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RESEARCH ARTICLE

STUDIES OF *IN-VITRO* AMLODIPINE AND ARSENIC DISPLACEMENT INTERACTION AT BINDING SITES OF BOVINE SERUM ALBUMIN

Md. ASHRAFUL ALAM, Md. ABDUL AWAL, NUSRAT SUBHAN, MAHMDUL HASAN SIKDER, S.M. MAHBUBUL ALAM, MAHBUB MOSTOFA and Shaikh JAMAL UDDIN

For author affiliations, see end of text.

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ABSTRACT

In this study, the binding of amlodipine (a Calcium channel Blocker) and arsenic (metalloid) to bovine serum albumin (BSA) was studied by equilibrium dialysis(ED) method in order to have an insight into their binding chemistry to BSA. Free amlodipine concentration was increased due to addition of arsenic which reduced the binding of the compounds to BSA. However, the free fraction was not increased to a level as it was expected from direct competitive displacement. The free amlodipine concentration was increased according to increasing the amlodipine concentration when only the BSA was present. When the binding sites were blocked by sufficient amount of arsenic, the increment of free concentration of amlodipine was prominent. When no arsenic was added, the free concentration of amlodipine was only 6.6% to 10.3%; whereas this release was 7.65% to 13.65% when arsenic was added with an increasing concentration from $1x10^{-5}$M to $12x10^{-5}$M. This suggests that in the presence of arsenic, the amlodipine is slowly displaced from its high affinity binding site.

Keywords: *Equilibrium dialysis, Amlodipine, Association constant, Binding site, Arsenic-amlodipine interaction, Calcium channel blockers*

Amlodipine was approved by FDA for use in 1996, and is indicated in the treatment of essential hypertension, chronic stable angina, vasospastic angina, myocardial ischemia, congestive heart failure, and Raynaud's disease [1]. It also decreases left ventricular mass in hypertensive patients with cardiac hypertrophy, decreases proteinuria in Type 2 diabetics, and increases vasodilatation of the pulmonary arteries in patients with pulmonary hypertension. However, it does not have FDA approval for these latter uses.

Amlodipine is 93% protein bound in the plasma. It has a volume of distribution of approximately 12-20 L/kg [2], and thus partitions extensively into the tissues. Once at the receptor site, it exhibits relatively slow association and dissociation with receptors. In an *in vitro* study by Burges *et al*., amlodipine's association and dissociation rates are up to 3 times slower those of other dihydropyridines [3]. This contributes to amlodipine being cleared more slowly, and having a longer duration of action. Normal therapeutic serum levels are 3-15 ng/ml [1,4,5].

Serum albumin, the most abundant protein in the blood, plays a very important role in the binding phenomenon and serves as a deport protein and transport protein for numerous endogenous compounds [6]. 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. Various investigators have suggested that human serum albumin (HSA) has limited number of binding sites [7-9]. The primary structure of HSA was explained by Meloun *et al*. and Brown [10, 11]. HSA is folded into three domains, each of which is built of three loops. HSA is competitively a large multi-domain protein.

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 concentra-

tion; which leads to higher toxicity or short duration of action of the related drug [12]. The ability of one drug to inhibit the other is a function of their relative concentration, binding affinities and specifically of binding [13]. 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 drug that is bound to proteins could produce considerable increase in its activity. 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, 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 [14].

Arsenic has a strong tendency for binding to protein. Our rural people are at high risk of arsenic ingestion in their daily water; which increases the blood arsenic concentration. Considering this factor, the purpose of our work is to see the effect of arsenic on the free concentration of the common antihypertensive drug, amlodipine (a Ca^{++} Channel blocker). BSA and HSA have structural similarity [11]. In this study, BSA was used in lieu of HSA, because of its low cost and easy availability.

MATERIALS AND METHOD

Drug and reagents used in the experiment

Amlodipine besylate (General Pharmaceutical Ltd., Bangladesh)**.** Diazepam, (Incepta pharmaceuticals Ltd., Bangladesh) Warfarin Sodium (ACI, Bangladesh), Disodium hydrogen phosphate (Na_2HPO_4), Potassium dihydrogen phosphate (KH_2PO_4), Cellulose Nitrate Membrane (Medicell International Ltd., London, England), Bovine Serum Albumin (BSA) (fatty acid free, fraction V) from Sigma Chemical Ltd., St Louis, USA, Arsenic Oxide(As_2O_3), and Na-arsenate.

