PharmacologyOnLine December 30, 2015

Archives • 2015 • vol.3 • 1-4

DRUG-DRUG INTERACTIONS BETWEEN GRISEOFULVIN AND NORTRIPTYLINE AT BINDING SITES OF BOVINE SERUM ALBUMIN

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Abstract

The study of drug-drug interaction between Griseofulvin and Nortriptyline hydrochloride at binding sites of Bovine Serum Albumin (BSA) was studied by equilibrium dialysis (ED) method. During concurrent administration of these drugs, Griseofulvin (antifungal) and Nortriptyline hydrochloride (antidepressant) had been found to increase the respective free concentration of one another causing reduced binding to BSA. The increment in free concentration of was more prominent in presence of site-I specific probe (warfarin sodium) than in absence of it. The binding of Griseofulvin and Nortriptyline HCl was found to be enhanced to site-II (low affinity binding site) in presence of site-I specific probe. This modified form of displacement has tentatively been referred to as site-to-site displacement.

Key words: Griseofulvin, Nortriptyline hydrochloride, warfarin sodium, drug-drug interaction, BSA, equilibrium dialysis.

Introduction

The primary structure of human serum albumin (HSA) was conducted by Meloun *et al*., 1975 and Brown, 1976. It is folded into three domains, each of which is built of three loops. HSA is comparatively a large multi-domain protein. Bovine serum albumin (BSA) and HSA has structural similarity. Among the plasma proteins, albumin is mostly bound to ligands or drugs [1, 2, 3].

The reduction in the extent of binding of a drug to protein occurred by the presence of other drugs is termed as drug-drug interaction or drug displacement. Competitive displacement and Non-Competitive displacement at binding sites may take place. As a consequence the free concentration of the displaced drug increases and may even lead to higher pharmacological as well as toxic effects [4].

Protein of a drug is not a phenomenon particular to the plasma. Plasma protein binding properties are related to plasma clearance, elimination half-life, apparent volume of the distribution and area under the curve. Though the information resource regarding the binding of drugs to HSA is extensive, the mechanism of drug binding to HSA is still a subject of speculation and controversy [5]. Combination therapy is common practice now a day. During the concurrent use of drugs all the drugs may exert their effects independently or may interfere or interact with each other in biopharmaceutical, biochemical or in pharmacological point of view. Keeping this consideration in mind, Griseofulvin, an antifungal & Nortriptyline HCl that is an antidepressant have been used in the study to observe the site to site displacement of these two drugs.

Method & Materials

Drugs & probes used in the study

Griseofulvin (The IBN Sina phamaceuticals Ltd.), Nortryptyline HCl (The IBN Sina phamaceuticals Ltd.) and Warfarin Sodium (Site-I) (Incepta Pharmaceuticals Ltd.).

Reagents used

Disodium hydrogen phosphate (Na_2HPO_4), Potassium dihydrogen phosphate (KH_2PO_4), Borax ($NaB_4O_7.H_2O$) (Analytical grade, Glaxo), Cellulose Membrane (Medical International Limited. Liverpool Road, London; mol wt 1200 Daltons), Bovine Serum Albumin (BSA) (fatty acid free, fraction V, 96-98%, Mol.Wt 66500 and purchased from the Sigma Chemical Co).

Instruments used

pH Meter (HANNA Microprocessor pH Meter, Portugal), SP8-400 UV/VIS Spectrophotometer (Thermospectronic, England.), Metabolic Shaking Incubator (Clifton Shaking Bath, Nickel Electro Ltd., England.), Micro syringe (Well. Liang.Jin. Yang.q.I., Chaina.). Equilibrium Dialysis method was employed in the study.

