Relative In Vitro Protein Binding Characteristics of Diclofenac Sodium, Pantoprazole and Gatifloxacin With Human Serum And Plasma

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Abstract:

Drug-plasma protein binding is one of the many factors which influence bioavailability of a drug. Efficacy, distribution and elimination of a drug can be affected by the reversible binding of the drug to plasma proteins, such as albumin and acid glycoprotein. The purpose of the study was to study the relative in vitro protein binding characteristics of various drugs like diclofenac sodium, pantoprazole and gatifloxacin in the presence of human plasma and serum proteins. Two ml solution of these drugs was mixed with 2 ml of serum and plasma separately and was allowed to dialyze through eggshell membrane and analyzed in a UV- Visible spectrophotometer at respective absorption maxima wavelength. The result of the studies revealed that pentoprazole showed maximum binding with proteins followed by diclofenac sodium and gatifloxacin.

Key Words: protein binding, gatifloxacin, diclofenac sodium, pentoprazole

Introduction:
The nature and magnitude of drug-protein binding has important pharmacokineyic and pharmacodynamic implications, because it is the unbound moiety that readily diffuses across biological membranes, reaches the receptor site to produce pharmacological effect, and is most readily available for elimination from the body.\(^{1,2}\) The interaction of a drug with protein may cause, the displacement of body hormones or a co administered agent or a confirurational change in the protein, the structurally altered form of which is capable of binding a coadministered agents or the formation of a drug-protein complex that itself is biologically active \(^3,4,5\). Diclofenac sodium is a potent NSAID; pentoprazole is a drug in proton pump inhibitor category while gatifloxacin is a 4\(^{th}\) generation fluoroquinolone with a broad spectrum of antimicrobial activity. These drugs are quite common in various clinical conditions to

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treat various diseases or disorders like pain, gastric ulcers, inflammation, microbial infections.\(^6\)\(^,\)\(^7\) Keeping the above in view attempt was made to study the protein binding characteristics of these drugs with human serum and plasma.

A number of methods are available for studying protein binding of drugs. Apart from satisfying the general criteria from any analytical technique, they must not upset the equilibrium dialysis, Dynamic dialysis, ultra filtration and Electrophoresis are the classic techniques used in recent years other methods such as gel filtration and nuclear magnetic resonance have been used with satisfactory results. All have their own inherent advantages and disadvantages.\(^8\)\(^,\)\(^9\)

Here the dynamic dialysis method is used to study protein-binding characteristics of diclofenac sodium, pantoprazole and gatifloxacin.

**Materials and Methods:**
All chemicals used were of analytical grade. Gatifloxacin, diclofenac sodium and Pantoprazole were kind gift samples from Ranbaxy Ltd., Gurgaon.

The isolated eggshell membrane was used as a diffusion layer for the drug. One end of an open-ended glass cylinder was tied with the membrane and used as protein compartment. A glass beaker capacity of 250 ml was used as non-protein compartment. The non-protein compartment was filled with 100 ml of distilled water, and the drug solution (1mg/ml) of 4 ml was placed in the inner tube and it was immersed into the beaker and care was taken to maintain the level of the drug solution coincide with a stand. This whole set-up was kept on a magnetic stirrer and the outer compartment was stirred continuously with an optimal sped. The temperature was maintained at 35±2\(^\circ\)c for the whole experiment.

Each time 10 ml of sample from the non-protein compartment were withdrawn at 5, 10, 15, 30, 60, 90 minutes time interval and every time the sample was replaced with same volume of fresh distilled water. The concentration of diclofenac sodium, pantoprazole and gatifloxacin was determined spectrophotometrically in each sample. The studies were conducted separately for each drug with serum and plasma. The experiment was repeated for each drug by using 2 ml of human blood serum and 2 ml of drug solution (2 mg/ml) in the protein compartment and the percentage of drug released from the protein compartment into the non-protein compartment were determined in the same time periods as above. This experiment was repeated for each drug by using 2 ml of human blood plasma and 2 ml of drug solution (2 mg/ml) in the protein compartment and the percentage of drug released was determined spectrophotometrically in UV-Visible spectrophotometer at absorption maxima 276 for diclofenac, 291 for pantoprazole and 286 for gatifloxacin.

**Results and discussion:**
The release study of three drugs was studied through eggshell membrane at different time intervals. The results are shown in table 1 and figure 1. It is revealed from the figure that cumulative percentage release of drugs after 90 min is very less in presence of serum and plasma than that of the release of
drug without any proteins. This represents that the plasma proteins, serum proteins having the great affinity towards the drug and bind with it.

While comparing the drug release in presence of serum and plasma, the percentage of drug release in presence of plasma is less than that of serum. Since the serum lacks the clotting factors (including fibrinogen) that are normally present in plasma. The release order of the drugs in different conditions is as follows- Drug release without any protein > Serum > Plasma and the drug release is gatifloxacin > diclofenac sodium > pentoprazole.

**Conclusion:**
The results clearly showed that in the presence of the plasma these drugs showed maximum protein binding and among these gatifloxacin showed maximum protein binding followed by diclofenac and pentoprazole.

**References:**


Table 1: The Percentage of Drug Release in Different Time Intervals with and Without Proteins

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>TIME (IN MIN.)</th>
<th>% Drug release</th>
<th>Control (Without any protein)</th>
<th>With serum</th>
<th>With plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>D1</td>
<td>P1</td>
<td>G1</td>
</tr>
<tr>
<td>1.</td>
<td>5</td>
<td></td>
<td>13.0</td>
<td>6.2</td>
<td>44.5</td>
</tr>
<tr>
<td>2.</td>
<td>10</td>
<td></td>
<td>19.5</td>
<td>13.75</td>
<td>45.5</td>
</tr>
<tr>
<td>3.</td>
<td>15</td>
<td></td>
<td>25.0</td>
<td>14.5</td>
<td>48.0</td>
</tr>
<tr>
<td>4.</td>
<td>30</td>
<td></td>
<td>40.5</td>
<td>24.0</td>
<td>56.0</td>
</tr>
<tr>
<td>5.</td>
<td>50</td>
<td></td>
<td>56.0</td>
<td>33.0</td>
<td>71.0</td>
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<tr>
<td>6.</td>
<td>70</td>
<td></td>
<td>62.0</td>
<td>42.0</td>
<td>76.0</td>
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<tr>
<td>7.</td>
<td>90</td>
<td></td>
<td>63.5</td>
<td>47.0</td>
<td>71.0</td>
</tr>
</tbody>
</table>

**Abbreviations:**
- **D1** - Diclofenac sod. Without any protein
- **D2** - Diclofenac sod. With serum
- **D3** - Diclofenac sod. with plasma
- **P1** - Pantoprazole without any protein
- **P2** - Pantoprazole with serum
- **P3** - Pantoprazole with plasma
- **G1** - Gatifloxacin without any protein
- **G2** - Gatifloxacin with serum
- **G3** - Gatifloxacin with plasma

Figure 1: Relative in vitro drug release characteristics (after 90 min) of diclofenac, pantoprazole and gatifloxacin from protein binding sites (i.e. in the presence of serum and plasma)