

University of Groningen

New receptor targeted drugs

Lub-de Hooge, Marjolijn Nicolette

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version

Publisher's PDF, also known as Version of record

Publication date:

2006

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Lub-de Hooge, M. N. (2006). *New receptor targeted drugs: pharmaceutical aspects and molecular imaging*. s.n.

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

SUMMARY

A new generation of innovative receptor targeted drugs rapidly enters the market. However, since these drugs target a specific receptor, they are not of value for all cancer patients, but only for specific tumor types or a subset within a tumor type. This makes it increasingly important to select the patients that might benefit from the targeted therapy and offers a potential role for receptor imaging with radiolabeled variants of these drugs.

This thesis aimed to study receptor targeted drugs and focused on recombinant human TNF-related apoptosis inducing ligand (rhTRAIL) and trastuzumab and their radiolabeled counterparts, intended to target and visualize the death receptor and the human epidermal growth factor receptor 2 (HER2/erbB2) respectively.

TRAIL is a member of a subfamily within the TNF-superfamily of cytokines, also called the death receptor ligands or death factors, that are able to induce apoptosis upon binding to their death receptors. Trastuzumab is a humanized IgG1 monoclonal antibody directed against HER2. HER2 is a receptor for regulation of cell survival. The HER2 receptor is overexpressed in a wide variety of human cancers, including 25-30% of primary breast cancers. Trastuzumab, by blocking the growth signal, and rhTRAIL, by directly initiating apoptosis, are both in different ways able to change the apoptosis balance in tumor cells after binding to their cognate cell surface receptor.

In **chapter 2** an overview is presented concerning regulation of cell death and cell survival by death receptor ligand and HER signaling pathways. Attention is paid to rhTRAIL and trastuzumab and to novel opportunities for receptor imaging. Data regarding death receptor imaging and imaging of HER-receptor family have been reviewed and clearly illustrate that molecular imaging techniques can be used in staging and restaging, evaluation and prediction of treatment response and characterization of lesions, as they permit total body imaging in a non-invasive way. With the development of these new generation of targeted agents, molecular imaging may become an important part of future clinical studies and clinical practice, supporting individualized cancer therapy.

The first part of the thesis (**chapters 3-6**) is dedicated to TRAIL. To learn more about the physiological role of TRAIL we have studied soluble (s)TRAIL concentrations in two non cancer settings namely, sepsis and systemic lupus erythematosus (SLE).

Intensive research has brought us an enormous body of knowledge about sepsis and the mechanisms behind it. The involvement of the immune and the coagulation system has received much attention. Currently, dysregulation of the immune system is considered to be the most important factor that determines outcome. The role of apoptosis in the regulation of the immune response is well recognized, but its role in the dysfunction of other organs and in the mortality due to sepsis is less clear. TNF α is one of the bridges between the inflammatory reaction and apoptosis. The role that the other death ligands Fas ligand (FasL) and TRAIL play, is far less known. There are however increasing indications that TRAIL is also involved in inflammatory processes. These data raised the question whether sTRAIL is involved in sepsis.