Effect of Nortriptyline on Griseofulvin binding to BSA in absence of site-I specific probe Warfarin Na was studied as follows

3ml of previously prepared 2×10^{-5} Molar BSA solution was taken in each of 7 cleaned and dried teat tubes.6 µl of 1×10^{-2} Molar Griseofulvin solution was taken in each of seven cleaned and dried test tubes. So that final ratio between protein and Griseofulvin was 1:1 (2×10^{-5} Molar: 2×10^{-5} Molar) in each of six test tubes. The seventh test tube containing only BSA solution was marked as blank. Nortriptyline was added with an increasing concentration in to five out of six test tubes containing 1:1 mixture of protein- Griseofulvin to make the final ratio of protein, Griseofulvin and Nortriptyline 1:1:0, 1:1:1, 1:1:2, 1:1:4, 1:1:6, 1:1:8, 1:1:10. Nortriptyline was not added to one test tube. After mixing the solution was pipette out into seven different semi-permeable membrane tubes both end of the membrane tubes were clipped ensured there were no leakage. The tubes containing drug mixtures were immersed in seven 50 mL conical flask containing 30 mL of phosphate buffer solution of pH 7.4. After shaking in a metabolic shaker at 25°C and 120 rpm for about 10 hours, absorbance of Griseofulvin buffer solution were measured by a UV spectrophotometer (Pye Unicum, England) at a wave length of 291 nm (BP).

In presence of site-I probe: Nortriptyline was added into five out of six test tubes containing 1:2:1 mixture of protein- Warfarin - Griseofulvin to make the final ratio of protein, Warfarin, Griseofulvin and Nortriptyline 1:2:1:0, 1:2:1:1, 1:2:1:2, 1:2:1:4, 1:2:1:6, 1:2:1:8 and 1:2:1:10.

Effect of Griseofulvin on Nortriptyline binding to BSA in absence of site-1specific probe Warfarin Na

2ml of previously prepared 2×10^{-5} Molar BSA solution was taken in each of seven cleaned and dried teat tubes.6 µl of 1×10^{-2} Molar Nortriptyline solution was taken in each of seven cleaned and dried test tubes. So that final ratio between protein and Nortriptyline was 1:1 (2×10^{-5} Molar: 2×10^{-5} Molar) in each of six test tubes. The seventh test tube containing only BSA solution was marked as

blank. Griseofulvin was added with an increasing concentration in to five out of six test tubes containing 1:1 mixture of protein-Nortriptyline to make the final ratio of protein, Nortriptyline and Griseofulvin 1:1:0, 1:1:1, 1:1:2, 1:1:4, 1:1:6, 1:1:8, 1:1:10. Griseofulvin was not added to one test tube. After mixing the solution was pipette out into seven different semi-permeable membrane tubes both end of the membrane tubes were clipped ensured there were no leakage. The tubes containing drug mixtures were immersed in seven 50 mL conical flask containing 30 mL of phosphate buffer solution of pH 7.4. After shaking in a metabolic shaker at 25°C and 120 rpm for about 10 hours, absorbance of Nortriptyline solution were measured by a UV spectrophotometer (Pye Unicum, England) at a wave length of 291 nm (BP). *In presence of site-I probe*: Griseofulvin was added into five out of six test tubes containing 1:2:1 mixture of protein-Warfarin - Griseofulvin to make the final ratio of protein, Warfarin, Nortriptyline and Griseofulvin 1:2:1:0, 1:2:1:1, 1:2:1:2, 1:2:1:4, 1:2:1:6, 1:2:1:8 and 1:2:1:10.

Results

In the study of Griseofulvin- Nortriptyline hydrochloride interaction the effects of Griseofulvin on Nortriptyline hydrochloride in absence and in presence of site-I specific probe, warfarin sodium, are shown in figure 1. Free concentration of Nortriptyline hydrochloride bound to BSA (1:1; 2x 10^{-5}M: 2x 10^{-5} M) upon the addition of Griseofulvin hydrochloride in absence of warfarin sodium was increased from 13.5% to 61.0% when Griseofulvin to BSA ratio was 10 while in presence of warfarin sodium (4x 10^{-5} M) the increment was from 19.5% to 62.4% when Griseofulvin to BSA ratio was also 10. Again, the effect of Nortriptyline HCl on Griseofulvin hydrochloride in absence and in presence of site-I specific probe warfarin sodium is shown in Fig.2. Free concentration of Griseofulvin HCl bound to BSA (1: l: 2 x 10^{-5} M: 2x 10^{-5} M) upon the addition of Nortriptyline hydrochloride in absence of warfarin was increased from 20.5% to 49.5% when Nortriptyline to BSA ratio was 10 whereas in presence of warfarin the increment of Griseofulvin was from 24.0 % to 53.5% when the Nortriptyline HCl to BSA ratio was also 10.

