SULFATION OF BUDESONIDE BY HUMAN CYTOSOLIC SULFOTRANSFERASE, DEHYDROEPIANDROSTERONE-SULFOTRANSFERASE (DHEA-ST)

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ABSTRACT:
Budesonide, a synthetic glucocorticosteroid, is used in the treatment of asthma and allergic reactions, rhinitis, and inflammatory bowel disease. It is distributed as a mixture of two epimers, 22R and 22S, and has a high ratio of topical to systemic activity due to extensive first-pass metabolism to metabolites with minimal activity. Previous studies have shown that the epimers are metabolized by the cytochrome P450 monoxygenase system. Metabolism and inactivation of the epimers by the phase II enzymes has not been well characterized. This study describes the conjugation of budesonide by human cytosolic sulfotransferases (SULTs). Seven human SULTs were analyzed to determine which were capable of catalyzing the sulfation of the epimers of budesonide. Only dehydroepiandrosterone-sulfotransferase (DHEA-ST, SULT2A1) was capable of forming a sulfated budesonide product. The epimeric forms of budesonide display different kinetic activities with the 22R epimer having a 3.5-fold greater rate of sulfation activity than the 22S epimer. The structure of budesonide shows two hydroxyl sites that are potential sites for sulfate conjugation, but analysis by mass spectrometry indicates the formation of only a monosulfated budesonide product. A modeling approach was used to define the site of sulfation as that of the 21-hydroxyl group. Although sulfation of budesonide by DHEA-ST may not be an important factor in its use as an antiasthmatic, intestinal and hepatic sulfation will be important for its proposed systemic use as an anti-inflammatory agent.

Budesonide (Fig. 1) is a synthetic glucocorticosteroid used in the treatment of asthma, rhinitis, and inflammatory bowel diseases. It has a high ratio of topical to systemic activity partly due to extensive first-pass metabolism in the liver to metabolites with minimal activity (Clissold and Heel, 1984). Budesonide is distributed as a mixture of two epimers, 22R and 22S. The epimeric mixture is a 1:1 ratio whereby the 22R epimer has been reported to have a potency that is 2- to 3-fold greater than the 22S epimer based on topical activity (Brattsand et al., 1982).

Previous in vitro studies with human liver microsomes have demonstrated that the budesonide epimers are differentially metabolized by the cytochrome P450 monoxygenase system. Budesonide 22R is metabolized by CYP3A4 to two major metabolites, 16α-hydroxybudesonide and 6β-hydroxybudesonide, whereas the 223 epimer is only metabolized to one major metabolite, 6β-hydroxybudesonide (Jonsen et al., 1995). These metabolites are also present in high amounts in plasma and urine after administration of budesonide to humans (P. Anderson, AstraZeneca, unpublished results). In contrast to phase I metabolism, only limited information on the metabolism of budesonide by the phase II drug-metabolizing enzymes is available. However, a previous report describes that budesonide undergoes reversible conjugation (apparently in the 21 position) with fatty acids in human lung and liver. This mechanism is thought to prolong the duration of the pharmacological effects of budesonide in the lung (Tunek et al., 1997). Another report (Pacifici et al., 1994) has shown that budesonide can be conjugated with sulfate in human liver and to some extent in lung, but the site of conjugation in the molecule, as well as the sulfotransferase (SULT1) isoenzymes involved, is not known. Of particular interest in this study is the ability of human cytosolic SULTs to catalyze the sulfate conjugation of the budesonide epimers. Generally, conjugation of steroids with a sulfonate moiety results in a loss of biological activity and an increase in water solubility and excretion (Falany, 1997).

After administration of budesonide by inhalation, a large part of the dose is absorbed from the lung (Ryrfeldt et al., 1982). The localization of the human cytosolic SULTs in the human lung has been controversial (Luu-The et al., 1995; Hume et al., 1996). Identification of the human SULT isoform(s) involved in budesonide sulfation is therefore important in understanding and predicting its biological activity.

This study investigates the ability of the human SULTs to conjugate the epimers of budesonide. Furthermore, we also attempted to identify the site(s) of sulfate conjugation, since there are two hydroxyl groups in the budesonide molecule, in the 11 and 21 position, that potentially...
could be sulfated (Fig. 1). Seven of the eleven known human cystosolic sulfotransferases were analyzed to determine which SULT isoforms were capable of catalyzing the sulfate conjugation of the 22R and 22S epimers of budesonide. These studies provide information of the ability of the sulfating enzymes to be involved in promoting the excretion of budesonide and therefore eliminating its systemic side effects.

