaceutical Sciences, Paul Scherrer Institute, Villigen, Switzerland; [3]Pharmaceutical Science, University of Geneva, Geneva, Switzerland.

In rapidly growing tumors such as glioma, the nucleoside analogue 5-lodo-2-deoxyuridine is used as efficient diagnostic agent and provides a means to determine the proliferative activity of the tumor. On the other hand, (radiolabeled) 5-ethyl-uridine analogues (targeting the viral thymidine kinase 1: HSV1 TK) can be used to assess the viral replication and the tumor response to therapy in "suicide gene therapy" and/or to inhibit gene therapy. Thus there is a prominent interest for inexpensive ^{99m}Tc-based nucleoside analogues for non-invasive diagnosis.

In this study we present the first quantitative *in vitro* and the first *in vivo* evaluation rhenium/technetium-tricarbonyl complexes of thymidine. Furthermore, *in silico* molecular dynamic and molecular docking experiments are described based on the structure of the structure of viral TK with selected compounds.

Thymidine was functionalized at position C5′. For tridentate coordination of $[M(H_2O)_3(CO)_3]^+$(M = Re, ^{99m}Tc), **1**, an iminodiacetic acid chelating system was chosen and spacer entities of different length have been introduced between the pharmacophor and the metal core. Enzyme kinetic studies revealed inhibition exclusively of the human cytosolic thymidine kinase (hTK1). The compounds revealed different K_i-values (7-334 µM). On the other hand, complex **2** with a spacer of ~30 Å (see figure) evinced also inhibition of the HSV1 TK (K_i = 2 µM for HSV1 TK and 30 µM for hTK1). Molecular docking and dynamics (MD) studies corroborate these observations. Stable simulations of HSV1 TK–Re-thymidine complex were only found in case of **2**. *In vitro* studies with compound **2** (M = ^{99m}Tc) using two different osteosarcoma cell lines expressing (TK+) or lacking (TK-) HSV1 TK revealed statistical differences in internalization (0.5 % vs. 2.5 % of total activity 4 h post incubation). *In vivo* studies in athymic nu/nu mice bearing TK+/- osteoblastoma cells, however, revealed no differences in tumor uptake (0.2±0.1 % ID/g in both cases). On the other hand in MG63 osteoblastoma cells expressing hTK1 displayed a significant higher uptake in tumor tissue (0.8±0.4 % ID/g, 3 h p.i.). Route of clearance was predominantly via the hepatobiliary pathway due to the pronounced lipophilicity of complex **2** (liver: 4.8±1.3 %, intestines: 2.6±0.9 % ID/g, 3 h p.i.). Radioactivity in other organs and tissues was < 0.4 % ID/g except for the stomach (0.9±0.6 % ID/g). *In vivo* experiments with more hydrophilic complexes and different cell lines expressing hTK1 are currently in progress.

At the present time our data preclude the use of ^{99m}Tc-thymidine derivatives functionalized at position C5′ for use in suicide gene therapy because of low tumor uptake or lack of selective interaction with HSV1 TK. However, the compounds can potentially be employed to monitor tumor proliferation. *In vivo* studies with different other tumor cell lines are currently in progress. MD experiments based on the recently published structure of human TK will be presented. These calculations should aid to develop more potent and more selective ^{99m}Tc-based nucleoside inhibitors and substrates respectively.



Keywords: Thymidine Kinase, Organometallic, Tricarbonyl

A NEW STRATEGY FOR LABELING BIOMOLECULES THAT CONTAIN DISULFIDE BONDS WITH RHENIUM AND TECHNETIUM

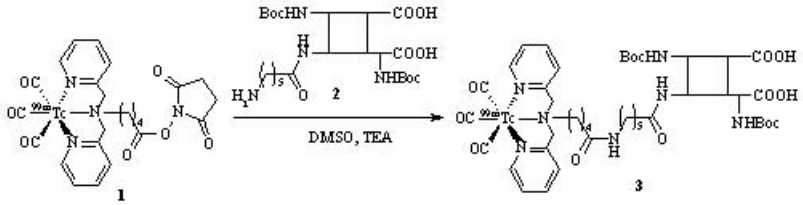
K.J. Guenther,[1] P. Schaffer,[1] S. Yoganathan,[1] R.V. Chirakal,[2] M. Dolovich,[3] J. Zubieta,[4] S.R. Banerjee,[4] J.W. Babich,[5] J.F. Valliant.[6]

[1]Chemistry, McMaster University, Hamilton, ON, Canada; [2]Radiology, Hamilton Health Sciences Centre, Hamilton, ON, Canada; [3]Medicine, Hamilton Health Sciences Centre, Hamilton, ON, Canada; [4]Chemistry, Syracuse University, Syracuse, NY, United States; [5]Molecular Insight Pharmaceuticals, Cambridge, MA, United States; [6]Chemistry and Medical Physics, McMaster University, Hamilton, ON, Canada.

Labeling biomolecules that contain disulfide bonds with radiometals like ^{99m}Tc and ^{188}Re is problematic. Typical labeling procedures often result in the reduction of disulfide bonds, which disrupts the structure and hence bioactivity of the parent compound. Even indirect labeling procedures, which are of use for basic research studies, can disrupt disulfide bonds through redox type reactions.

The objective was to develop a robust strategy for radiolabeling biomolecules that contain disulfides using organometallic complexes of ^{99m}Tc(I). Appropriately designed complexes of Tc(I) should not be overly redox active thereby preventing unwanted reduction of disulfide bonds. The model compound selected for initial studies was insulin because the hormone is readily available and it contains two reactive disulfide groups that must remain intact in order to maintain bioactivity. This feature provides an additional handle from which to evaluate any new labeling strategy.

The initial labeling methodology was build on an indirect strategy involving conjugation of a $Tc(CO)_3$-dipyridyl active ester complex (**1**), through a spacer chain, to insulin at the B1 amino acid residue (**2**). The prosthetic group **1** was prepared in high yield in two steps from the corresponding dipyridyl acid chelate. Subsequent conjugation to insulin was facile and there was no sign of degradation of the parent hormone. A particular highlight of the synthetic strategy is that the entire synthesis was completed without the need of HPLC purification. The synthesis, characterization, biological evaluation of the conjugates will be presented along with a discussion of the potential general utility of the methodology for tagging other biomolecules that contain disulfide bonds.



Keywords: Radiopharmaceutical Chemistry, Technetium-99m, Insulin