Instruments

SP8-400 UV/VIS Spectrophotometer (Thermospectronic, England), Metabolic Shaking Incubator (Clifton Shaking Bath, Nical electro Ltd., England)

Methods

Equilibrium Dialysis method was employed in the study [15,16]. We used various site-specific probes to enhance our understanding of the drug-BSA interaction and thereby characterization of binding sites of the drugs used in the study on the BSA molecule.

These two site-specific probes were used for the identification of the binding sites of the drug on BSA:

(i) Warfarin sodium (site-I specific probe)

(ii) Diazepam (site-II specific probe)

In direct procedure, the ratio of BSA and probe (either warfarin or diazepam) is 1:1 (2×10^{-5}M: 2×10^{-5}M) and various concentrations of drug may be added. In the reverse procedure, the ratio of BSA and drug will be 1:1 (2×10^{-5}M: 2×10^{-5}M) and various concentrations of probe (site-I-specific: warfarin sodium or site-II-specific: diazepam) will be added. After conducting equilibrium dialysis, the free concentration of probe will be determined in direct procedure and reverse procedure respectively.

Estimation of association constant of amlodipine at pH 6.4 and 27°C

To determine the association constant of amlodipine at pH 6.4, the following steps were performed. Ten clean and dried test tubes were taken and 3 ml of previously-prepared 2×10^{-5} M BSA solution at pH 6.4 was taken in each of them. Amlodipine stock solution (1×10^{-2} M or 1×10^{-3} M) was added in different volumes to nine out of 10 test tubes to have the following concentrations: 0.5×10^{-5} M, 1×10^{-5} M, 2×10^{-5} M, 3×10^{-5} M, 4×10^{-5} M, 5×10^{-5} M, 6×10^{-5} M, 7×10^{-5} M, 8×10^{-5} M and 9×10^{-5} M. The tenth test tube containing only BSA solution at pH 6.4 was marked as "control". After mixing the solutions, they were allowed to stand for a while for maximum binding of amlodipine to BSA. Two ml from each test tube was pipetted out and poured into previously prepared semi-permeable membranes tubes and finally both sides of the tubes were clipped properly so that there was no leakage. The membrane tubes containing the drug-protein mixture were immersed in ten 50-mililiter flasks containing 30 ml of phosphate buffer solution (pH 6.4). The mouths of the council flasks were covered by foil paper. These conical flasks were then placed in a metabolic shaker for dialysis for 10 hours at 27°C and 20 rpm. Buffer samples were collected from each flask after complete dialysis. Free concentrations of amlodipine were measured by a UV spectrophotometer at a wavelength of 238 nm [17].

Estimation of association constant of amlodipine at pH 7.4 and 27°C

To determine the association constant of amlodipine at pH 7.4, a similar protocol as for estimation of the association constant of amlodipine at pH 6.4 was followed using the buffer solution with pH 7.4. When dialysis was completed, buffer solutions were collected from each conical flask and the free concentration of amlodipine was measured by a UV spectrophotometer at a wave length of 238 nm [17].

Determination of binding site of amlodipine using warfarin sodium as a site-I-specific probe

To perform the experiment, the following successive steps were followed. From the previously-prepared 2×10^{-5} M BSA solution, 3 ml was taken in each of the eight cleaned and dried test tubes. Warfarin solution (1×10^{-3} M) was added to the seven out of 8 test tubes so that the final ratio of protein and warfarin was 1:1 (2×10^{-5} M: 2×10^{-5} M) in each of these seven test tubes. The eighth test tube containing only BSA solution was marked as "Blank" or "Control". These mixtures were

