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IN-VITRO STUDIES OF THE EFFECT OF ARSENIC ON THE BINDING OF METRONIDAZOLE AT THE BINDING SITES OF BOVINE SERUM ALBUMIN

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Abstract: The effect of arsenic on the binding of metronidazole (an anti-amoebic agent) to bovine serum albumin (BSA) was studied by equilibrium dialysis (ED) method in order to justify the nature of binding capability of the drug and arsenic to BSA. There was decrease in metronidazole concentration due to addition of arsenic. But the free metronidazole concentration was increased with increasing the metronidazole concentration when only the BSA was present. The result obtained when no arsenic was added the free concentration of metronidazole was 41% whereas this release was only 7% when arsenic was added with an increasing concentration from $0.5x10^{-5}$ to $10x10^{-5}$M. But the free concentration of metronidazole was 36% to 71% when only metronidazole was added to BSA from $0.5x10^{-5}$ M to$12x10^{-5}$M. This result suggests that in presence of arsenic, metronidazole shows a greater binding to BSA and results in a decreased serum drug concentration. It could be suggested that in the presence of arsenic, metronidazole may form complex with arsenic, arsenic may increases the binding affinity to its sites or may form complex to BSA and metronidazole.

Key words: Equilibrium dialysis, metronidazole, arsenic, arsenic-metronidazole interaction

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 comparitively a large multi-domain protein. Bovine serum albumin (BSA) and HSA has structural similarity (Brown, 1977a). Among the plasma proteins, albumin is mostly bound to ligands or drugs.

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 (Rahman, 1993).

Plasma protein binding properties are related to plasma clearance, elimination half-life, apparent volume of the distribution. 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 (Jiunn *et al*., 1987).

Now we are at a higher risk of cancer from arsenic ingestion than previously thought. People live in Bangladesh become worried when they came to know that underground water in parts of the country is tainted by deadly arsenic. Arsenic is a naturally occurring element and ubiquitous in the environment in both organic and inorganic forms. Arsenic commonly occurs in insecticides, fungicides and herbicides. The three major bio-chemical actions of arsenic are coagulation of proteins, complexation with co-enzymes, uncoupling of oxidative phosphorylation reaction. The US Environmental Protection Agency (EPA) established the current maximum contaminant level (MCL) for arsenic 50 ppm. Arsenic also has high tendency to deposit in the body for a long time in the tissues, nail, hair, and in some protein.

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The interaction of drugs with plasma or tissue proteins or other macromolecules such as melanin and DNA, with the formation of a drug macromolecule complex, is called drug protein binding. It may be reversible or irreversible depending on the nature of chemical bonding. Plasma protein binding is one of the pharmacokinetic parameters of a drug and takes its place along with those relating to absorption, distribution, biotransformation and excretion.

Serum albumin, the most abundant protein in the blood, plays an important role in the binding phenomenon and serves as a depot protein and transport protein for numerous endogenous compounds (Krag-Hansen, 1981). Displacement of drug is defined as reduction in the extent of binding of a drug to protein caused by competition of another drug, the displacer. This type of interaction may occur when two drugs, capable of binding to proteins, are administered concurrently. Competitive displacement is more significant, when two drugs are capable of binding to the same sites on the protein. From different investigations, it has been suggested that human serum albumin (HSA) has limited number of binding sites (Fehske *et al.,* 1979; Hansen, 1981; Nahar *et al.,* 1997*).* BSA and HSA has structural similarity (Brown, 1976). Among the plasma proteins, albumin is mostly bound to ligands or drugs. Since number of protein binding sites is limited, competition will exist between two drugs and the drugs with higher affinity will displace the other, causing increased free drug concentration leading to higher toxicity (Rahman, 1993) or short duration of action. As a part of our on going drug-drug interaction study, we now report the effect of arsenic on the binding of metronidazol, a most commonly used antiameobic drug, at the binding site of bovine serum albumin. In this study BSA, in lieu of HSA, was used because of its low cost and easy availability.