**Experimental Procedures**

**Materials.** The budesonide epimers, 22R and 22S, were provided by AstraZeneca Pharmaceuticals LP (Södertälje, Sweden) and were 97.5 and 96.5% pure, respectively. [35S]-phosphoadenosine 5'-phosphosulfate (PAPS) (2 Ci/mmol) was purchased from PerkinElmer Life Sciences (Boston, MA). LK60DF silica gel 60A thin-layer chromatography (TLC) plates were purchased from Whatman Inc. (Clifton, NJ).

**Methods.** Sulfotransferase assays. Budesonide sulfation activity was assayed using both nonradioactively labeled 22R and 22S budesonide. [35S]PAPS was included in the reactions, and [35S]budesonide sulfate was resolved by TLC as described previously (Falany et al., 1994). Initial reactions involved screening 22R and 22S for activity with each SULT at 25 μM and 150 μM budesonide. Positive controls using known substrates at optimal concentrations for each SULT isoform analyzed were included in the reactions. Typical reactions contained 1 to 20 μM budesonide in 50 mM Tris-HCl, pH 7.4, and 20 μM [35S]PAPS in a final volume of 62.5 μL and were linear with respect to time and protein. Control reactions were identical except that budesonide was not added. The substrates were resolved using an 85:15:5 methylene chloride, methanol, ammonium hydroxide solvent system. After TLC, autoradiography was used to localize sulfated products. The conversion to radioactive product was determined by scintillation counting after scraping the specific radioactive budesonide product bands from the TLC plate. Control reactions contained all products of the reaction mixture except for substrate to detect background sulfation. The bacterial expression, purification, and isolation of the expressed human SULTs, monoamine-sulfating form of phenol SULT (MPST, SULT1A3, accession number P50224), phenol-sulfating form of phenol SULT (PST-1, SULT1A1, AAA99892), dehydroepiandrosterone sulfotransferase (DHEA-ST, SULT2A1, Q06520), estrogen sulfotransferase (EST, SULT1E1, AAB34601), SULT2B1a (AAC75534), and SULT2B1b (AAC78554) have been reported previously (Falany et al., 1989; Kakuta et al., 1998; Pedersen et al., 2000). Hydrogen atoms and Kollman-all charges were added to the protein whereas the atoms of DHEA were held as fixed aggregates during the protein minimization by the Powel algorithm using the Tripos force field with a gradient termination of 500 iterations as the convergence criteria.

**Docking Procedure.** The automated docking package FlexiDock, a module of SYBYL 6.4, was used in the docking studies. Individual budesonide epimers were constructed, given Gasteiger-Hückel charges, and optimized by energy minimization using the Powel algorithm. These structures were fit into the position occupied by DHEA in the DHEA-ST-PAPS complex using the Multifit option in SYBYL 6.4. The resultant structure was used as the initial conformation for the docking procedure. This methodology provided a set of solutions that represented the optimum ligand geometry in the active site of the DHEA-ST model.

**Results**

**Sulfation Activity.** Budesonide sulfation was initially analyzed using expressed isoforms of the major members of both the SULT1 and SULT2 families. The SULT1 family was represented by PPST-1, MPST, EST, and SULT1B2. The SULT2 family was represented by DHEA-ST, SULT2B1a, and SULT2B1b. Budesonide sulfation activity was assayed under varying conditions, and budesonide sulfation was only observed with DHEA-ST. This was the only SULT that demonstrated budesonide activity that was greater than background. Figure 2 is a representative figure of sulfation activity shown by only DHEA-ST. In preliminary studies, the epimeric forms of budesonide were not sulfated at the same time. The scan showed peaks corresponding to the nonsulfated parent compound and the negative ion peak for budesonide nonsulfate. Since budesonide possesses hydroxyl groups at the 11 and 21 positions, the site of sulfation was investigated. Further analysis of budesonide sulfate by MS-MS was SYBYL version 6.4 molecular-modeling package (Tripos Inc., St. Louis, MO) was used for all the modeling procedures. For the calculation of the minimum energies of the two epimers and four singly sulfated products of budesonide, each molecule was assigned Gasteiger-Hückel charges and minimized by the Powel algorithm using the Tripos force field with a gradient termination of 0.01 kcal/mol as the convergence criteria.