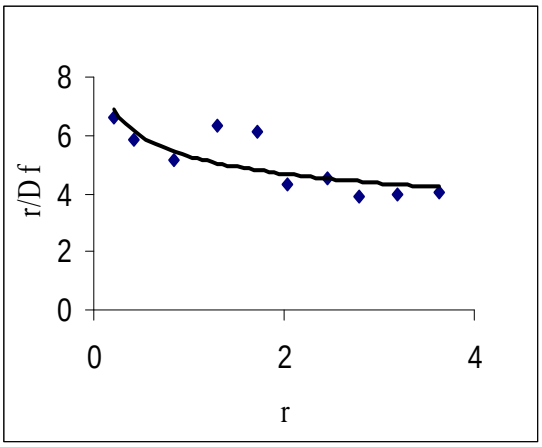


Fig 1. Scatchard plot for amlodipine bound to BSA at pH 7.4

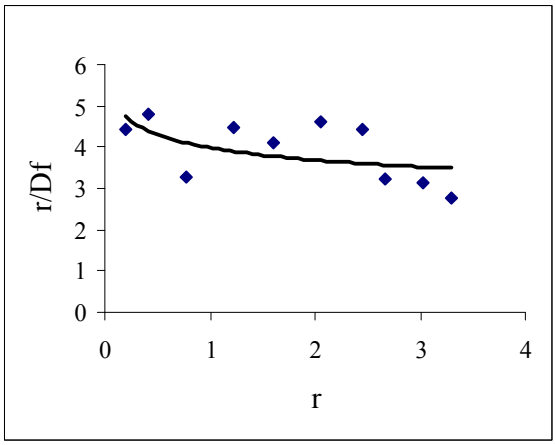


Fig 2. Scatchard plot for amlodipine bound to BSA at pH 6.4

stand for 10 minutes to allow binding of the warfarin to its particular binding site. Amlodipine solutions (either 2×10^{-2} M or 2×10^{-3} M) was added with increasing con

centrations into six out of seven test tubes containing 1:1 mixture of protein-warfarin. The final ratios of protein: warfarin: amlodipine were 1:1:0, 1:1:1, 1:1:2, 1:1:3, 1:1:4, 1:1:5 and 1:1:6. Amlodipine was not added into the first test tube which contained only protein-warfarin mixture (1:1). After pipetting, the solution was properly mixed and allowed to stand for 10 minutes to ensure maximum binding of warfarin to site-I and thereby displacing the probe from site-I on BSA. From each test tube, 2 ml of solution was taken into eight different semi-permeable membrane tubes. Both ends of the membrane tubes were clipped and it was ensured that there was no leakage. The membrane tubes were then immersed in eight separates 50-mililiter conical flasks containing 30 ml of phosphate buffer solution (pH 7.4). The conical flasks were then placed in a metabolic shaker for dialysis at 27°C and 20 rpm and shaking was continued for 10 hours. At the end of dialysis, samples were collected from each flask. The free concentrations of warfarin were measured by a UV spectrophotometer at λ_{max} 308 nm.

Determination of binding site of amlodipine using diazepam as a site-II-specific probe

To perform the experiment, the previously-described procedure has been followed successively using diazepam solution. The final ratios of protein: diazepam: amlodipine were 1:1:0, 1:1:1, 1:1:2, 1:1:3, 1:1:4, 1:1:5, 1:1:6 and 1:1:9. Amlodipine was not present into the first test tube which contained only protein-diazepam mixture (1:1). At the end of dialysis, samples were collected from each flask. The free concentrations of diazepam were measured by a UV spectrophotometer at a wave length of 235 nm.

Binding of amlodipine to BSA at pH 7.4 and 27°C

From the previously-prepared $2x10^{-5}$M BSA solution at pH 7.4, 3ml was taken in each of the ten cleaned and dried test tubes. Amlodipine stock solution ($1x10^{-3}$ M) in different volumes was added to the nine out of ten test tubes to have the following concentration: $0.5x10^{-5}$M, $1x10^{-5}$ M, $2x10^{-5}$ M, $3x10^{-5}$ M, $4x10^{-5}$ M, $5x10^{-5}$ M, $6x10^{-5}$ M, $7x10^{-5}$ M, $8x10^{-5}$ M, $9x10^{-5}$ M, $10x10^{-5}$ M, and $12x10^{-5}$ M. The tenth test tube contained only BSA solution and was marked as" blank" or "control". After pippeting, the solution was properly mixed and allowed to stand for 10 minutes to ensure maximum binding of amlodipine to BSA. From each test tube, 2 ml of solution was taken into ten semi-permeable membrane tubes. Both ends of the membrane tubes were clipped and it was ensured that there was no leakage. The membrane tubes were then immersed in ten separated 50-mililiter conical flasks containing 30 ml of phosphate buffer solution (pH 7.4). The conical flasks were then placed in a metabolic shaker for dialysis at 27° C and 20 rpm and shaking was continued for 6 hours. At the end of the dialysis, samples were collected from each flask. The free concentration of amlodipine was measured by a UV spectrophotometer at λ_{max} of 238 nm [17].