A human endotoxemia model is available in which some aspects of sepsis can be studied. In **chapter 3**, the effect of endotoxin administration (4 ng/kg body weight (10,000 endotoxin U/ μ g) as a 1-min infusion) on the response of sTRAIL, and the role of p38 MAP kinase (K) inhibition was studied in 21 human volunteers. p38 MAPK inhibitors are considered potential drugs in inflammatory diseases. Thirty minutes before the endotoxin infusion, the volunteers received a single oral dose of placebo or the selective p38 MAPK inhibitor drug, RWJ-67657. We tested the RWJ-67657 dose levels 1400 mg (n = 4), 700 mg (n = 6), 350 mg (n = 5), and 0 mg (n = 6). Blood for sTRAIL analysis was drawn predose, and 1, 1.5, 2, 2.5, 3.5, 4.5, 6.5, 8, and 24 hours after endotoxin infusion. Plasma levels of sTRAIL were determined with a validated solid-phase sandwich enzyme-linked immunosorbent assay (ELISA). At t = 0 before the endotoxin infusion, the median sTRAIL level in the placebo group was 681 \pm 86.8 pg/mL (mean \pm SEM, n = 6). This level can be considered as the normal value in young resting males in the morning. Plasma sTRAIL increased 10-fold to 6564 \pm 511 pg/mL after 2.5 hours. This increase was blocked completely by the highest dose of RWJ-67657 (1400 mg). The sTRAIL levels were already lowering 1 hour later (p=0.003, Student's *t* test) and were normalized 6.5 hours after endotoxin infusion (p=0.98). After a dose of 350 mg or 750 mg RWJ-67657, sTRAIL levels 24 hours after endotoxin infusion were elevated. The peak levels (t = 3.5 h) were 2729.2 \pm 328.0 pg/mL and 3155.4 \pm 645.3 pg/mL respectively. However no dose response relation was found. In conclusion, sTRAIL is responsive to endotoxemia. However, the precise role in sepsis remains to be elucidated. If sTRAIL turns out to participate in the pathogenesis of sepsis, there is already a tool available, namely p38 MAPK inhibition, to inhibit the sTRAIL response.

Increased apoptosis may induce autoimmune conditions. SLE is an autoimmune disease with a wide spectrum of clinical and immunological abnormalities. The presence of autoantibodies, especially those directed to double stranded DNA, is characteristic for the disease. SLE may affect different organ systems, including the skin, joints, central and peripheral nervous system, kidneys, and liver. The etiology of SLE remains unknown. There is however increasing evidence that the presence and accumulation of apoptotic cells play a role in autoimmunity. Apoptosis is induced by binding of death receptor ligands to their cognate receptors. The Fas-FasL pathway has been studied extensively in relation to SLE. However, other death pathways are also considered important and we hypothesized that secreted sTRAIL might be important in the induction of apoptosis in SLE.

The aim of **chapter 4** was to assess sTRAIL concentrations in sera of SLE patients. Forty SLE patients were studied (20 with active disease and 20 with inactive disease). Serum sTRAIL levels were measured by a validated ELISA. Serum sTRAIL levels in SLE patients were compared with those in patients with rheumatoid arthritis (n=20), Wegener's granulomatosis (n=20) and healthy controls (n=20). Mean serum sTRAIL concentration (\pm SEM) in SLE patients (936.0 ± 108.2 pg/mL) was higher than in healthy controls (509.4 ± 33.8 pg/mL, $p < 0.01$) and in disease control patients with rheumatoid arthritis (443.8 ± 36.1 pg/mL, $p < 0.001$) or Wegener's granulomatosis (357.1 ± 32.2 pg/mL, $p < 0.001$). The mean serum sTRAIL concentration was 1010.2 ± 168.0 pg/mL for patients with inactive disease and 861.8 ± 138.7 pg/mL for patients with active disease. Soluble TRAIL values were not correlated with specific manifestations of the disease, such as leucopenia or lymphopenia, or with SLE disease activity index.

In conclusion, this study reports that serum sTRAIL concentrations are increased in SLE patients. This seems to be disease specific and could indicate a role for TRAIL in SLE pathophysiology.

Of the death receptor ligands, especially TRAIL might be an interesting candidate to facilitate the apoptotic process in combination with chemotherapeutic drugs, as it can induce apoptosis in transformed cell lines, but not in normal cells, by binding to its cell membrane receptors. A non-commercial production of rhTRAIL was set up to allow a clinical phase I study.