**Modification of DHEA-ST structure.** The structure of DHEA-ST, [protein database bank (PDB) file 1EFH] was minimized after including PAPS, DHEA, and hydrogen atoms. For the addition of PAPS, the structure of a proposed mimic of the transition state intermediate of the SULT reaction, PAP-vanadate, was extracted from (PDB file 1BO6) (Kakuta et al., 1998) that contained the coordinates for the estrogen sulfotransferase-PAP-vanadate complex. Replacing the vanadate with a sulfuryl group modified the PAP-vanadate moiety to result in a PAPS molecule. The PAPS molecule was then fit into the position occupied by PAP in the active site of the DHEA-ST model using the Multifit option in SYBYL 6.4. For the addition of DHEA, the estradiol molecule was extracted from the (PDB file 1AQU) (Kakuta et al., 1997) that contained the coordinates for the estrogen sulfotransferase-PAP-estradiol complex. The structure of estradiol was modified to result in a DHEA molecule. The DHEA molecule was fit into the substrate-binding pocket of the DHEA-ST structure based on structural analogy with estradiol and estrogen sulfotransferase. The estrogen sulfotransferase-PAP-estradiol complex was used because it contains a substrate that was co-crystallized with the enzyme, unlike the crystal structure for DHEA-ST (Kakuta et al., 1998; Pedersen et al., 2000). Hydrogen atoms and Kollman-all charges were added to the protein whereas the atoms of DHEA and PAPS were assigned Gasteiger-Hückel charges. DHEA and PAPS were held as fixed aggregates during the protein minimization by the Powel algorithm using the Tripos force field with a gradient termination of 500 iterations as the convergence criteria.
isomer, which might indicate higher affinity for the 22S epimer are shown in Table 2. For both the 22R and 22S epimers, sulfate conjugation at position 21 generates more stable sulfated products than conjugation at position 11.

The availability of the coordinates for the structure of DHEA-ST (Pedersen et al., 2000) allows for the analysis of the probable interaction of the budesonide epimers with DHEA-ST in the presence of PAPS. Using FlexiDock methodology, only position 21 of budesonide showed binding in a suitable geometry for sulfate transfer. In none of the binding models did position 11 associate closely with the active site histidine (Pedersen et al., 2000). Comparison of the binding of the budesonide epimers indicated that the 22S epimer (Fig. 3A) could be docked in the active site with better geometry than the 22R epimer (Fig. 3B). The results are consistent with the lower K_m for the 22S isomer, which might indicate higher affinity for the 22S isomer (Table 1).

**Discussion**

In this report, DHEA-ST has been shown to be the SULT isoform primarily responsible for the sulfation of budesonide in human tissues. DHEA-ST is capable of conjugating both the 3α- and 3β-hydroxyls of hydroxysteroids, the 3-phenolic hydroxyl of estrogens, as well as the 17β-hydroxyl of testosterone and β-estradiol (Falany et al., 1989). This observation is consistent with the report of Pacifici et al. (1994) that human liver budesonide sulfation was inhibited by testosterone and correlated with testosterone sulfotransferase activity. To date, DHEA-ST is the only human SULT reported to sulfate testosterone.

Budesonide is administered by inhalation for the treatment of asthma and is also used in nasal preparations for the treatment of rhinitis and in oral preparations for the treatment of inflammatory bowel disease (e.g., Crohn’s disease; Spencer and McTavish, 1995). DHEA-ST is highly expressed in both the human adrenal cortex and liver and is present in the gastrointestinal tract indicating a probable role in the first-pass and systemic metabolism of budesonide (Her et al., 1996; Tashiro et al., 2000). DHEA-ST expression in human lung is less well characterized. Luu-The et al. (1996) did not detect DHEA-ST message in adult lung RNA, whereas Hume et al. (1996) report the immunolocalization of DHEA-ST in lung tissue of young rodent species.

**Table 1**

*Apparent K_m value and the budesonide sulfation activities of the sulfation of 22R and 22S epimers by DHEA-ST*

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Budesonide Sulfation Activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>K_m (M) pmol/min/mg</td>
<td>513.9 ± 25.3</td>
</tr>
<tr>
<td>Budesonide 22R</td>
<td>20.9 ± 3.9</td>
</tr>
<tr>
<td>Budesonide 22S</td>
<td>11.1 ± 3.0</td>
</tr>
</tbody>
</table>

**Table 2**

*Energies of the sulfated products of 22R and 22S epimers of budesonide*

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Energy (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Budesonide 22R</td>
<td>30.55</td>
</tr>
<tr>
<td>Budesonide 22R, 11 sulfate</td>
<td>25.58</td>
</tr>
<tr>
<td>Budesonide 22R, 21 sulfate</td>
<td>21.17</td>
</tr>
<tr>
<td>Budesonide 22S</td>
<td>30.84</td>
</tr>
<tr>
<td>Budesonide 22S, 11 sulfate</td>
<td>26.03</td>
</tr>
<tr>
<td>Budesonide 22S, 21 sulfate</td>
<td>20.62</td>
</tr>
</tbody>
</table>

