Development of ready to use kit formulation for trastuzumab radioimmunoconjugates and identification of radiochemical purity as the first step in quality control of the final product

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Aim

The aim of this study is to present the part of our project dedicated to obtaining a stable, ready to use freeze dried kit formulation of antibody radioimmunoconjugates (trastuzumab immunoconjugates labelled with ^{90}Y and ^{177}Lu). As the first step in on-going *in vitro* stability of the final product and radiochemical purity determination, we used ITLC-SG method with different mobile phases.

Methods

Radioactive labelling of trastuzumab was performed with ^{90}Y and ^{177}Lu via DOTA, DTPA and 1B4M-DTPA in molar ratio 1:20. The specific activity of 1.425 mCi (^{90}Y) and 8.150 mCi (^{177}Lu) was achieved, using a solutions of 0.04 M HCl. Radiolabeling is performed by adding 8.5 µL of ^{90}Y at pH 4.5-5 and 5 µL ^{177}Lu at pH 6. Solutions with Tr-DTPA and Tr-1B4M-DTPA were incubated at room temperature for 30 min, while Tr-DOTA was incubated at 40 °C for 1 hour.
Radiochemical purity of radioisotopes was tested with ITLC-SG using three mobile phases: 0.9% NaCl, 0.4 M methanol/sodium-acetate (1:1) and 0.1 M acetic buffer.
The stability of radioimmunoconjugates was tested in 0.9% NaCl (^{177}Lu) and 0.4 M methanol/sodium-acetate (1:1) (^{90}Y), after incubation at room temperature for 1, 24, 48 and 72h.

Results

After choosing the most suitable mobile phase for determination of radiochemical purity by ITLC-SG of conjugates labeled with ^{90}Y (99.87%) we used 0.4 M methanol/sodium acetate (1:1), and those with ^{177}Lu (100%) with 0.9%. NaCl. Examination of radiochemical yield of radioimmunoconjugates showed the presence of radioactivity only at the start of the strip, due to the high Mw of Tr. The absence of radiolabeled fragments of the antibody, as well as radiolabeled chelators and free radioisotopes, proved that the stable radioimmunoconjugates were formulated. The highest yield of labeling with ^{90}Y (>96%) is achieved in 0.4 M methanol/sodium-acetate (1:1), while with ^{177}Lu (>99%) in 0.9% NaCl.
Test stability after 24h showed the highest stability of ^{90}Y-DOTA-Tr (>92.40%) and ^{177}Lu-DOTA-Tr (>99.14%), with minimum released $^{90}Y^{3+}$ (<7.60%) and $^{177}Lu^{3+}$ (<0.86%). After 72h, the highest stability was determined for ^{90}Y-1B4M-DTPA-Tr (>84.90%) and ^{177}Lu-DOTA-Tr (>98.52%), with minimum released $^{90}Y^{3+}$ (<15.10%) and $^{177}Lu^{3+}$ (<1.48%).

Conclusions

After obtaining the final ready to use kit formulation, the results of the determination of radiochemical purity using ITLC-SG show a high radiolabeling efficiency (>95%), using both isotopes. However, radioactive yield with ^{177}Lu (99%) was higher compared with ^{90}Y (>96%). This method was used to monitor the stability of radiolabeled conjugates and after 72 hours of incubation, a small amount of free radioisotopes was released from radioimmunoconjugates (<5% of ^{177}Lu and <25% of ^{90}Y).
The next planed step includes *in vivo* examinations in healthy mice and in a mouse model of HER2 positive breast tumor after the *i.v.* injection of radiolabeled trastuzumab radioimmunoconjugates in order to monitor and determine their pharmacokinetics and biodistribution in the whole body and critical organs/tumor.

References

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