

# A chemical model suggesting the maximum radiolabeled somatostatin analogue tumor uptake, in the presence of unlabeled somatostatin

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## Abstract

Somatostatin analogues (SSA), both radiolabeled and unlabeled play an important role in the management of carcinoid tumors. They are often administered in parallel, the unlabeled analogue for treating the carcinoid tumors' symptoms and the radiolabeled one for imaging tumors foci. There is a debate about when is the optimum time for a somatostatin receptor scintigraphy during treatment. Opinions are divided, with some authors suggesting stopping SSA treatment, while others do not. *Our aim* was to try to explore pharmacokinetics behind the radiolabeled peptide administration in the presence of circulating in blood unlabeled SSA, by using a model of "law of mass". *Applying the pharmacokinetic data* from the manufacturers' Prescription Information Sheet in a formula describing competitive binding, led to a reduced uptake for the radiolabeled peptide in the presence of the unlabeled peptide, in comparison with standalone radiolabeled peptide administration, regardless of the total number of available receptors. *We provide data* that unlabeled somatostatin should be withdrawn for no less than 14 days before the labeled SSA is administered, because biotherapy agents interfere with both diagnostic and therapeutic nuclear medicine procedures. Further research is needed to reach secure conclusions on patient medication management before diagnostic scans or therapeutic administrations in nuclear medicine. *In conclusion*, by waiting at least 6 half-lives (14 days), after the unlabeled SSA administration, the radiolabeled receptor uptake increased two-fold to three-fold, as compared to simultaneous administration of radiolabeled and unlabeled peptides depending on which SSA was used.

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## Introduction

Carcinoid tumors were first described by Lubarsch in 1888, who found multiple tumor foci at the ileum during autopsy in two patients [1]. The term "karzinoide tumoren" was first used by Oberndorfer in 1907 to describe a tumor similar to adenocarcinoma but less aggressive and histologically different [2]. Carcinoid tumors are neuroendocrine tumors (NET) originating from enterochromaffin cells (Kulchitsky cells), which are dispersed in the body. They may appear anywhere in the body, but they usually originate from the intestine.

The annual incidence for carcinoid tumors is about 1-2 cases per 100,000 population, rising after autopsy up to 0.5%-1% per 100 autopsies, mainly localized in the small intestine [1]. Five years survival has improved in the last years [3-5] after the use of somatostatin analogues (SSA) as part of treatment. The main indication for the use of SSA is treatment of functioning NET causing hormone-related clinical syndromes, where SSA might block the release of hormone related agents. In nonfunctioning NET, available data for the effect of SSA are still controversial [6].

Although administration of radiolabeled SSA is becoming a useful nuclear medicine procedure for diagnostic and therapeutic purposes [7-10], according to the European Association of Nuclear Medicine Guidelines it has not been clarified whether the administration of SSA should be discontinued before pentreotide scintigraphy, as some authors report better diagnostic results without SSA withdrawal [11]. European NET consensus guidelines for peptide receptor radiotherapy (PRRT) with high doses of radiolabeled SSA suggest that, long-acting SSA (octreotide or lanreotide) 6 weeks before PRRT should be switched to short-acting formulations, up to 1 day before PRRT [10]. Nevertheless, the therapeutic outcome of this schedule is uncertain.

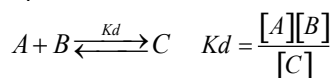
One of the reasons for the above discrepancies could be the suboptimum ratio between labeled and unlabeled SSA in the serum of these patients, unable to cause the optimum diagnostic or therapeutic effect.

The aim of this article was to study a chemistry model that suggests the maximum radiolabeled SSA tumor uptake using various combinations of synchronous and delayed administration of unlabeled and labeled SSA according to the "law of mass" and the principles of competitive binding. This model could also apply for the administration of other radiopharmaceuticals.

## Methods

To explore the hypothesis of how unlabeled SSA treatment interferes with the labeled peptide administration, several assumptions were made. The first was that although there are 5 types of somatostatin tumor receptors (SSTR) [12], with different affinity for each peptide-receptor, our model assumed that there was only one kind of receptor. Second, that there was equilibrium around the cell population, so that the unlabeled and labeled SSA concentrations were constant and that no nonspecific binding occurred. In addition, for nuclear medicine purposes we were interested only in binding, making use of the work of others for competitive binding [13] and not in the behavior of SSA as agonists or antagonists.

