Chapter 7

A BRIEF STUDY OF SYSTEMIC EFFECTS OF PY-IM POLYAMIDE TARGETED TO HYPOXIA-RESPONSE ELEMENT

Introduction

Oxygen regulation is essential for maintaining homeostasis in mammals. As a result, many human diseases are affected by hypoxic gene expression and in some cases these diseases can be alleviated, or prevented, by inhibiting the response to hypoxia. In particular, inhibition of hypoxic signaling could be useful in treatment of cancers, age-related macular degeneration, liver and kidney fibroses, systemic hypertension associated with apnea, and some aspects of a chronic heart disease (1, 2). In our *in vivo* studies Py-Im polyamides were administered systemically and were found in many organs at micromolar levels, often higher than those found in tumors (see chapter 5, (3, 4)). Additionally, we found that the FITC-conjugates of Py-Im polyamides were present in the nuclei of tested organs, such as livers, kidneys, or lungs (see chapter 5, (5, 6)). This raised a question if that gene expression in the tested organs can be regulated by Py-Im polyamides, and if the disease of those organs could potentially be alleviated with use of Py-Im polyamides.

Results and discussion

Py-Im polyamide 1 uptake in mouse tissues. The compound 1 (Fig 7.1A) was injected into balb/c mice intraperitoneally at 15 nmoles per mouse and the tissues were harvested after 24 hours. They were subsequently dissolved in Solvable reagent and analyzed for concentration as previously reported (7). The concentrations of 1 were analyzed for 14 organs and tissues and were the highest for liver, spleen, lungs, and kidneys (Fig 7.1B). We found comparatively low amounts of 1 in blood, brain, and both cardia and skeletal muscles (Fig. 7.1B).

These results suggested, that upon systemic administration of 1 we should expect more pronounced changes in gene expression in tissues such as liver, kidney, spleen, or lungs.

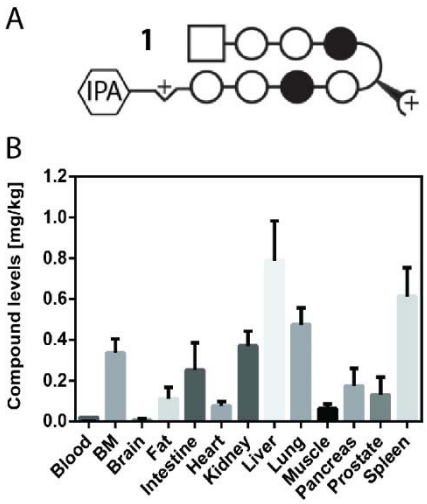

Figure 7.1 Uptake of 1 in tissues of Balb/C mice.A) Compound **1** used in the study in ball and stick notation – open circles represent N-methyl-Pyrroles, filled circles N-methyl-Imidazoles and open square 3-Chlorothiophene, as described in the Chapter 1. B) Levels of a C-14 labeled polyamide **1** in tested tissues of Balb/C mice (n=5). (Raskatov JA, 2014, unpublished data)

Gene expression changes in mouse tissues. We decided to analyze gene expression in three of the tested tissues: liver and kidneys that showed good uptake of **1,** and muscles, that showed significantly lower amounts of **1**. A panel of genes was used to determine gene expression changes in the tissues upon treatment of C57BL6 mice (NSG mice in case of muscles) with **1** (2 x s.c. inj., on day 1 and 3 at 6.8 mg/kg, tissues harvested on day 5). The organs were frozen immediately upon harvesting and RNA extracted using Trizol as described previously (see Chapter 4, Materials and Methods). RNA was then reverse-transcribed to cDNA and its levels quantified by RT-qPCR. The two tissues that have shown compound uptake levels above 0.2 mg/kg showed significant changes in gene

expression levels, while muscle, with lower uptake levels, showed no significant gene expression changes.