Effect of arsenic on amlodipine binding to BSA

Effect of arsenic on amlodipine binding to BSA was studied as follows; from previously-prepared $2x10^{-5}$M BSA solution, 3ml was taken in each of the nine cleaned and dried test tubes. Amlodipine solution was added to the eight out of nine test tubes so that the final ratio of protein and amlodipine was 1:1($2x10^{-5}$ M: $2x10^{-5}$ M) in each of these eight test tubes. The ninth test tube containing only BSA solution and was marked as" blank" or "control". Arsenic solution (either $2x10^{-2}$ or $2x10^{-3}$M) was added with increasing concentration into seven out of eight test tubes containing 1:1 mixture of BSA- amlodipine. The final ratios of BSA: amlodipine: arsenic were 1:1:0, 1:1:2, 1:1:4, 1:1:6, 1:1:8, 1:1:10 and 1:1:12. Arsenic was not added into the first test tube which contained only BSA-Amlodipine mixture. After pippeting, 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 semi-permeable membrane tubes. Two ends of the membrane tubes were clipped and it was ensured that there was no leakage. The membrane tubes were then immersed in nine separated 50-mililiter conical flasks

Table 1. Binding parameter of amlodipine bound to BSA at different pH values.

pH	Association constant		Number of binding site	
	K_1 (High affinity) × 10^{-5} M	K_2 (Low affinity) × 10^{-5} M	n_1 (High affinity)	n_2 (Low affinity)
6.4	5.26±0.113	4.93±0.143	1.9±0.114	3.73±0.118
7.4	7.8±0.114	7.06±0.143	2.2±0.173	2.9±0.151

Values are represented as mean ± standard deviation

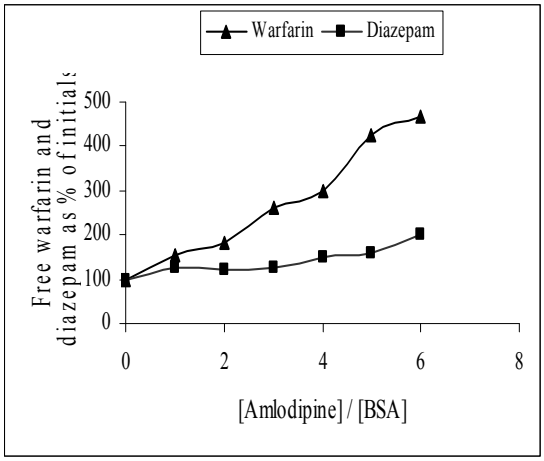


Fig 3. Free concentration of warfarin and diazepam bound to BSA upon the addition of amlodipine.

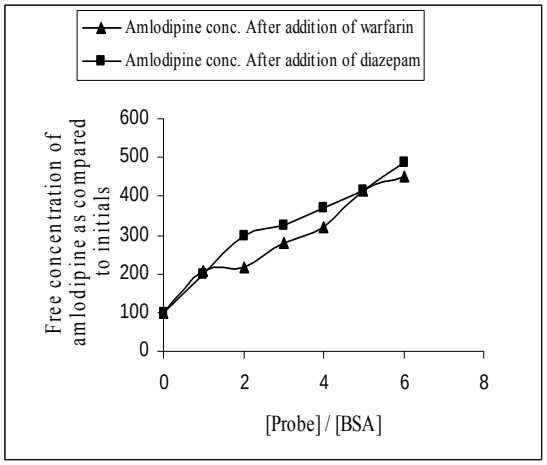


Fig 4. Free concentration of amlodipine when used with diazepam and warfarin sodium bound to BSA

containing 30 ml of phosphate buffer solution (pH 7.4). The conical flasks were then placed in a metabolic shaker for dialysis at 27°C and 20 rpm and shaking was continued for 10 hours. At the end of the dialysis, samples were collected from each flask. The concentration of free amlodipine was measured by a UV spectrophotometer at 238 nm [17].

