A novel radiotherapy approach based on $^{188}$Re-hyaluronic acid for the liver-focused treatment of primary and metastatic tumors

A. Banzato¹, M. Bello²,³, D. Bernardini², P. Boccaccio¹, D. Bollini⁵,⁶, F. deNotaristefani⁷,⁸, M.C. Giron⁹, U. Mazzi¹⁰, L. Melendez-Alafort¹⁰, G. Moschini²,³, M. Rondina¹, A. Rosato¹,¹¹, E. Zangoni¹⁰.


INTRODUCTION

As previously reported [1], a simple method of labelling hyaluronic acid with Rhenium-188 is well established, and suitable samples of the labelled compound are available for radiotherapy studies in mice.

In this paper the preliminary studies on the efficacy of $^{188}$Re radiation towards hepatocellular carcinoma (HCC) cells in mice are reported.

Until now only $^{131}$I-lipiodol treatment showed up to be the most effective in experiments with HCC-bearing rats, since this is the only method that leads to a prolonged improvement of the survival[2]. It is predictable that Rhenium-188, owing to better physical and chemical properties, such as higher-energy beta-ray emission, shorter half-life and low-intensity high-energy gamma-ray emission in its decay, which allows for imaging during therapeutic treatment, should be a promising candidate for therapy treatment.

On the other hand, the biodistribution studies in healthy mice of the analogue $^{99m}$Tc-HA radiolabelled complex showed that 25 minutes after the intravenous administration, more than 80% of the radiopharmaceutical is found in the liver and the spleen due to the selective binding of the hyaluronic acid to specific receptors [3]. Based on these results, hepatocellular carcinoma was induced in mouse livers and treated with $^{188}$Re-HA complex.

RESULTS AND DISCUSSION

The HA was radiolabelled using the addition of 100 µl of $^{188}$Re-perrhenate solution with gamma-ray activity of 20 MBq to a vial containing HA and a SnCl₂ solution and the pH was adjusted to 4.

The biodistribution of $^{188}$Re-HA was studied in healthy female C57BL/6 black mice injected with 50 µL (3 MBq) of the purified complex in the tail vein.

Thirty minutes after the injection the accumulated dose in the liver and spleen reached maximal levels and remained constant for more than 72 h without renal clearance.

To assess the therapeutic efficacy of $^{188}$Re-HA against liver metastases, C57BL/6 mice were injected i.v. with M5076 tumor cells, a fibrosarcoma which peculiarly metastasizes at liver, and treated 7 days later with 9.2, 7.4, 4.5, and 2.2 MBq of $^{188}$Re-HA. Two weeks later, mice were sacrificed to evaluate the therapeutic impact. While livers of untreated mice disclosed a large increase of weight (Fig. 1) and exhibited a massive neoplastic infiltration (Fig. 2) organs from treated animals were macroscopically normal.

![FIG. 1: The figure shows the weight (mean ± SD) of spleen and liver from mice bearing M5076 tumor metastases and treated with 120 µCi or 60 µCi of $^{188}$Re-HA.](image)

Few metastatic foci were only visible in livers of mice receiving the lowest activity. Similar results were also obtained in a xenogenic mouse model of human colon carcinoma liver metastases employing HT-29 tumor cells implanted in SCID (Severe Combined Immune Deficiency) mice (data not shown). Noteworthy, $^{188}$Re-HA treatment brought about an increase in survival in this mouse model. Overall, $^{188}$Re-HA radiotherapy approach was well tolerated and associated to mild liver and bone marrow toxicity. We are currently testing the therapeutic potentiality of multiple administrations of low activities of the radiopharmaceutical to afford a long-lasting curative effect, in order to set-up all experimental conditions for potential transfer to the clinical setting.
FIG. 2: The figure shows the pictures of livers and spleens explanted from mice injected with M5076 tumor cells at day 0 and treated at day +7 with saline (A), 60 µCi (B) or 120 µCi (C) of 188Re-HA. Animals were sacrificed at day 21 from tumor injection.

CONCLUSIONS

The biodistribution studies demonstrated that the 188Re-HA complex was more stable than the Re-188-HDD/lipiodol which is explored at present in the preclinical trials. Moreover, on the contrary of Re-188-HDD/lipiodol, which must be administered via intrarterial route, the 188Re-HA can be administered by a simple intravenous injection and is rapidly concentrated in the liver and the spleen without clearance thus reducing the risk of damage to other organs. The treatment of liver metastases in mice revealed that the conjugate exhibited a strong therapeutic effect even at the lowest activity employed. This is a remarkable observation as such activity overlaps those already used in clinical trials. Furthermore, the therapeutic efficacy of the treatment was also confirmed in a xenogenic mouse model of human coloncarcinoma liver metastases employing HT-29 tumor cells implanted in SCID mice, and not associated with relevant liver and bone marrow toxicity.

Overall, 188Re-HA appears as a promising therapeutic tool to approach primary and metastatic liver tumors.