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Induced-fit docking of mometasone furoate and further evidence for glucocorticoid receptor 17α pocket flexibility

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ABSTRACT

An induced-fit docking method was used to characterize the interactions of the glucocorticoid receptor binding-site with mometasone furoate, a glucocorticoid with a lipophilic ester at the C17α position. Two validation studies demonstrated that the protocol can reproduce crystal structures of nuclear receptors, and is appropriate for modeling ligand binding to the glucocorticoid receptor. Key hydrogen bonding interactions between mometasone furoate and the glucocorticoid receptor, as well as favorable hydrophobic interactions between the furoate group and the 17α pocket, contribute to high affinity and specificity of this ligand for the receptor. Using the glucocorticoid des-ciclesonide, which has an even larger moiety at the 16,17α position, induced-fit docking demonstrates the ability of the 17α pocket of the receptor to expand even further to accommodate the ligand.

1. Introduction

Since Hench's pioneering research in the 1950s using hydrocortisone for the treatment of rheumatoid arthritis, glucocorticoid (GC) compounds have become the foundation of management of many inflammatory diseases, including those of the lung and upper respiratory tract such as asthma and allergic rhinitis [1–5]. Older oral GCs such as dexamethasone (Dex) and prednisolone are effective at suppressing inflammation, but produce negative side effects such as hyperglycemia and osteoporosis, particularly with long-term administration [6]. The more recent development of GCs administered by inhalation or intranasal application marked a major advance in the management of upper respiratory inflammatory diseases by providing local drug delivery with minimal systemic absorption. Pharmaceutical research has continued to search for high-affinity GC compounds with minimal toxicity, and the newest GCs for inhalation/intranasal administration (*i.e.*, mometasone furoate [MF], fluticasone furoate [FF], fluticasone propionate [FP] and ciclesonide) have greatly improved benefit-risk ratio.

Glucocorticoid pharmacologic activity is mediated through interaction with the glucocorticoid receptor (GR), a member of the nuclear-receptor family of ligand-activated transcription factors that has been shown to suppress the inflammatory response in the context of asthma [7]. Similar to other members of this family, GR is characterized by three major domains: an N-terminal activation function-1 domain (AF-1), a central DNA-binding domain, and a C-terminal ligand-binding domain (LBD) [8]. An accurate understanding of the structure of the LBD could have tremendous ramifications for glucocorticoid research and pharmaceutical development, but unfortunately this domain is difficult to express in recombinant form and is not easily purified or crystallized [8,9]. To date, three human GR-LBD structures have been documented in the Protein Data Bank (PDB) [8–10]. These structures reveal that GR has a unique side pocket bounded by helices 3, 6, and 7. This pocket can accommodate large substituents at position C17α of GCs that are characteristic of clinically effective compounds. Experimental evidence shows that the GR binding site is extremely flexible and adaptive in its interactions with GCs.

Most modeling of ligand–receptor interactions has been done via docking methods that use rigid receptor structures obtained either from crystallography or homology modeling. Ligands that

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Abbreviations: des-CIC, desisobutyryl-ciclesonide; Dex, dexamethasone; FF, fluticasone furoate; FP, fluticasone propionate; GC, glucocorticoid; GR, glucocorticoid receptor; IFD, induced-fit docking; LBD, ligand-binding domain; MF, mometasone furoate; PDB, Protein Data Bank; PR, progesterone receptor; RBA, relative binding affinity; RMSD, root-mean-square deviation; vdW, van der Waals.

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require conformational changes in the receptor for binding limit the usefulness of these traditional methods. In fact, receptor flexibility is one of the greatest challenges for structure-based drug design [11,12]. The binding-site conformational changes induced by different ligands can range from modest to striking, depending on their interactions with the receptor. In a recent study, Boström *et al.* showed that the binding sites of pairs of proteins complexed with structurally similar ligands differed in 83% of cases [13]. Side-chain movements were observed in half of the pairs, whereas backbone movements rarely occurred. By adopting different rotamers, protein side-chains can cause changes in the shape, size, and electrostatic character of the receptor binding-pocket. Therefore, the use of a single rigid protein structure is in many cases too primitive for accurately docking ligands into receptors [14], and rigid-receptor docking has failed to produce reasonable models when the protein must be "induced" into the correct binding conformation for a given ligand.

Induced-fit docking (IFD) can be used to model and characterize binding-site geometries while taking into account both ligand and receptor flexibility [15–19]. Schrödinger's IFD protocol combines the use of a rigid-receptor docking program (Glide) [17] with a protein structure prediction and refinement module (Prime) [20] to allow accurate prediction of ligand-binding modes and concomitant structural changes in the receptor. Glide was designed to explore the positional, orientational, and conformational space of the ligand within the protein binding-site, while Prime took care of side-chain conformational changes as well as limited backbone changes in protein loop regions. IFD has the potential to produce a structure that more accurately reflects binding interactions by mutually accommodating the receptor and ligand to each other. Furthermore, IFD was shown to be able to generate reasonable binding structures for ligands known to be active but unable to be docked in an existing structure of the receptor using the rigid approach [16].

