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Effect of Amitriptyline an Antidepressant Drug on Structural and Functional Properties of Brain Cystatin

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Abstract

Cystatins are the thiol proteinases inhibitors of prime physiologic importance. They are ubiquitously present in mammalian body. They prevent unwanted proteolysis and play important role in several diseases. Regulation of cysteine proteinases and their inhibitors are of utmost importance in diseases like Alzheimer, amlyoid angiopathy and other neurodegenerative disease. Proteinase anti-proteinase imbalance accelerates disease progression. Amitriptylin an antidepressant helps to relieve depression and pain. It is often used to manage nerve pain resulting from cancer treatment. Such injury to nerves causes a burning, tingling sensation. This medication, usually given at bedtime, helps patients to sleep better

In this paper interaction of brain cystatin (BC) with Amytriptyline (AMY) has been studied by UV absorption and fluorescence spectroscopy. In the present study, the effect of drug has been studied to explore AMY induced changes in functional and structural integrity of the cystatin. The fluorescence quenching data is indicative of complex formation between the protein and drug which confirmed the binding of Amytriptyline with brain cystatin. Stern-Volmer analysis of Amytriptyline binding with brain cystatin indicates the presence of static component in the quenching mechanism. The thermodynamic parameters ΔG° (Free energy change) -36.966 KJ/mol indicated that both hydrogen bonds and hydrophobic interactions played a major role in the binding of AMY with BC. Binding investigations give in this work, gives significant information about the conformational changes in cystatin due interaction with the drug.

Keywords: Purification of buffalo brain cystatin; Thiol proteinases inhibitors; Amytriptyline; Depression

Abbreviation

BC: Brain Cystatin

Introduction

Cysteine proteinases such as cathepsins B, H, L and S [1], have myriad of biologic activities attributed to them, like proliferation, differentiation, ageing and cancer. Cystatins, the ubiquitous non-covalent competitive inhibitors are involved in the regulation and protection of cells from unwanted proteolysis [2]. Cystatins also play a vital role in the homeostasis of intracellular and extra cellular matrix whose recycling is important otherwise it may cause a number of neurodegerative diseases like Alzheimer and amlyoid angiopathy [3].

An antidepressant is a psychiatric medication used to alleviate mood disorders, such as major depression insomnia, drossiness muscle stiffness, nervousness and loss of appetite. The Drugs including the monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs), and serotonin-norepinephrine reuptake inhibitors (SNRIs) are most commonly used for psychiatric problems drugs. Amytriptyline Hydrochloride is a tricyclic antidepressant drug. It is a white, odorless, crystalline compound which is freely soluble in water. In terms of its mechanism of action, amytriptyline inhibits serotonin degradation and noradrenaline reuptake almost equally. Amytriptyline (Figure1) is also approved for pain associated with the nerves (neuropathic pain), and to prevent migraine headaches. The recommended therapeutic concentration of amitriptyline is 75 mg/day [4] and the concentration of amitriptyline in brain after crossing the blood –brain barrier is 1500 ng/g – 2000 ng/g wet brain tissue [5]. Typically lower dosages 10 to 50 mg daily are required for pain control [6].

The marginal stability of the native conformation of proteins is also a delicate balance of various interactions in proteins (van der Waals, electrostatic, hydrogen bonds and disulphide bridges) [7] which is affected by pH, temperature or addition of small molecules such as substrates, coenzymes, inhibitors and activators that bind especially to the native state and thus alter this fragile equilibrium leading to a number of slightly different conformational states of proteins which differ drastically in their function. In this regard, most drugs exert their activity on interaction with proteins. Moreover drug accumulation at off-target sites in the body leads to unintended adverse reactions [8,9] ,however drug/ligand induced protein structure conformational alterations [10] are prime problems complicating drug medical therapy by affecting the cellular proteins. Keeping in view the importance of cystatins in maintenance of proper brain function, the effect of Amytriptyline on the integrity of a newly purified cysteine proteinase inhibitor from buffalo brain has been explored. Most of the antidepressants drugs target neurotransmitters serotonin, although norepinephrine is affected to some degree as well [11]. In fact antidepressant medications are the first-line treatment for people meeting current diagnostic criteria for major depressive disorder. Depression is associated with reduced levels of the monoamines in the

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brain, such as serotonin. Most antidepressants are designed to enhance the level of serotonin. The selective serotonin and noradrenaline re-uptake inhibitors (SNRIs) are thought to restore the levels of these neurotransmitters in the synaptic cleft by binding at their re-uptake transporters preventing the re-uptake and subsequent degradation of serotonin and noradrenaline. This re-uptake blockade leads to the accumulation of monoamines in the synaptic cleft and their concentration then returns to within the normal range. This action of SNRIs is thought to contribute to the alleviation of the symptoms of depression. Since antidepressants are spread widely throughout the body may also influence serotonergic processes in the periphery. The serotonin transporter also plays a crucial role in the homeostatic regulation of serotonin in the gut and plasma (Figure 1 and 2).

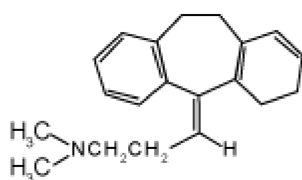

Figure 1: Structure of Amytriptyline hydrochloride [6].