Dicussion

Adequate knowledge about composition, size and location of binding sites as well as the probable interactions at binding sites at HSA along with all the binding parameters of plasma protein is required for proper explanation of pharmacokinetic aspects of drugs. It is important, for the rational understanding of drug- serum albumin binding during concurrent administration and its consequences in drug actions. During concurrent administration, Griseofulvin displaced Nortriptyline from its high affinity binding site-I. Thus free concentration of Nortriptyline increased from 13.5% to 61.0%. This free Nortriptyline may bind to its low affinity-binding site, site-II. When warfarin sodium (a site-1 specific probe) was used sufficiently to block site-I, the free concentration of Nortriptyline was further increased from 19.5% to 62.4% (Figure1). On the other hand, Nortriptyline displaced Griseofulvin from its high affinity binding site-I. Thus free concentration of Griseofulvin increased from 20.5% to 49.5%. This free Griseofulvin may bind to its low affinity binding site, site-II**.** When warfarin sodium (a site-1. specific probe) was used sufficiently to block site-l, the free concentration of Griseofulvin was further increased from 24.0 % to 53.5% (Figure 2). This little more increment of release than that of absence of probe can be justified and it might be due to the inhibition of released drug from further binding to site-I, which is blocked by warfarin sodium or might be due to the displacement by one another for co-operative binding between them and the probe. During concurrent administration of these two drugs site-to-site displacements will take place and may change pharmacodynamic properties of the drugs. So care should be exercised during concurrent administration of Griseofulvin and Nortriptyline hydrochloride.

Acknowledgement

The authors are thankful to Khulna University, Khulna and Daffodil International University, Dhaka for providing the necessary facilities for the study.

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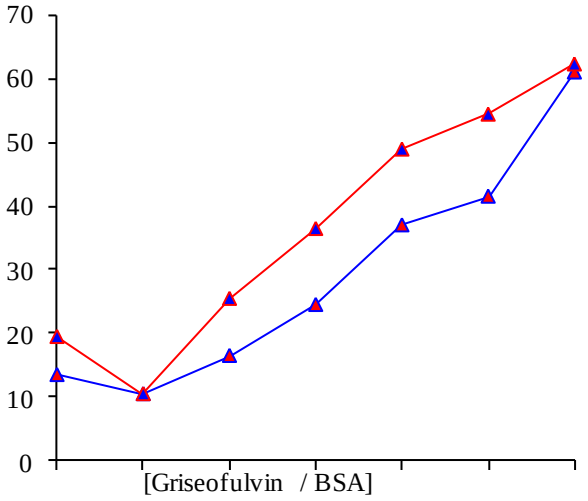

Figure 1. Effect of Griseofulvin on Nortriptyline HCl binding to BSA in absence (Blue line) and in presence (Red line) of site-I specific probe, warfarin Na upon the addition of increasing concentration of Nortriptyline HCl from 0x10 to 20 x10^{-5} M

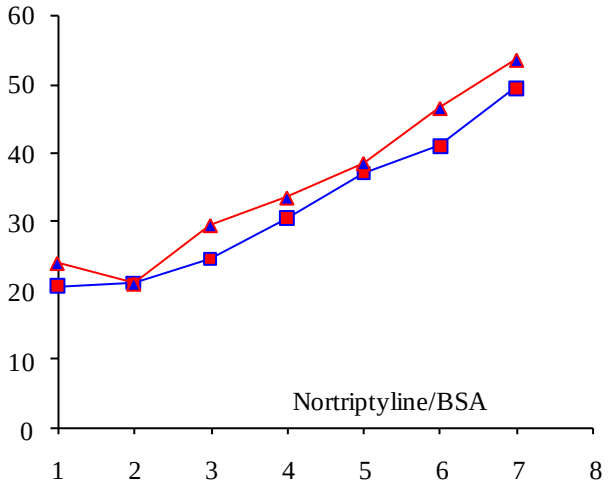

Figure 2. Effect of Nortriptyline HCl on Sulfa- methoxazole binding to BSA in absence (Blue line) and in presence (Red line) of site-I specific probe, warfarin Na upon the addition of increasing concentration of Nortriptyline HCl from 0x10 to 20 x10^{-5} M