Materials and Methods

Drugs and reagents used in the experiment: Metronidazole (IBN Sina Pharmaceutical Ltd.). Disodium hydrogen phosphate (Na_2HPO_4), Potassium di-hydrogen phosphate (KH_2PO_4), Borax (NaB_4O_7,H_2O), Cellulose Membrane (Medicel International Ltd. Liverpool Road, London; mol. Wt. 1200 Daltons), Bovine Serum Albumin (BSA) (fatty acid free, fraction V, Mol. Wt. 66500 from Sigma Chemical Ltd.), Arsenic Oxide (As_2O_3), and Na-arsenate.

Instrument used: p^H Meter (HANNA Microprocessor p^H Meter, Portugal), SP8-400 UV/VIS Spectrophotometer (Thermospectronic, England), Metabolic Shaking Incubator (Clifton Shaking Bath, Nical electro Ltd., England), Micro Syringe (well. Liang. Jin. Yang. q.I., China.)

Method used: Equilibrium Dialysis method was employed in the study (Signals, 1987a, b).

Binding of Metronidazole to BSA at pH 7.4 and 37 °C: From the previously prepared $2x10^{-5}$M BSA solution at P^H 7.4 3ml was taken in each of the nine cleaned and dried test tubes. $0.5x10^{-3}$ M metronidazole stock solution in different volumes was added to the eight out of nine test tubes to have the following concentration: $0.5x10^{-5}$ M, $1x10^{-5}$ M, $2x10^{-5}$ M, $4x10^{-5}$ M, $6x10^{-5}$ M, $8x10^{-5}$ M, $10x10^{-5}$ M and $12x10^{-5}$ M. The ninth test tube containing only BSA solution was marked as 'blank' or 'control'. After pipetting, the solution was properly mixed and allowed to stand for 10 minutes to ensure maximum binding of metronidazole to BSA. From each test tube 2 ml of solution was taken into nine different semi-permeable membrane tubes. To end of the membrane the tube were clipped and was ensured that there was no leakage. The membrane tubes were then immersed in ten separated 50 ml conical flasks containing 30 ml of phosphate buffer solution of P^H 7.4. The conical flasks were then placed in a metabolic shaker for dialysis at 37 $^{\circ}$C and 20 rpm and shaking was continued for 10 hours. At the end of the dialysis, samples were collected from each flask. The free concentration of metronidazole was measured by a UV spectrophotometer at of 278 nm (BP).

Drug-drug displacement study

Effect of arsenic on metronidazole binding to BSA: From the previously prepared $2x10^{-5}$ M BSA solution 3 ml was taken in each of the nine cleaned and dried test tubes. Metronidazole solution was added to the eight out of nine test tubes so that the final ratio of protein and metronidazole was 1:1($2x10^{-5}$ M: $2x10^{-5}$ M) in each of these eight test tubes. The ninth test tube containing only BSA solution was marked as 'blank' or 'control'. Arsenic solution (either $2x10^{-10}$ or $2x10^{-3}$M) was added with increasing concentration into seven out of eight test tubes containing 1:1 mixture of protein- metronidazole. The final ratios of protein: metronidazole: arsenic were 1:1:0.5, 1:1:1, 1:1:2, 1:1:4, 1:1:6, 1:1:8 and 1:1:10. That is arsenic was not into the first test tube which contained only protein-metronidazole mixture. After pipetting, the solution was properly mixed and allowed to stand for 15 minutes to ensure maximum binding and from each test tube 2 ml of solution was taken into

nine different semi-permeable membrane tubes. Two end of the membrane a tube were clipped and was ensured that there was no leakage. The membrane tubes were then immersed in nine separated 50 ml conical flasks containing 30 ml of phosphate buffer solution of P^H 7.4. The conical flasks were then placed in a metabolic shaker for dialysis at 37 °C and 20 rpm and shaking was continued for 10 hours. At the end of the dialysis, samples were collected from each flask. The free concentrations of test drugs were measured by a UV spectrophotometer at 278 nm (BP).

Results

The complex of the drug, which forms with the protein, governs protein binding of a drug. Strong affinity binding to a small number of sites and weak affinity binding to a large number of sites. Since binding is almost exclusively to albumin and the number of sites available is limited, the protein of some drugs depends on the plasma albumin concentration.