Certain features of corticosteroid structure–activity relationships appear to be common to all glucocorticoids [21]. For instance, carbonyl groups at C3 and C20, a β-hydroxyl group at C11, and a $\Delta^{4,5}$ double bond are essential for good GR binding [22]. A double bond at C1 generally increases selectivity for GR versus the mineralocorticoid receptor and improves anti-inflammatory activity, as does the combination of halogenation (either chlorine or fluorine) at C6 or C9 and an α- or β-methyl group at C16 [23]. Halogenation at either C6 or C9 increases receptor-binding affinity, but halogen substitutions at both positions do not give further increases in potency [22]. Many glucocorticoids have incorporated substituents at the 17α position to increase binding affinity and lipophilicity. Mometasone furoate (9,21-dichloro-11[β],17-dihydroxy-16[α]-methylpregna-1,4-diene-3,20-dione 17-[2-furoate]), a corticosteroid formulated for inhalation and intranasal use, was the first marketed corticosteroid to incorporate the lipophilic furoate ester at the 17α position (Fig. 1). MF has a high affinity for GR, with reported relative receptor affinity (RRA) values ranging from 1200 to 2900 [23–26]. While the furoate ester has contributed to these characteristics, it has not been demonstrated how the structural features of MF contribute to its high binding affinity, since no experimental or modeled structure of this molecule in complex with GR has been reported to date.

Our primary goal in this study was to apply IFD methodology to gain a detailed understanding of the nature of the MF–GR interactions. This corticosteroid-GR complex is of particular interest because the contacts between the highly desirable furoate moiety of MF and the GR 17α pocket have not yet been fully elucidated. In order to gain further perspective into the conformational flexibility of this pocket, we conducted a similar IFD analysis

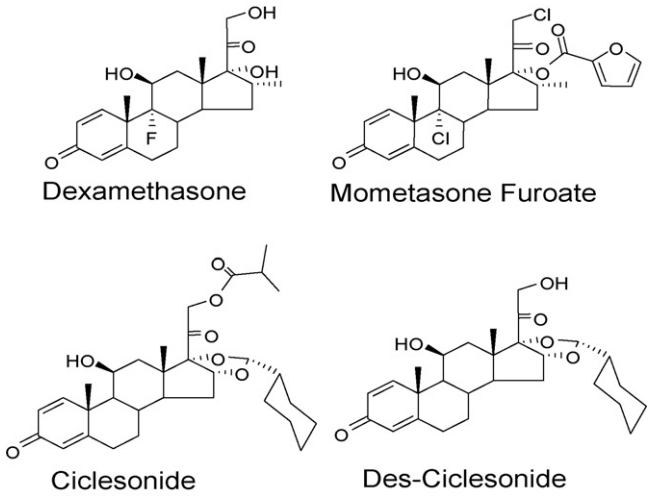

Fig. 1. Molecular structures of the compounds studied.

with the glucocorticoid desisobutyryl-ciclesonide (des-CIC), which has an even larger moiety at the 16,17α position (Fig. 1). des-CIC is the active metabolite of the pro-drug ciclesonide ([R]-11[β],16[α],17,21-tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16,17-acetal with cyclohexanecarboxaldehyde 21-isobutyrate), another corticosteroid formulated for inhalation/intranasal administration. Ciclesonide is converted to des-CIC by esterases at the site of action (*e.g.*, lung/nasal tissue), and the active metabolite has an approximately 100-fold greater RBA for GR than its pro-drug [27,28].

2. Methods

2.1. Protein structures

The protein structures used in this study were retrieved from the PDB [29] and included the following: GR-LBD in complex with Dex (PDB IDs: 1p93 and 1m2z), GR-LBD in complex with deacylcortivazol (PDB ID: 3bqd), progesterone receptor (PR)-LBD in complex with progesterone (PDB ID: 1a28) [30], and PR-LBD in complex with MF (PDB ID: 1sr7) [31]. All chains in these structures were extracted and aligned using LSQMAN [32] with 1p93 chain A as the template (The brute force option was used to align the structures). Additionally, a GR-LBD structure in complex with FP was documented in a recent patent [33].

For structures with multiple chains, only chain A was retained and prepared for docking studies. Protein preparations were carried out with Maestro [34] and involved the following steps: assign bond orders and add hydrogen atoms to the ligand molecule; add hydrogen atoms to protein heavy atoms and charge the Asp, Glu, Arg and Lys residues; optimize the orientation of hydroxyl groups on Ser, Thr and Tyr residues; optimize the side chains of Gln and Asn residues; and determine the state of His residues. The ligand and water molecules in each structure were retained throughout the protein preparation process. Water molecules and cofactors were removed before docking studies. The binding pocket volume was calculated with VOIDOO using default parameters [35].

2.2. Compound preparation

The two-dimensional structures of the compounds used in this study are shown in Fig. 1. Corresponding three-dimensional structures were generated using the Concord program [36]. These