The presence of gene expression changes that correlated with uptake of compound 1 into the tissues suggested that action of 1 *in vivo* could be tissue specific, likely due to differences in tissue distribution. Significant uptake into livers, kidneys, or spleens suggests a possibility of regulating gene expression in those organs at lower levels than used in our previous xenograft studies. Thus studies with orthotopic xenografts or animal models of disease could show better therapeutic index of **1** than would be expected for subcutaneous xenografts used in our previous study (see Chapter 5).

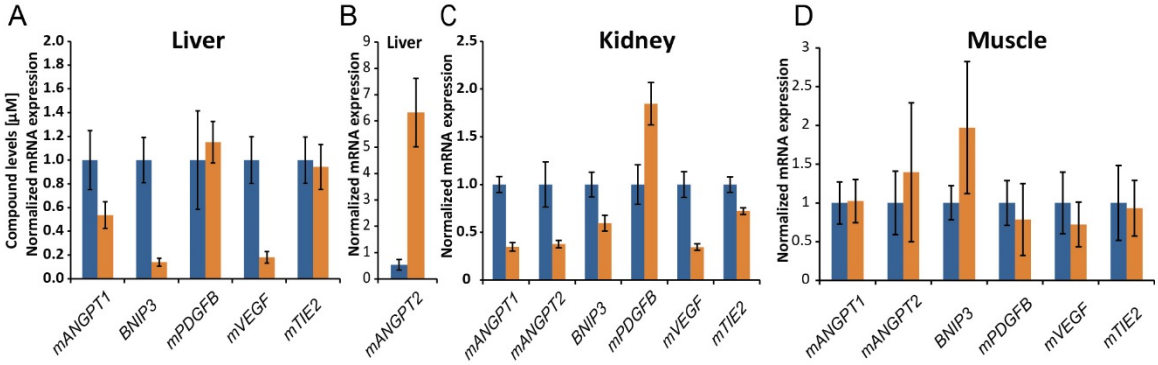

Figure 7.2 Gene expression changes in select organs. A) Proangiogenic gene were regulated by **1** in livers. Three of the tested genes, BNIP3, ANGPT1, and VEGF were downregulated, while ANGPT2 (B) was upregulated, possible due to liver toxicity (8). C) Similarly, in kidneys all genes were downregulated by **1** with the exception of PDGFB. D) In muscles, we observed no significant changes in gene expression, which could be explained by low amounts of compound **1** found after IP injection (Fig 7.1B)

References

1. Semenza GL (2014) Oxygen sensing, hypoxia-inducible factors, and disease pathophysiology. *Annu. Rev. Pathol.* 9:47-71.
2. Semenza GL (2014) Hypoxia-inducible factor 1 and cardiovascular disease. *Annu. Rev. Physiol.* 76:39-56.
3. Raskatov JA, Puckett JW, & Dervan PB (2014) A C-14 labeled Py-Im polyamide localizes to a subcutaneous prostate cancer tumor. *Bioorg. Med. Chem.*
4. Raskatov JA, Szablowski JO, & Dervan PB (2014) Tumor xenograft uptake of a pyrrole-imidazole (Py-Im) polyamide varies as a function of cell line grafted. *J. Med. Chem.* 57(20):8471-8476.
5. Nickols NG*, et al.* (2013) Activity of a Py-Im polyamide targeted to the estrogen response element. *Mol. Cancer Ther.* 12(5):675-684.
6. Yang F*, et al.* (2013) Animal toxicity of hairpin pyrrole-imidazole polyamides varies with the turn unit. *J. Med. Chem.* 56(18):7449-7457.
7. Raskatov JA, Puckett JW, & Dervan PB (2014) A C-14 labeled Py-Im polyamide localizes to a subcutaneous prostate cancer tumor. *Bioorg. Med. Chem.* 22(16):4371-4375.
8. Hadem J*, et al.* (2012) Angiopoietin-2 in acute liver failure. *Crit. Care Med.* 40(5):1499-1505.