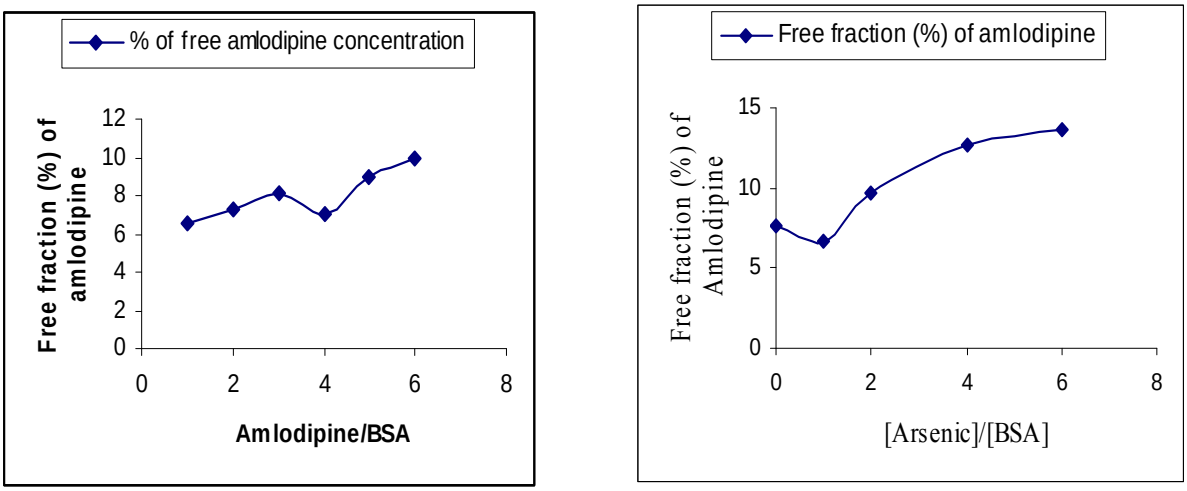
RESULTS

Standard curves were prepared using drugs and their corresponding concentrations at pH 6.4 and 7.4. UV spectrophotometric scanning of amlodipine, showed maximum absorbance of the UV light at 238 nm. Amlodipine showed linearity at a concentration range of $1x10^{-5}$M - $8x10^{-5}$ M with a confidence level of 0.9285 and 0.9861 at pH 6.4 and 7.4 with linear equation (Y=0.1501 X) and (Y=0.1774 X) respectively. Similar standard curves were also prepared for diazepam and warfarin, and the concentration of those drugs was calculated using corresponding linear equations. Both association's constant (k_a) and number of binding sites (n) of amlodipine were determined using the Scatchard plot. To estimate the binding parameters of amlodipine, equilibrium dialysis (ED) method was used and the subsequent non-linear shape of the Scatchard plot describes both high and low affinity binding site of the drugs on the protein molecule.

Determination of association constant and number of binding sites

Amlodipine is characterized by a high affinity association constant (k_1) to BSA and the value at pH 7.4 is $7.8x10^{-5}$ M (Table 1 and Fig 1) while the low affinity association constant (k_2) for amlodipine is nearly 1 times lower than that of higher affinity constant (k_1). For this drug, the number of high affinity and low affinity binding sites are 2.2 ± 0.173 and 2.9±0.151 respectively at ph 7.4. In case of amlodipine bound to BSA, the high affinity association constant (k_1) was found to be decreased when pH was changed from 7.4 to 6.4 and the value for k_1 and k_2 are 5.26±0.113 and 4.93±0.143 respectively (Table-1 and Fig 2).

