

Discussion

Chapter - 5

The analysis of drug-protein interaction in case of tuberculosis and cardiovascular diseases sometimes are of great importance. The multidrug therapy from high effectiveness carries the risk of side effects and an increase of therapeutic action of free concentration of drugs. This can be dangerous when exhibiting toxic effect. Thus, the present study was designed to explore, the side effects of rifampicin, Atorvastatin (statins- a class of drugs used to lower cholesterol level) and pesticide malathion on cysteine proteinase inhibitor isolated from buffalo liver. All the three substances taken have liver as a primary site of metabolism and activation.

Rifampicin (RF) is very toxic to liver, this having been found both in the treatment of tuberculosis and cholestasis (Prince MI et al., 2002; Bertolami, 2005; Sodhi CP et al., 1997). When it was used together with pyrazinamide, a 5.8% incidence of severe liver injury was reported, 2.6% with isoniazid and 1.1% alone (Sharma SK, 2004). The pathogenesis of hepatotoxicity is poorly understood although limited evidence has been obtained. Histopathological examination has showed dose-related hepatic necrosis, ballooning degeneration and inflammatory infiltrates (Sharma SK, 2004).

In the present study RF (rifampicin) was taken at low concentration in the range of 0.01 to 1 μM and made to interact with BLC. Fluorescence spectroscopy shows a marked decrease in the intensity followed by a red shift. UV-visible study also shows interaction between BLC and rifampicin (fig 8.5). The fluorescence data analysis revealed that the RF interacts with BLC and has one binding site for rifampicin. However, the binding constant for RF with BLC shows a higher value of around $3.31 \times 10^5 \text{ M}^{-1}$ (fig 8.3 and fig 8.4). The activity of buffalo liver cystatin against papain decreased as the RF concentration increased (Tab 2.0). The fluorescence data and UV-Vis spectroscopy data unambiguously reveals the denaturation of BLC at higher concentration of rifampicin (app 0.5-1 μM) which is complimented with the inhibitory activity data in Table. 2. While at lower concentrations there is a quenching of fluorescence of buffalo liver cystatin (BLC) which is accompanied by the red shift of fluorescence maximum (fig 8.2). This indicates the increase in polarity of the chromophore environment, probably due to the hydrogen bonds formed between rifampicin (RF) and NH₂, OH and SH groups in the inhibitor (BLC) which stabilizes the complex (Bures et al., 1990).

It is well known fact that tuberculosis is a tissue destructing disease which progresses by means of ulceration and caesation. Hence, the role of lysosomes and their battery

of hydrolytic enzymes can rightly be suspected in such diseases which involve breakdown of tissue by the action of hydrolytic enzymes. Moreover, lysosomal cysteine proteinases i.e. cathepsins B, L and H when secreted can be very harmful for their environment resulting in pathological conditions (Kettelhu et al., 1994). Since, cystatins are specific inhibitors of lysosomal cathepsins suggest an important role of these inhibitors to reduce the aggravating role of cathepsins in the diseased conditions.

3-Hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase inhibitors, commonly known as statins, are cholesterol-lowering drugs that are the second most prescribed therapeutic drug class in the United States, after pain killers such as acetaminophen (Gonyeau and Yuen, 2010). Statin therapy is highly recommended and used for the prevention of cardiovascular disease in men forty years or older, who incidentally also have the highest risk of developing PCa (Murtola et al., 2008). The most concerning adverse effects include hepatotoxicity and myotoxicity. Increases in serum liver enzymes are dose dependent and occur at a reported frequency of 1–33% (Maron et al., 2000). The majority of cases of clinically significant transaminitis occur within the first 3 months of therapy and therefore monitoring of liver enzymes is required. At the pharmacokinetic level (ie, absorption, distribution, metabolism, and excretion of a given drug), the available statins have important differences, including half-life, systemic exposure, maximum plasma concentration (C_{max}), bioavailability, protein binding, lipophilicity, metabolism, presence of active metabolites, and excretion routes (Corsini et al., 1999). Because the primary site of cholesterol synthesis is the liver, statins that are currently available have been selected for their capacity to target the liver and decrease cholesterol biosynthesis (Ahmed et al., 2011).

In this study, Atorvastatin has been taken from the “statin” class of drugs as it is mostly and extensively a drug of choice of with the commercial name “Lipitor”, in case of cardiovascular diseases and hypercholesterolemia. Fluorescence spectroscopy between atorvastatin and BLC reveals a decrease in the intensity of fluorescence maximum in concentration range of 0.1-0.6 μM range while above this concentration of atorvastatin, there is a red shift (5 nm) at 0.8 μM . Further increase in the concentration decreases the fluorescence intensity and there is a red shift in the peak of around 10 nm (fig 8.6). Figure 8.7 and fig 8.8 shows the binding constant of the drug atorvastatin with BLC which is $2.78 \times 10^6 \text{ M}^{-1}$ and the number of binding sites is slightly higher than one ($n \approx 1.5$). UV spectroscopy also shows a binding between the

drug and BLC as the concentration increases. An increase in the atorvastatin concentration gives blue shift which indicates changes in the structure of BLC as the drug atorvastatin binds to it (fig 8.9). The inhibitory activity of BLC against papain in the presence of the atorvastatin shows marked decrease vouching for the structural changes and changing affinity of BLC towards atorvastatin (Tab. 2.1).

Rifampicin has been shown as the potent inducer of p-glycoprotein. Rifampicin can therefore decline the therapeutic usefulness of other drugs if the patient is undergoing treatment for cardiovascular disease or hypercholesterolemia where more drugs or statins are pumped out of the cells. p-Glycoprotein has only recently been identified and thus the potential risk of p-glycoprotein-mediated drug interactions has probably been underestimated in the past leading to the induction of MDR1 (Multiple drug resistance) (Kyrklund C et al., 2000). Concomitant administration of rifampicin can lead to greatly reduced cholesterol lowering efficiency of simvastatin.

Malathion is one of the most widely used OP insecticides for agriculture and public health programs (Maroni et al., 2000). Malathion is soluble in lipids and is stored in liver and other lipophilic tissues (Garcia-Repetto et al., 1995). The quenching of fluorescence of buffalo liver cystatin (BLC) when treated with malathion, in vitro, with concentrations ranging from 0.1 ppm to 50 ppm, is accompanied by the red shift of fluorescence maximum (fig 9.0), followed by decrease in the fluorescence intensity. This indicates the increase in polarity of the chromophore environment, probably due to change in the microenvironment of the inhibitor (BLC) leading to complete denaturation of the protein at 50 ppm of malathion. UV spectroscopy (fig 9.1) and anti-papain activity (Tab. 2.2) complements the results of fluorescence spectroscopy showing changes in the secondary structure and in turn the function of BLC.

The ratio of cathepsin B and the inhibitory activity of endogenous cystatins (CPIs) in the mammalian body in some diseases are significantly shifted in favour of increased cathepsin activity. Malathion exposure can lead to the functional inactivation of cystatins in the liver, besides causing other harmful effect to hepatocytes and conformational changes in this protein inside the cells, perhaps lead to the free radical generation which along with the low level of cystatins can trigger tumour formation.

Bibliography