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Beta-adrenergic receptor imaging with positron emission tomography

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IX

Summary and Concluding Remarks

In vivo quantification of β -adrenoceptors in the brain, heart and lung could be very valuable for the investigation of these receptors in neuropathologies, myocardial and pulmonary diseases, and for monitoring the effects of treatment. The aim of this thesis was the development of a method to image β -adrenoceptors in the human brain and heart with PET and the validation of a tracer kinetic model to *in vivo* quantify receptor densities.

Cerebral β -adrenoceptor imaging

For quantification of β -adrenoceptors in the brain, the radioligand *S*-1'-[^{18}F]fluorocarazolol seemed to be an excellent candidate. It is a fluorinated analog of the potent, non-subtype selective β -adrenoceptor antagonist carazolol, and exhibits specific *in vivo* binding to cerebral (14), myocardial and pulmonary (15) β -adrenoceptors in humans. In order to assess (cerebral) β -adrenoceptor densities (B_{max}) quantitatively with PET and *S*-1'-[^{18}F]fluorocarazolol, a tracer kinetic model (5) is required. **Chapter II** demonstrated that *S*-1'-[^{18}F]fluorocarazolol meets all the requirements for a tracer-kinetic model such as proposed by Delforge *et al* (5). These include low metabolite levels in brain, saturability and reversibility of the receptor binding in rats.

S-1'-[^{18}F]fluorocarazolol levels in brain were relatively low. This affects the count statistics and therefore the quality of the PET images. In **chapter III** the possibility to enhance the cerebral uptake of *S*-1'-[^{18}F]fluorocarazolol, without affecting its regional distribution, was demonstrated by modulating the transmembrane protein P-glycoprotein with Cyclosporin A in the blood-brain barrier (BBB) of rats. Coadministration of P-gp modulators may therefore improve the quality of PET images of β -adrenoceptors in the brain. However, the modulator Cyclosporin A cannot be used in healthy volunteers, because of its toxicity (12). More potent and highly specific P-gp modulators, such as LY335979 (2) and the non-immunosuppressive cyclosporin D analog SDZ PSC 833 (10) have already been reported. Because of their potency and specificity, such compounds may be suitable for application in human PET studies.

Since the application of a tracer kinetic model requires multiple injections and the addition of carrier to the radioligand, a toxicological study of S-1'-[¹⁸F]fluorocarazolol (see **chapter IV**) was performed. Unfortunately, the introduction of fluorine in the β -blocker carazolol yielded a compound with strong mutagenic potency as measured by the Ames test. Therefore, carrier-added studies with S-1'-[¹⁸F]fluorocarazolol in humans are precluded. Further research to elucidate if this radioligand is a risk to humans should address the genotoxic and/or carcinogenic potency of S-fluorocarazolol in mammalian cells and in (long term) *in vivo* experiments.

Toxicity testing of newly developed radioactive pharmaceuticals differs markedly from most medicinal products, because mostly small, not pharmacologically active quantities are involved and repeated studies are exceptional. Therefore, repeated dose toxicity studies of novel radiopharmaceuticals may be omitted, but single dose toxicity and genotoxicity studies should be included, before the test drugs are administered to humans. This approach was recently demonstrated for the radioligand β -CIT (7). To properly assess the possible genotoxicity of a newly developed radioligand, a battery of tests is recommended by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH guidelines S2B). This includes a test for gene mutation in bacteria, an *in vitro* test with cytogenic evaluation of chromosomal damage (e.g. human lymphocytes) and an *in vivo* test for chromosomal damage (rodent hematopoietic cells). This battery of tests should be performed not only before carrier-added doses of the new compound are administered (multi-injection protocols for receptor density assessment), but also before PET studies at the non-carrier added level are initiated, since genotoxicity is not a linear but a stochastic process. A real dose-risk assessment for humans can never be made with the data from the genotoxicity tests.

To find alternatives for S-1'-[¹⁸F]fluorocarazolol, five new radiotracers with high affinity for the β -adrenoceptor and moderately high lipophilicity were synthesized (**chapter V**). Evaluation of these radiotracers for *in vivo* imaging of β -adrenoceptors in the brain, together with [¹²⁵I]iodocyanopindolol (standard ligand for autoradiography), revealed that cerebral uptake of these ligands was either too low or not related to β -adrenoceptors, even though some radioligands did display specific binding in lung and heart. Although a high K_D and (moderately) high lipophilicity are often used as prerequisites for the suitability of a compound for *in vivo* imaging of cerebral receptors, these criteria, combined with available pharmacokinetic information, proved not sufficient to select a suitable β -adrenergic ligand for cerebral β -adrenoceptor imaging. Possible reasons include transport by P-glycoprotein, metabolism, protein binding and uncertainties in the K_D values, as determined in *in vitro* assays.

Another candidate for cerebral β -adrenoceptor imaging with favorable affinity and lipophilicity, (S)-[¹⁸F]fluoroethylcarazolol could be successfully synthesized for animal studies (**chapter VI**). Evaluation in rats demonstrated its potency and specific binding to

cerebral β -adrenoceptors *in vivo* and *in vitro* in rats. This novel radioligand is therefore of potential use for β -adrenoceptor imaging in the brain with PET. Whether (S)-[^{18}F]fluoroethylcarazolol is genotoxic like (S)-[^{18}F]fluorocarazolol, remains to be examined. The mutagenic properties of carazolol analogues are hard to predict since (S)-fluorocarazolol showed mutagenic activity in the Ames test whereas (S)-carazolol did not (6).