During the administration of metronidazole in a patient contaminated with arsenic, adequate knowledge about composition, size and location of binding as well as the probable interactions at binding sites at HSA along with all the binding of plasma protein is required for proper explanation of pharmacokinetic aspect of drugs.

Administration of metronidazole in arsenic treated albumin, site to site displacement take place and arsenic increases the binding of metronidazole significantly (*i.e.* decreases free concentration of captopril) (Fig. 1). But in the absence of arsenic, increment of metronidazole to BSA (Fig. 2), free concentration of metronidazole was more prominent. This displacement may be due to saturation of the binding site of metronidazole and increasing the metronidazole concentration increase free drug concentration. Whereas in the presence of arsenic, metronidazole may form complex with arsenic or arsenic may increases the binding affinity to its sites or arsenic may form complex to BSA and metronidazole. Concurrent administration of arsenic and metronidazole, the decrement of binding of metronidazole was from 41 % to 7 % with the increase of arsenic concentration from $0x10^{-5}$ M to $10x10^{-5}$ M whereas in the absence of arsenic the free concentration of metronidazole was from 36 % to 71 % with the increases of metronidazole from $0.5x10^{-5}$ M to $12x10^{-5}$ M. This result suggests that arsenic may increases the binding affinity of metronidazole or may form arsenic-protein- metronidazole complex.

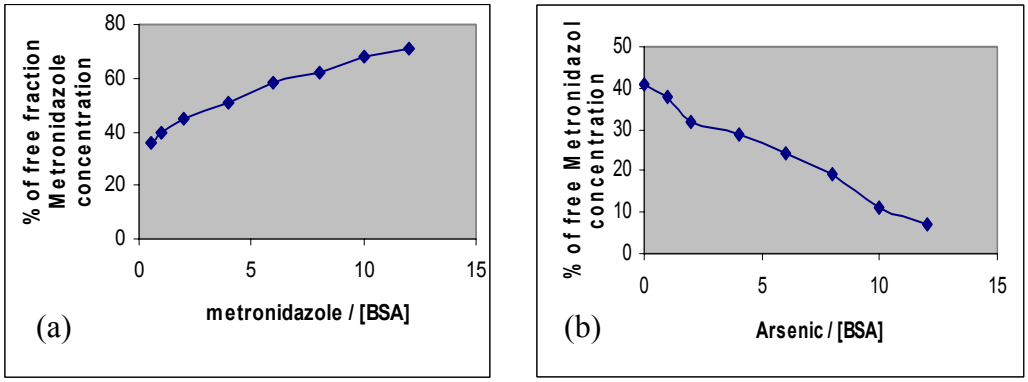

Fig. 1. Free fraction of Metronidazole bound to BSA in the absence or presence of arsenic (a): when [BSA] = $2x10^{-5}$, [Metronidazole] = 0.5 to $12x10^{-5}$M and [Arsenic] = 0.; (b): when [BSA] = $2x10^{-5}$M = [Metronidazole] and [Arsenic] = 0.5 to $10x10^{-5}$ M.

Discussion

The ability of one drug to inhibit the other is a function of their relative concentration, binding affinities and specificity of binding (Koch-Weser *et al.,* 1976). Since only a small fraction of the drug would ordinarily be available in the free from, the displacement of even a small percentage of the amount that is bound to proteins could produce considerable increase in activity. Thus when studying with arsenic-drug interaction, more specifically the drug displacement, the possibility of the occurrence of site-to-site displacement should also be considered, as there will be a difference between the free concentration of a displaced drug with or without site-to-site displacement. Moreover protein binding 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 HAS is still a subject of speculation and controversy (Jiunn *et al.,* 1987). Again arsenic has a tendency strongly bind to protein and our rural people at higher risk of arsenic ingestion in their daily water, which increases the blood arsenic concentration. Here arsenic decreases the free concentration of metronidazol to a greater extent, so arsenic probably increases the binding affinity to the binding site of the albumin or may form a arsenic-albumin-metronidazol complex.

