Determining normal tissue toxicity of non-radioactively Lu/Y-labeled rituximab-conjugates in rat animal model

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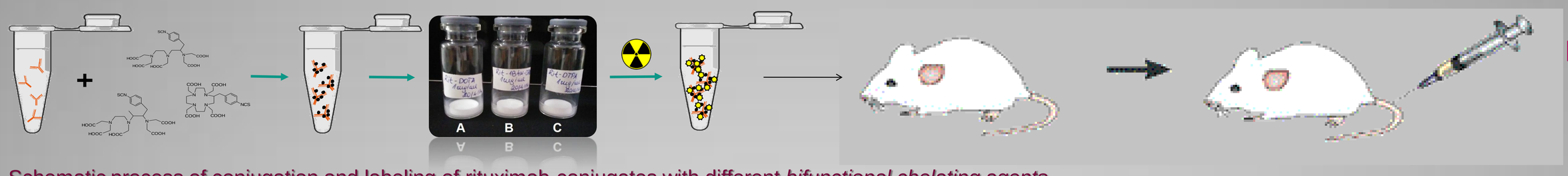
INTRODUCTION

Antibodies are slowly eliminated by the reticuloendothelial system where the resulting effect on toxicity towards healthy tissue during treatment with preparations containing them arises. Radiolanthanides that dissociate from the conjugate *in vivo*, can form colloids in the blood stream, increasing the uptake in the liver, or can accumulate in bones due to high affinity of the metal ions to the phosphate anion which results in myelotoxicity. Radiolabeled monoclonal antibodies (mAbs) intended for radioimmunotherapy (RIT) of cancers in humans should first be evaluated by preclinical toxicological studies in animal models. Kinetics, distribution and induced effects in healthy mice/rats for normal tissue toxicity and in animals with implanted tumor are followed. Generalized and gastrointestinal toxicity, liver toxicity and haemopoietic toxicity are followed. Haemopoietic toxicity, if present, is usually seen within 2-3 weeks after injection of the radiopharmaceutical and resolves within 6-8 weeks, while liver and renal toxicity may require a longer period of observation (4 - 8 weeks). Ideal radiotherapeutic agent would demonstrate specific anti-tumor effects with minimal to moderate toxicity to normal tissues. Concerning these facts, *in vivo* examination of the behavior of Lu- and Y-rituximab-conjugates in healthy animal models (rats) with particular reference to haematotoxicity was performed.

METHODS



Schematic process of conjugation and labeling of rituximab-conjugates with different *bifunctional chelating agents*.

Application protocol:

In the experimental study six groups (I-VI) of normal rats with 5 animals in each group were used . Each group receives different formulation:

I. Lu- *p*-SCN-Bn-DOTA-rituximab
II. Lu- *p*-SCN-Bn-DTPA-rituximab
III. Lu-1B4M-DTPA-rituximab
IV. Y- *p*-SCN-Bn-DOTA-rituximab
V. Y- *p*-SCN-Bn-DTPA-rituximab
VI. Y-1B4M-DTPA-rituximab

RESULTS

The results from blood analysis showed decrease in value for RBC in all samples from all groups (without exception) where the lowest value detected was RBC value determined in the group treated with Y-*p*-SCN-Bn-DOTA-rituximab.

This result is in consistence with the confirmed myelosuppressive activity of rituximab itself and the affinity of the yttrium to the bone marrow.

On average, in half of the tested samples thrombocythemia (thrombocytosis) was also observed. It is important to note that after the completion of treatment (4 weeks after administration of the last dose) results showed normalization of blood parameters, i.e. RBC values approaching almost normal values.

Additional *in vivo* tests for evaluation of rituximab-conjugates in tumor-bearing animal model are required in order to make a final characterization for qualification of this formulation for possible use in RIT for Non-Hodgkin's lymphoma.

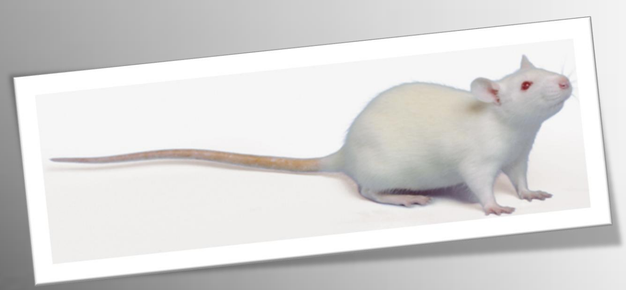


Table 1. Results from the blood analysis of animals treated with non-radioactive Lu/Y-labeled immunoconjugates of rituximab.

Sample	RBC	WBC	PLT
	Referent values		
	7.21 – 8.45x10^{12}/L	7.2 – 12.6x10^{9}/L	250 – 1200x10^{9}/L
Mean value for Group I	3.40	7.8	1496.5
Mean value for Group II	3.05	8.06	558
Mean value for Group III	3.69	9.375	984
Mean value for Group IV	2.83	10.74	770.66
Mean value for Group V	3.288	9.66	1802
Mean value for Group VI	3.575	9.10	1332.5

Table 2. Results from the blood analysis of animals treated with non-radioactive Lu/Y-labeled immunoconjugates of rituximab after 4 weeks from the last dose.

Sample	RBC	WBC	PLT
	Referent values		
	7.21 – 8.45x10^{12}/L	7.2 – 12.6x10^{9}/L	250 – 1200x10^{9}/L
Mean value for Group I	6.90	6.50	652
Mean value for Group II	5.39	1.80	71
Mean value for Group III	7.05	3.80	963
Mean value for Group IV	4.21	0.70	30
Mean value for Group V	6.80	7.10	824
Mean value for Group VI	6.98	3.30	1075

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