As a consequence, at pH 6.4, the number of high affinity and low affinity binding sites for amlodipine are 1.9± 0.114 and 3.73±0.118 respectively. When physiologic pH changes, BSA undergoes conformational alteration. This is generally termed as N-B transition. BSA remains almost entirely in neutral from at pH 6 and in basic form at pH 9. When the protein is in the B-conformation, fewer protons are bound to BSA than that in the N-conformation. Thus the high affinity and low affinity binding of amlodipine is affected by pH change. These differences induced by pH alterations may be due to the structural modification of protein molecule. For this reason, at a specific pH, the binding site for amlodipine is more suitable or properly accommodated and



(A) **(B)**

Fig 5. (A) Free concentration of amlodipine bound to BSA. **(B)** Free concentration of amlodipine bound to BSA upon the addition of arsenic tri oxide in absence of site-I-specific probe, warfarin sodium.

at other pH values the binding sites become less accommodating to the drugs in concern.

Determination of binding sites

Well-established probes, which are specific for particular sites on the albumin molecule, are used for identification of binding site of the drugs on the protein molecule. If a drug is able to displace a probe from its binding site, it is assumed that the drug also binds to that particular site. Thus, the binding site as well as the specificity and relative strength of binding to albumin of amlodipine has been determined by this principle. Warfarin sodium as site-I specific probe and diazepam as site-II specific probe were used. To characterize the binding site of amlodipine, the free concentration of warfarin sodium (site-I specific probe) was measured upon the addition of amlodipine. It was found that the free concentration of warfarin sodium was increased from 100% (as % of initial) to 468 % when the ratio of amlodipine to BSA was increased to 6 (Fig 3). In contrast, under the same experimental conditions, when in lieu of warfarin sodium, diazepam was used as site-II specific probe; the increment of the free concentration of diazepam by amlodipine was from 100% (as % of initial) to 200% (Fig 3).

From these data this is evident that the increment of free concentration of warfarin sodium is obviously greater than that of diazepam by amlodipine. It can be concluded that amlodipine preferentially binds to site-I. Again as the displacement of diazepam is quite remarkable, it can be also suggested that amlodipine binds to site-II on the BSA molecule to a lower extent.

In the reverse experiment, the free concentration of amlodipine was increased from 100% (as % of initial) to 452.22% when warfarin to BSA ratio was 6 as shown in Fig 4. On the other hand, the free concentration of amlodipine was increased from 100% (as % of initial) to 413.33% when the ratio of diazepam to BSA was also 6 as shown Fig 4. It is clear that the increment of free amlodipine due to displacement by Warfarin (site I probe) is higher than that of that when displaced by diazepam. Thus, these reverse experiments also agree with that of previous experiment.

Displacement of amlodipine due to the effect of arsenic

During concurrent administration of amlodipine and arsenic (Fig 5), site to site displacement took place and arsenic displaced the amlodipine from its binding sites more slowly (i.e. released small free concentration of amlodipine). In the absence of arsenic along with increment of amlodipine to BSA (Fig 5 (A)), free concentration of amlodipine was more prominent. This displacement may be due to reduction in the binding sites of amlodipine and increasing the free drug concentration, whereas in the presence of arsenic, amlodipine may form complex with arsenic, or arsenic may increase the binding affinity of amlodipine to its sites, or arsenic may form complex to BSA. As observed from our proposed model (Fig 6), during concurrent administration, arsenic displaced amlodipine from its high affinity binding site-I. Thus, free concentration of amlodipine increased from 7.65% to 13.65%. This free amlodipine may bind to its low affinity binding site, site-II. Thus, it can be suggested that arsenic displaced amlodipine from its binding site I and at the same time, a sufficient portion of the free drug amlodipine might have bound to site-II.

DISCUSSION

Recent studies indicate that we are at a high risk of cancer from arsenic ingestion. People live in Bangladesh become terror-stricken when they understand that