In **chapter 5** we described an approach for the production of clinical grade rhTRAIL meeting requirements of Good Manufacturing Practice and regulatory guidelines of the European Agency for the Evaluation of Medicinal Products assuring quality and safety of the product. RhTRAIL was produced by recombinant DNA techniques in *Escherichia coli* BL21-SI cells. Competent BL21-SI cells were transformed with pET15b-TRAIL114-281 plasmid, that harbors the gene encoding soluble human TRAIL, corresponding to the extracellular part of the TRAIL molecule (i.e. amino acids 114-281). Soluble rhTRAIL was recovered from host BL21-SI cell lysates by sonication and was subsequently purified by SP-Sepharose Fast Flow cation exchange chromatography, nickel-nitrilotriacetic acid affinity chromatography and dialysis. Three consecutive production batches were run. An extensive range of in-process and characterization tests was performed to show batch-to-batch consistency with regard to identity, purity and biological activity. The recombinant protein was then formulated in a TRIS buffer containing zinc, glycerol, NaCl, and human serum albumin and stored in polypropylene vials at -80 °C.

Because of the shown anti-tumor activity in tumor xenograft models and the lack of systemic toxicity in mice, cynomolgus monkeys and chimpanzees, rhTRAIL is now under investigation as a therapeutic agent. The physiological role of TRAIL and the pharmacokinetic behavior of rhTRAIL are complex and still not fully understood. Availability of radiolabeled rhTRAIL would offer the possibility of molecular imaging in humans to study biodistribution and whole body pharmacokinetics. Furthermore imaging enables to illustrate whether the drug reaches the target.

In **chapter 6** radioiodinated rhTRAIL was developed to study in-vitro and in-vivo death receptor targeting and pharmacological behavior in a human tumor bearing mice model. In order to develop a tracer suitable for imaging in patients, much attention was paid to optimization and validation of the labeling process, to stability testing and to show maintenance of receptor binding properties.

RhTRAIL radioiodination was optimized using chloramine T as oxidizing agent. Radiochemical purity and stability of ¹²⁵I-rhTRAIL in TRIS-buffer and human serum were determined by size exclusion chromatography HPLC. Immunoreactivity and receptor binding properties were examined using different TRAIL receptor positive tumor cell lines.

^{125}I -rhTRAIL biodistribution was assessed in a biodistribution study using athymic mice bearing human SKBR3 or SW948 xenografts with different TRAIL receptor expression levels.

^{125}I -rhTRAIL (labeling yield $70.3 \pm 1.1\%$, radiochemical purity $> 98\%$, specific activity $0.75 \pm 0.05\text{ MBq}/\mu\text{g}$) was stable in TRIS-buffer and human serum for 24 h although free iodine and high molecular weight compounds gradually developed (16 and 19 % respectively after 72 h). The immunoreactive fraction, determined using the Colo320 tumor cell line, was 0.80 ± 0.08 ($n=4$). A concentration of $9.3 \times 10^{-8}\text{ M}$ rhTRAIL was needed to reduce ^{125}I -TRAIL binding with 50%. Due to competition with soluble DR5 (5 μg) binding of ^{125}I -rhTRAIL (5 ng) to various cell lines was considerably reduced with 56% in SW948 and up to 74% in Colo320. ^{125}I -rhTRAIL biodistribution is characterized by fast renal elimination. Uptake rapidly decreased in all normal tissues. SW948 tumor uptake however, although only 1.1% of the injected dose/g, increased over time.

In conclusion, rhTRAIL can be efficiently radioiodinated. Radioiodinated rhTRAIL binds to the TRAIL receptors in-vitro and in-vivo and can be used in humans for death receptor imaging.

The second part of this thesis (**chapters 7 and 8**) is dedicated to the anti-HER2 monoclonal antibody trastuzumab. Trastuzumab is an exciting new targeted drug with a unique mechanism of action, that is currently approved for the treatment of patients with HER2 overexpressing metastatic breast cancer. In addition to its use in the metastatic breast cancer setting, impressive results of large randomized multicenter trials, designed to evaluate the use of adjuvant trastuzumab, were recently reported. The studies showed highly significant reductions in the risk of recurrence, prolongation of disease free and overall survival in the adjuvant treatment of patients with HER2 positive breast cancer.