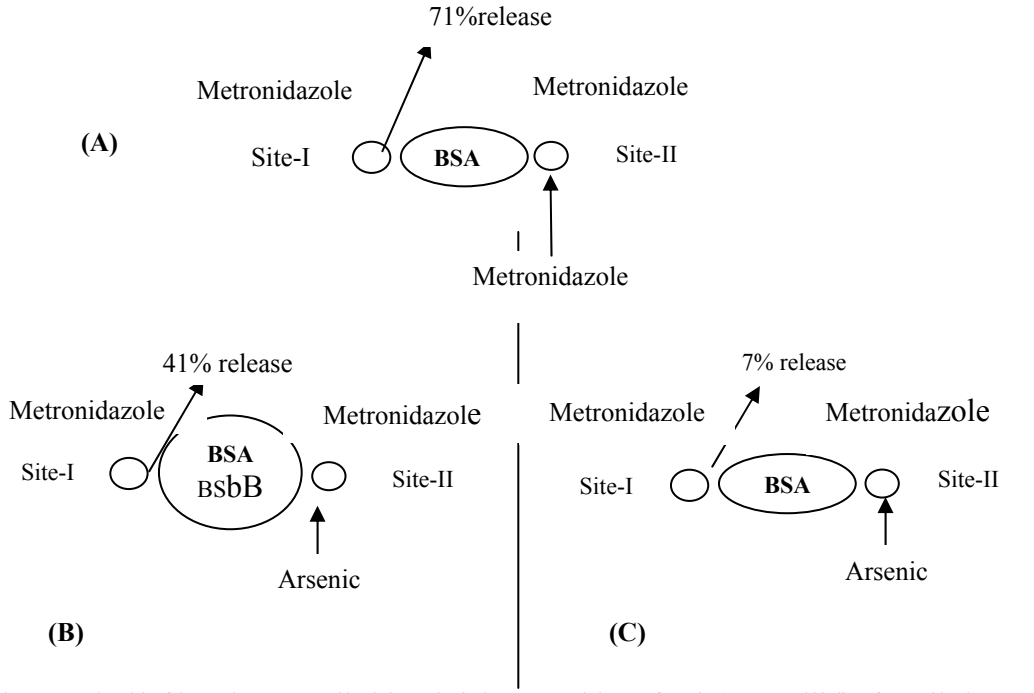

Fig. 2. Proposed models of the arsenic-BSA- Metronidazole interaction in the presence and absence of arsenic: (A) = Normal binding of Metronidazole to BSA, when [BSA]= $2x10^{-5}$M, [Metronidazole] =$12x10^{-5}$M and [Arsenic] = 0.; (B) = Normal binding of Metronidazole to BSA, when [BSA] = $2x10^{-5}$M=[Metronidazole], and [Arsenic] = 0.; (C) = Effect of arsenic on Metronidazole to BSA, when [BSA] = $2x10^{-5}$M= [Metronidazole] and [Arsenic] = 0.5 to $10x10^{-5}$M.

Conclusion

Drugs are bound to plasma protein at sites located on the surface of the protein. The idea of binding sites is suggested by the relative size of the drugs and proteins. Protein binding of a drug is governed by the complex of the drug, which forms with the protein. There are two main type of protein binding. Strong affinity binding to a small number of sites and weak affinity binding to a large number of sites. Since binding is almost exclusively to albumin and the number of sites available is limited, the protein of some drugs depends on the plasma albumin concentration.

Here arsenic reduced the free concentration of drug, so in the highly arsenic affected affected area, arsenic contaminated people, who suffers from chronic dysentery or any amoebic infection taking metronidazole may not give desired pharmacological action or may not rapidly excreted from the body or give repetitive action i.e. give prolong action or may remain always under the MEC (Minimum effective concentration) in the normal doses. Such patients require more doses of metronidazole to cure the disease, which increases the cost of treatment or may be dangerous to their health. Due to arsenic changes the pharmacokinetics of metronidazole during concurrent administration of arsenic and the drug, care should be taken for prescribing of drug to the arsenic affected people. Higher research may reveal the reason of reducing the free concentration of metronidazole in the presence of arsenic to BSA.

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