The G protein-coupled receptors (GPCR) constitute the largest family of cell-surface receptors involved in signal transmission [14]. They regulate the function of most cells in the body using reversible reactions by responding to a wide variety of structurally diverse ligands. The dissociation constant ( $K_d$ ) is commonly used to describe the affinity between a ligand (A) and a binding site (B). This constant,  $K_d$  is expressed in molar units (M) and shows how firmly a ligand binds to a particular protein. Smaller dissociation constant means that the ligand is more tightly bound, or that it has higher affinity for the corresponding protein [15]. Ligand-protein affinities are influenced by non-covalent intermolecular interactions between these two molecules such as hydrogen bonding, electrostatic interactions, hydrophobic and Van der Waals forces. The dissociation constant in a reversible reaction where A and B unite in reversibly forming a complex C is defined as:



Competitive reversible antagonism is defined as the condition in which the agonist and antagonist bind reversibly to the same recognition sites on the receptor and, thus, compete for them when simultaneously present [16]. According to the model of others [13], the following equilibria are considered:



R is the free receptor, A: the radiolabeled peptide, B: the unlabeled peptide, and  $K_A$  and  $K_B$ : the equilibrium dissociation constants for the AR and BR ligand-receptor complexes, respectively.

If the total receptor concentration is  $[R_0]$ , then some of the receptors will be bound with A ( $[AR]$ ), some will be bound with B ( $[BR]$ ) and some will be left free ( $[R]$ ):

$$R_0 = [AR] + [BR] + [R]$$

Reversing and multiplying with  $[AR]$  gives the fractional receptor occupancy by the labeled peptide in the presence of the unlabeled one:

$$\frac{[AR]}{[R_0]} = \frac{[AR]}{[AR] + [BR] + [R]}$$

Solving the equation gives fractional receptor occupancy (percentage of bound labeled peptide) as:

$$\frac{[AR]}{[R_0]} = \frac{[A]}{[A] + K_A \left(1 + \frac{[B]}{K_B}\right)}$$

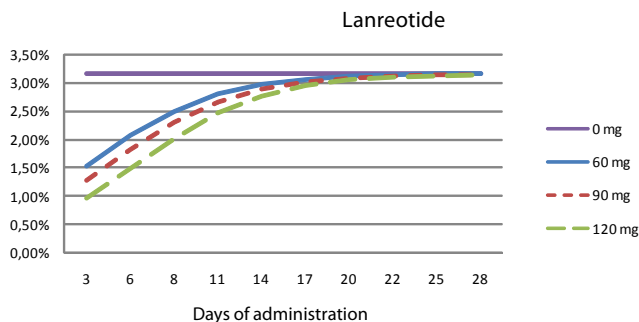
*Equation 1:* Interpretation of this equation leads to the following conclusions: a) the fractional receptor occupancy by the radiolabeled peptide decreases as the concentration of the unlabeled one increases. b) The fractional receptor occupancy by the radiolabeled peptide decreases as the affinity of the unlabeled one for the receptor increases. c) The total number of receptors is indifferent.

## Applying data

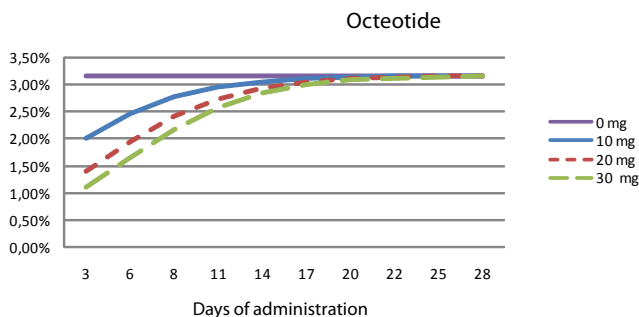
Several authors have worked on assessing affinity profiles for commercially available SSA or experimental peptides, unlabeled or radiolabeled [12, 17]. Data from these affinity profiles' tables, IC50 can be used for calculations in equation 1. The experimental values vary, but what is of importance, according to the assumptions already made, is the rank order and not the exact IC50 values. In fact, due to the simplicity of this binding model, the  $K_d$  values can be considered equal to IC50. The pharmacokinetic data available by the manufacturer, for each of the commercially available drugs will be used for the concentration in this equation. According to these data [18], serum concentrations in patients after deep subcutaneous injections of lanreotide (somatuline depot; Ipsen Pharma Biotech, France) every 28 days were  $1.8 \pm 0.3$ ,  $2.5 \pm 0.9$ , and  $3.8 \pm 1.0$  ng/mL (1.64, 2.28, 3.46 nM) at 60, 90, and 120 mg doses, respectively. Also, in patients with carcinoid tumours, the mean (and median) steady-state serum concentrations of octreotide (sandostatin LAR; Novartis Pharmaceuticals Corp., Switzerland) after multiple deep intramuscular intragluteal injections of 10 mg, 20 mg or 30 mg of sandostatin LAR, given at 4 weeks intervals were 1.2 ng/mL, 2.6 ng/mL and 3.9 ng/mL, or 1.77, 2.55, 3.82 nM respectively [19]. As for the radiolabeled pentetreotide (octreoscan; Covidien, USA), 10 min after its intravenous administration of 10  $\mu$ g, one third would remain in the blood pool. Radiolabeled pentetreotide circulates unbound by plasma proteins [20], so it disperses in 2.7 L of plasma, assuming that human plasma consists on average of 55% of the 5 L whole blood, making a concentration of pentetreotide of approximately 1 ng/ $\mu$ L (0.71 nM). If the biological degradation of the unlabeled SSA is taken into account (half-life of 2.8 days [18, 19]) and applying their concentration to the formula, a graph can be drawn for each unlabeled-labeled peptide combination. (Fig. 1 and 2).