Unfortunately, even when strong HER2 overexpression (3+) is present, part of the patients do not respond to trastuzumab therapy. Moreover, during trastuzumab treatment patients can develop severe cardiac dysfunction. The mechanism of the trastuzumab-induced cardiac toxicity is still unclear, but might be related to specific binding to HER2 receptors expressed in the myocardium. A reliable test for the prediction of tumor response and the risk of developing cardiac failure is therefore urgently needed. This was the main motivation for the development of radiolabeled trastuzumab that is described in **chapter 7**.

The aim of this study was to develop clinical grade ^{111}In radiolabeled trastuzumab, to evaluate the stability and immunoreactivity of the tracer and to perform a biodistribution study in human tumor-bearing mice. Trastuzumab was radiolabeled with ^{111}In using cyclic anhydride diethylenetriamine pentaacetic acid (cDTPA) as a chelator. ^{111}In -DTPA-trastuzumab (labeling yield $92.3 \pm 2.3\%$, radiochemical purity $97.0 \pm 1.5\%$) is stable in phosphate buffered saline when stored at 4°C for more than 14 days. The immunoreactive fraction determined by cell-binding assays, using the HER2-overexpressing human ovarian SKOV3 tumor cell line, was 0.87 ± 0.06 . Biodistribution and tumor targeting were studied in HER2 receptor-positive and HER2-negative tumor-bearing athymic mice. The HER2-positive tumor showed ($9.77 \pm 1.14\%$ injected dose per gram) substantial uptake of the labeled antibody already after 5 hours. The difference in uptake between HER2-positive versus HER2-negative tumors was even more pronounced 3 days after injection ($16.30 \pm 0.64\%$ injected dose per gram), and was visualized by radioimmunoscinigraphy. Liver, spleen, and kidney showed marked tracer uptake.

In summary, we showed that trastuzumab can be efficiently radiolabeled with ^{111}In resulting in high labeling yields and high stability. ^{111}In -DTPA-trastuzumab selectively binds to the human HER2 receptor both in-vitro and in-vivo in animals. Therefore, ^{111}In -DTPA-trastuzumab appeared suitable for clinical use.

Chapter 8 described the clinical study that aimed to evaluate whether ^{111}In -labeled trastuzumab scintigraphy can predict cardiotoxicity and identify tumor lesions. In addition, we evaluated whether plasma markers for cardiac dysfunction can be used to predict cardiotoxicity.

Patients with HER2-positive metastatic breast cancer underwent gammacamera imaging from 15 minutes to 7 days after injection of 150 MBq ^{111}In -DTPA-trastuzumab, prior to and after 12 weekly trastuzumab doses and concomitant 3-weekly paclitaxel. Cardiac assessments were performed before treatment, after 4 and 6 cycles. Plasma N-terminal proB-type natriuretic peptide (NT-proBNP) and serum troponin I (Tnl) were measured with immunoassay.

Fifteen of the 17 patients were available for cardiac and tumor uptake analysis. Pre-treatment myocardial ^{111}In -DTPA-trastuzumab uptake was observed in one patient with pre-existent cardiac arrhythmias, who did not develop heart failure during treatment. Severe cardiotoxicity occurred in three patients, without initial myocardial ^{111}In -DTPA-trastuzumab uptake, while one showed weak myocardial uptake after 4

cycles. The detection rate of single tumor lesions was 45%. New tumor lesions were discovered in 13 / 15 patients. Pre-treatment plasma NT-proBNP levels were higher in patients with, than without heart failure (mean $534 \pm \text{SD } 236$ vs. 105 ± 79 ng/L, $p=0.009$).

In conclusion, radiolabeled trastuzumab scintigraphy was not valuable in predicting trastuzumab-related cardiotoxicity in metastatic breast cancer patients, but can identify HER2-positive tumors. Measurement of plasma NT-proBNP is promising regarding prediction of trastuzumab-related cardiotoxicity.