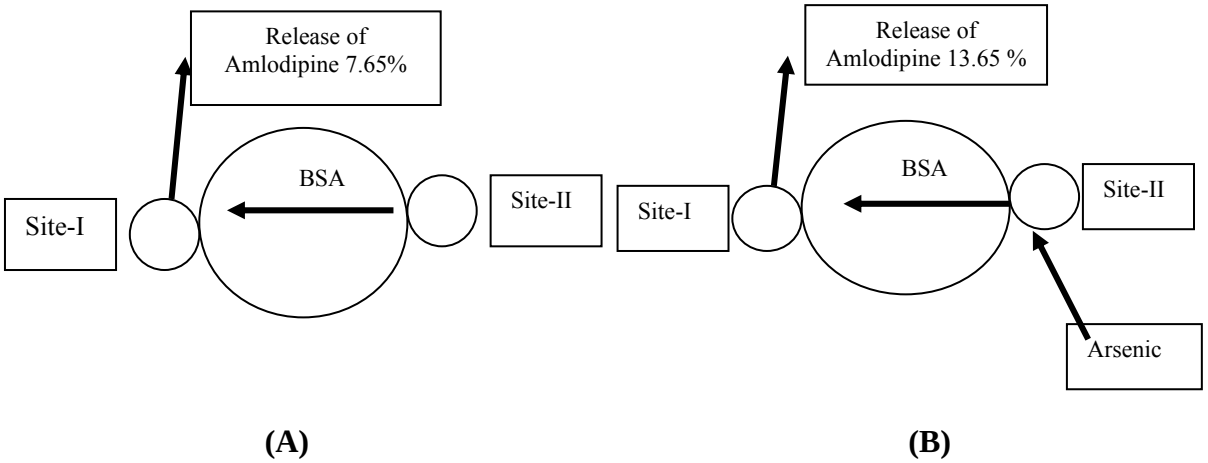


Fig 6. Proposed models of the Amlodipine-BSA- Arsenic interaction in absence of warfarin (site-I specific probe)
(A) Normal condition of binding
(B) Drug interaction in absence of probe

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 biochemical actions of arsenic are coagulation of proteins, complexation with co-enzymes, and uncoupling of phosphorylation. The US Environmental Protection Agency (EPA) established 50 p.p.m for the current maximum contaminant level (MCL) of arsenic. Arsenite exerts its acute toxicity by inhibiting enzymes containing vicinal sulfhydryl groups at their active centers [18]. Arsenate disrupts oxidative phosphorylation by substituting for phosphate in the formation of ATP [19]. This results in depletion of cellular energy stores.

Mammalian systems are believed to detoxify inorganic arsenic by methylation to less toxic compounds that are then excreted in the urine [20]. Arsenic also has high tendency to deposit in the body for a long time in the tissues, nail, hair, and some protein. Binding of inorganic arsenic to tissue proteins can be an additional or perhaps the first step in the detoxification of inorganic arsenic before methylation [21]. Arsenic–protein binding occurs mainly in the cytosol, most notably in liver tissue [22]. The binding of arsenic to proteins in plasma and packed blood cells was also observed by several authors, although most of these studies were based on animal models and the mechanism is not well understood. Vahter and Marafante observed a binding percentage up to 20% of arsenic to plasma proteins after intravenous administration of arsenite or arsenate to mice and rabbits [23]. Bertolero *et al*. (1981) found that about 10% and 50% of arsenic in plasma were bound to plasma proteins after 5- and 48-h intraperitoneal administration of 1 µg/kg of arsenic-(III) to rabbits, respectively [24].

Vascular diseases have long been noted to be associated with chronic arsenic exposures among German vineyard workers and inhabitants of Antofagasta in Chile [25,26]. Peripheral vascular diseases have been reported to be associated with the occurrence of arsenic in well waters in Taiwan [27-29]. Wu *et al*. (1989) found significant correlations of mortality rates due to peripheral vascular diseases and cardiovascular diseases with concentrations of arsenic in well water [30]. However, no significant association was observed for cerebrovascular accidents. A comparison of hypertension prevalence among subjects with and those without arsenic exposure through drinking water was conducted in Bangladesh. There was a significant dose-response relationship for both series of risk estimates (PÇ0.001). Using a linear regression model including age, sex and body mass index, there was a correlation between increase in the mean blood pressure and arsenic exposure. The mean blood pressure increase in both series of exposure categories was statistically significant (P<0.001). This study showed a dose-response relationship between inorganic arsenic exposure from drinking water and risk of hypertension [31].