As suggested by equation 1 and seen in both graphs, the fractional receptor occupancy for the radiolabeled ("hot") peptide depends on the temporal difference of unlabeled ("cold") and radiolabeled peptide administration. The presence of unlabeled peptide "inhibits" the binding of radiolabeled peptide, leading to a "suboptimum" for the purposes of nuclear medicine binding, as both peptides compete for the same binding site. That competition is irrelevant to the fact that the number of binding sites greatly exceeds the number of radiolabeled and unlabeled molecules. Maximum "hot" peptide uptake decreases approximately 50% to 300% depending on the substance used (octreotide or lanreotide)

and on the time elapsed since the last "cold" peptide administration. According to this model, there is a 50% decrease of SSTR fractional occupancy at standalone radiolabeled administration, compared to a synchronous 10mg octreotide and  $^{111}\text{In}$ -pentetreotide administration, or up to 300% for a synchronous 30mg lanreotide and  $^{111}\text{In}$ -pentetreotide administration.



**Figure 1.** Fractional receptor occupancy for octreoscan after standalone (0mg somatuline) or various synchronous administration with somatuline (60, 90 or 120mg).



**Figure 2.** Fractional receptor occupancy for octreoscan after standalone (0mg sandostatin) or various synchronous administration with sandostatin LAR (10, 20 or 30mg).

## Discussion

The chemical model described uses many assumptions but it pictures the interactions that occur when two substances compete for a unique receptor. There are authors who report better results in a somatostatin receptor scan without "cold" SSA withdrawal [11]. This may be because occupying some of the background tissue receptors with "cold" SSA increases the lesion-to-background ratio, enhances the image contrast by reducing the denominator in the tumor/background fraction, and leads to better visual results. There is another way of increasing this ratio: by waiting at least 6 half-lives (14 days), the receptor uptake (numerator) increases two-fold to three-fold, depending on which "cold" SSA is used. More research is needed to establish the optimum way of increasing the lesion-to-background ratio, by measuring the relative effect of background tissue blocking and uptake increase. The same principle of competitive binding may lie behind the pre dosing technique, used in radioimmunotherapy (RIT). Administration of unlabeled antibodies leads to reduced absorbed doses for the normal tissues, making possible a relative increase in the injected activity and thus the tumor absorbed dose. Up to now there has been no related study, to determine the op-

timium ratio of labeled to unlabeled SSA. Our model can also be studied in other cases where drugs interfere with radiopharmaceuticals' receptors.

This model does not provide a definite answer if "cold" SSA should be stopped or not, but suggests that SSA as biotherapy agents interfere with both diagnostic and therapeutic nuclear medicine procedures. This knowledge arouses the thought that a new practice should be tested: stopping the "cold" peptide administration before the injection of labeled peptide for SRS, and pre dosing with cold peptide before therapeutic administration of the radiolabeled one. Further research is needed to reach secure conclusions on patient medication management before diagnostic scans or therapeutic administrations in nuclear medicine.

*In conclusion*, by waiting at least 6 half-lives (14 days), after the unlabeled SSA administration, the radiolabeled receptor uptake increased two-fold to three-fold, as compared to simultaneous administration of radiolabeled and unlabeled peptides depending on which SSA was used.

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