If (S)-[^{18}F]fluoroethylcarazolol has proven to be non-toxic, pilot studies with this ligand can be performed in volunteers. To produce sufficient amounts of (S)-[^{18}F]fluoroethylcarazolol, the synthetic route via fluoro-ethylation of (S)-desisopropyl will be investigated. To measure B_{max} a tracer kinetic model (see chapter VII) might be applied. However, for receptor quantification in the brain binding potentials (BP) may be sufficient, which can be estimated using a region with mainly non-specific binding as reference tissue. For β -adrenoceptors this is slightly difficult because these receptors are widely distributed throughout the brain. White matter of the corpus callosum may be used as a reference region, since the β -adrenoceptor density in this region is known to be very low.

Subsequently, (S)-[^{18}F]fluoroethylcarazolol can be applied to quantify β -adrenoceptors in the brains of patients suffering from mood disorders (e.g. depression), degenerative diseases (e.g. Alzheimer's disease), and also in patients suffering from multiple sclerosis (MS). This last group of patients displays a lack of astrocytic β_2 -adrenoceptors in *post mortem* tissue, which may be involved in the induction or perpetuation of autoimmune reactions (3). Multiple *in vivo* measurements with PET and (S)-[^{18}F]fluoroethylcarazolol can probably be used to monitor disease progression and may allow differential diagnosis of MS and other neurodegenerative diseases.

Furthermore, PET and (S)-[^{18}F]fluoroethylcarazolol may prove to be a valuable technique to assess receptor occupancy of centrally acting β -blockers. This might reveal if the mechanisms of action of β -blockers in a variety of brain pathologies not initially associated with β -adrenoceptors, including migraine (16) and (1,8) sudden cardiac death (9), are related to β -adrenoceptor binding, serotonin receptor binding, non-specific membrane stabilizing properties or peripherally mediated activity (17). Moreover, (S)-[^{18}F]fluoroethylcarazolol-PET could elucidate the relationship between β -adrenoceptor occupancy and side effects, such as hallucination, depression, insomnia, fatigue, dizziness, impaired short-term memory and alertness (reviewed in (8,11)).

Myocardial and pulmonary β -adrenoceptor imaging

Chapter VII deals with the imaging of β -adrenoceptors in human heart. The radioligand [^{11}C]CGP 12388 produced excellent images of β -adrenoceptors in the heart, lung and spleen of healthy volunteers. Because of its favorable pharmacokinetic (e.g. low metabolism) and pharmacodynamic (e.g. specific binding with high association rates) properties, (S)-[^{11}C]CGP 12388 appears suitable for quantitative PET studies using a multi-injection protocol.

Tracer-kinetic modeling allowed the estimation of all model parameters of (S)-[¹¹C]CGP 12388 (**chapter VIII**). B_{\max} and K_D were reliably calculated in the whole left ventricle and regional differences could be plotted in a parametric polar map (120 different segments). We conclude that (S)-[¹¹C]CGP 12388 in combination with a tracer-kinetic model is useful for calculating regional B_{\max} values in human left ventricle *in vivo*.

Data from various injection protocols appeared to allow calculation of reliable B_{\max} values. The fit procedure appeared to be equally sensitive to changes in the value of the parameters. The ideal protocol however, should be most sensitive to the parameter B_{\max} , while the sensitivity to the other parameters is less essential. Simulation of different injection protocols (various injected masses and injection intervals), based on the acquired data, should reveal the optimal injection protocol and whether three injections are essential or not. Obviously, a protocol with a minimal number of injections is preferred because it is more convenient in a clinical setting.

The protocol may be further simplified by using the bloodpool (endocardium) as input function in stead of the arterial samples, which reduces the inconveniences for the patient. (S)-[¹¹C]CGP 12388, however shows substantial binding to red blood cells (**chapter VII**). Therefore, using the whole blood as input function to accurately model the kinetics of the ligand, is probably only feasible when the bloodpool data is (dynamically) corrected for red blood cell binding.

When the optimal protocol is found, the B_{\max} can be assessed in patients suffering from HCM, DCM or other myocardial deficiencies and compared to those found in healthy volunteers. Since tracer kinetic modeling in principle allows the identification of all model parameters while requiring only standard *a priori* assumptions, it could serve as a gold standard to validate simplified procedures including graphical models, which are based on many additional hypotheses and often allow only the identification of composites of parameters (e.g. B_{\max}/K_D , rather than B_{\max}).

β -Adrenoceptors were also visualized with (S)-[¹¹C]CGP 12388 in the lungs of healthy volunteers. This technique however is probably not suitable to detect differences in β -adrenoceptors in COPD, as was already demonstrated with (S)-[¹¹C]CGP 12177 in asthmatic subjects versus age-matched healthy controls (13). Intravenously administered radioligand mainly binds to alveolar receptors (> 90%), and much less (< 10%) to receptors on smooth muscle cells in the upper airways (see also (15)), which are involved in the regulation of airway caliber and thus contributes to the pathophysiology of COPD. Therefore, it would be far more interesting to image exclusively β -adrenoceptors in the upper airways. This might be possible by administering (S)-[¹¹C]CGP 12388 via inhalation. (S)-[¹¹C]CGP 12388 inhalation may also be used to assess β -adrenoceptor occupancy of β -mimetics and to determine the pattern of deposition of various inhalers and nebulizers.