Treatment of hypertension with calcium channel blockers in the arsenic-affected area, where people were bound to drink arsenic-containing well water, should have taken extra precaution. Arsenic displaces the *in-vitro* amlodipine binding to BSA and increases the free drug concentration which may be available for further action upon the calcium channel. Cardiac and vascular smooth muscle cells are dependent on calcium movement through specific ions channels for contraction. Amlodipine, like other CCBs, exerts its pharmacologi-

cal action by "inhibiting the transmembrane influx of calcium into myocardial fibers, cardiac pacemaker cells, and vascular smooth muscle cells [3]. The dihydropyridine class of CCBs, to which amlodipine belongs, is more selective for vascular smooth muscle than for cardiac cells. As such, amlodipine tends to cause fewer incidents of cardiac depression and fewer conduction disturbances, compared to verapamil or diltiazem. It acts on the L-type calcium channel and binds both dihydropyrodine and non-dihydropyridine sites. It does not tend to produce negative inotropic or chronotropic effects. Administration of amlodipine results in relaxation of vascular smooth muscle, producing decreased afterload, decreased systemic blood pressure and increased coronary vascular dilatation [2]. Amlodipine has a basic amino side chain that makes it very different from all other CCBs, and creates a very interesting pharmacologic, pharmacokinetic and toxicological profile. Calcium channel blockers exert their toxicity via saturation and blockade of calcium channels that are vital to heart and smooth muscle contraction. When this lack of contractility is potentiated due to toxic levels of the drug, refractory hypotension and reflex tachycardia ensue. This effect of hypodynamic shock, or “bottoming out”, is greatly exaggerated with amlodipine, due to its increased effect on vascular smooth muscle, increased binding to calcium channels via its basic amino side chain, and its extremely sluggish association and dissocation with these channels. Also, CCB toxicity produces hyperglycemia and lactic acidosis, which renders the patient even more unstable. Hyperglycemia is thought to be caused by several mechanisms. Firstly, there is an inhibition of calcium-mediated insulin release by pancreatic islet cells. Secondly, CCB toxicity produces myocardial and whole-body insulin resistance. Thirdly, the combination of poor tissue perfusion and severe acidosis impairs carbohydrate delivery and glycolysis in shock conditions. As a result, hyperglycemia develops, and myocardial energy transfer becomes inefficient. The cause of lactic acidosis is uncertain, but it is speculated that lactic acidemia is probably a manifestation of poor tissue perfusion. In addition, lactic acidosis may also be due to mitochondrial dehydrogenase inhibition during CCB-induced circulatory shock. In high concentrations, CCBs inhibit mitochondrial calcium entry at the sarcolemma and the mitochondrial membrane, which in turn can decrease pyruvate dehydrogenase activity. Pyruvate does not enter the Kreb’s cycle and lactate accumulates, producing metabolic acidosis [4].

CONCLUSION

Adequate knowledge about composition, size and location of binding sites as well as the probable interactions at binding sites at HSA and other plasma protein is required for proper explanation of pharmacokinetic aspects of drugs. This is important, for the rational understanding of drugs binding sites on serum albumin during concurrent administration of medicines which effects on drug actions. Protein binding of a drug is governed by the complex of the drug - 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 limited to albumin and the number of albumin sites available is limited, the binding properties of drugs depends on the plasma albumin concentration. Since arsenic increases the free amlodipine concentration, in the highly-arsenic affected people who have taken amlodipine to treat hypertension, the drug may give rapid fall of blood pressure. As arsenic changes the pharmacokinetics of amlodipine during concurrent administration of arsenic and the drug, care should be taken for prescribing amlodipine to the arsenic-affected people. More detailed research may reveal mechanism of increasing the free concentration of amlodipine in the presence of arsenic.

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CURRENT AUTHOR ADDRESSES

Md. Ashraful Alam, Department of Pharmacy, Stramford University, Bangladesh. E-mail: sonaliagun@yahoo.com (Corresponding author)
Md. Abdul Awal, Department of Pharmacology, Bangladesh Agricultural University.
Nusrat Subhan, Department of Pharmacy, Northern University, Bangladesh.
Mahmudul Hasan Sikder, Department of Pharmacology, Bangladesh Agricultural University.
S. M. Mahbubul Alam, Pharmacy Discipline, Khulna University.
Mahbub Mostofa, Department of Pharmacology, Bangladesh Agricultural University.
Shaikh Jamal Uddin, Pharmacy Discipline, Khulna University.