**Fig. 2.** Representative TLC autoradiograph showing sulfation of 25 μM 22R-budesonide with seven human SULTs.

Lane 1, 22R-budesonide with DHEA-ST; lane 2, no substrate with DHEA-ST; lane 3, 22R-budesonide with SULT2B1a; lane 4, 22R-budesonide with SULT2B1b; lane 5, 22R-budesonide with PPST-1; lane 6, 22R-budesonide with MPST; lane 7, 22R-budesonide with EST; lane 8, 22R-budesonide with SULT1B2.

**Fig. 3.** 22(S) (Fig. 3A) and 22(R) (Fig. 3B) budesonide docked into the coordinates of the active site of human DHEA-ST.

This enzyme structure was modeled with analogy to the coordinates for the mouse estrogen sulfotransferase (PDB code 1AQU). The labeled areas of the figure include the sulfate group of PAPS, the 11-hydroxyl, and the 21-hydroxyl groups of the budesonide epimers.
children. Therefore, the role of DHEA-ST in the metabolism of budesonide in different tissues still needs to be investigated.

Budesonide is administered as an equal mixture of two epimers, 22R and 22S (Ryrfeldt et al., 1982). The 22R epimer has a 2- to 3-fold more potent anti-inflammatory response and has a different pharmacokinetic profile (Brattsand et al., 1982). The greater potency of the 22R epimer is possibly due to a greater affinity for the glucocorticoid receptor (Derendorf et al., 1998). As shown in Table 1, DHEA-ST displays a 2-fold lower apparent \( K_{m} \) for the 22S epimer as compared with the 22R; however, the sulfation activity of the 22R epimer is 3.5-fold greater than the 22S epimer.

Attempts to identify the site of sulfate conjugation using MS-MS were unsuccessful. Sufficient fragmentation of the steroid nucleus was not obtained to allow for definitive identification of daughter fragments. The availability of the structural coordinates for EST and DHEA-ST (Kakuta et al., 1998; Pedersen et al., 2000) allowed for a molecular-modeling approach to predict the geometry of budesonide binding in the active site. Consistent with the activity data, the 11-hydroxy position did not bind in the active site close to the active site histidine (Pedersen et al., 2000). Also, the presence of the two angular methyl groups on the steroid backbone should provide considerable steric hindrance to sulfation of the 11-hydroxy group. In accordance with these observations, it has been shown that budesonide and hydrocortisone can be selectively acetylated chemically at the 21-hydroxy group without affecting the more sterically hindered 11β-hydroxy group (Paulson and Lindberg, 1991; Lindberg et al., 1992).

Moreover, budesonide is known to be conjugated with fatty acids in lung and liver apparently at the 21-hydroxy group (Tunek et al., 1997).

Studies of the metabolism of budesonide have not reported the formation of large amounts of sulfate conjugate; however, phase II metabolism has not been the focus of these reports (Andersen et al., 1982; Edsbacker et al., 1983, 1987; Jonsson et al., 1995). Previous reports of the in vitro metabolism of epimERIC budesonide by human liver microsomes have identified phase I metabolites resulting from the action of the oxidative cytochrome P450 enzyme family and principally, CYP3A (Jonsson et al., 1995). The two major metabolites of epimeric budesonide from in vitro studies using human liver homogenates are 6β-hydroxybudesonide and 16a-hydroxyprednisolone, although 16α-hydroxyprednisolone was not a detectable metabolite of the 22S epimer (Jonsson et al., 1995). Although sulfation might not be a major pathway in budesonide metabolism and inactivation following inhalation, it may have a significant role in budesonide metabolism following oral administration, especially if time-release formulations are used to increase delivery to the colon (Spencer and McTavish, 1995). Absorption from the gastrointestinal tract can also occur from the fraction of the given dose that is deposited in the mouth and swallowed after inhalation (Newman et al., 1981; Davies, 1982).

In conclusion, this study has shown the ability of DHEA-ST, a major human hepatic hydroxysteroid SULT, to catalyze the sulfation of both epimers of budesonide. Sulfation apparently occurs only at the 21-hydroxy moiety and shows differences in rate and affinity for the two epimers. Although, the CYP3A enzymes have the most important role in the budesonide deactivation, the significance of sulfation in the deactivation of budesonide may become more important in situations in which the CYP3A isoforms are inhibited by concomitant medication or by dietary factors (Ameer and Weintraub, 1997).

References