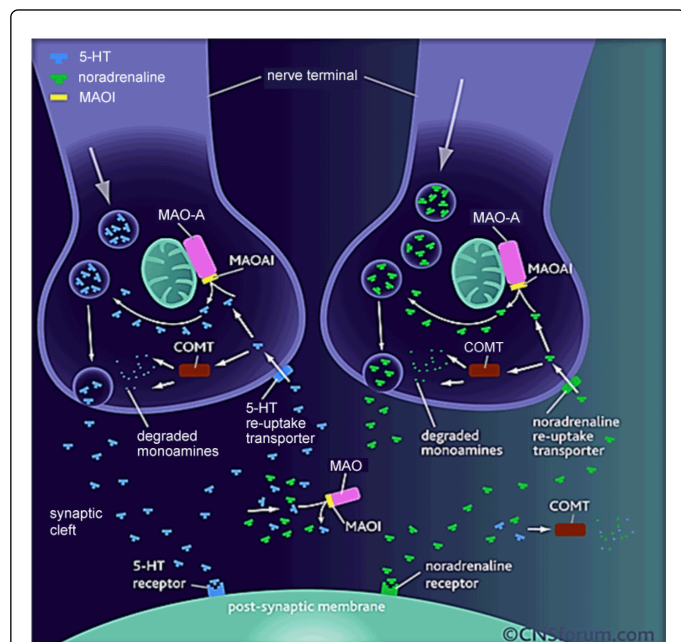

Figure 2: The mechanism of action of antidepressants (monoamine oxidase inhibitors) [12].

Since amitriptyline is believed to be a potent inhibitor of noradrenaline re-uptake at the adrenergic nerve endings [13]. The mechanism in figure 2 and 3 shows that it increases both serotonin and norepinephrine level and this helps in reducing depression.

We have already reported the purification and characterization of buffalo brain cystatin (BC) identified to be a variant of cystatin type-I [14]. BC is a double sub-unit structure having a molecular weight 44.2 kDa protein, Fluorescence spectroscopy was employed to assess any structural changes in brain cystatin on interaction with Amytriptyline. Functional changes were analyzed by assessment of the inhibitory potential of the drug-bound protein. Literature shows that amitriptyline binds to amino acids tyrosine, tryptophan, valine, leucine, isoleucine and phenylalanine our purified protein contains these amino acids thus amitriptyline after binding with purified protein leads to inactivation of cystatin thus it is a physiologic event [15].

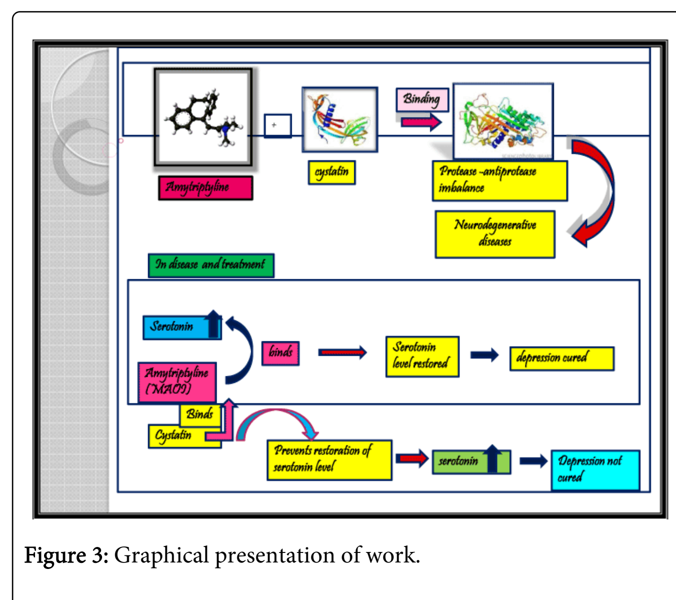

Figure 3: Graphical presentation of work.

Material and Methods

Experimental procedures

Materials

Papain (99% purity) and amytriptyline was obtained from Sigma Chemical Company (St. Louis, USA). The solutions were prepared in 50 mM phosphate buffer of pH 7.4. Salts were purchased from Merck (India). The protein concentration was determined spectro-photometrically. All other reagent was of analytical grade and double distilled water was used throughout.

Purification of Brain Cystatin

Fresh brain tissue (150 grams) was homogenized in 50 mM sodium phosphate buffer of pH 7.5 (30 ml) containing 1% NaCl, 3mM EDTA and 2% n-butanol. After centrifugation at 11000 rpm for 15 minutes at 4°C residue was discarded and the supernatant was further processed. The procedure involved a combination of alkaline treatment (pH 11.0), ammonium sulphate fractionation and gel filtration chromatography. Buffalo brain was homogenized and fractionated with ammonium sulfate between 40-60%, it was then dialyzed against 50 mM sodium phosphate buffer pH 7.4 containing 0.1 M NaCl. Elution profile showed two protein peak one major and one minor named as peak-I and peak-II. Peak-I corresponding to high molecular weight. Cystatin had significant inhibitory activity and protein content; however peak-II with insignificant proteins concentration and low inhibitory activity was not taken into consideration for further studies. Peak-I renamed as BC was then purified with fold purification of 384.72 and yield of 64.13%. Papain inhibitory fractions of peak-I were pooled, concentrated and checked for purity. Five milliliter

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fractions were collected and assayed for protein by the method of Lowry et al. (1951) and inhibitory activity against papain by the method of Kunitz [16]. Homogeneity of the preparation was investigated by 7.5% PAGE [14].

Spectroscopic studies

Fluorescence spectra of brain cystatin with amytriptyline

Brain cystatin (BC) (1μM) was incubated for 30 min with increasing concentrations of amytriptylinein 0.05 M sodium phosphate buffer pH 7.5 in a final reaction volume of 1ml at room temperature. Drug solutions were prepared in the same buffer. Fluorescence measurements were carried out on a Shimadzu Spectrofluorimeter model RF-540 equipped with a data recorded DR-3 at 298K. The fluorescence was recorded in wavelength range of 300-400 nm after exciting the protein at 280 nm. The slits were set at 10 nm for excitation and emission. The path length of the sample was 1 cm. The data was analyzed by stern-Volmer equation

Stern- volmer constant

The fluorescence quenching was analyzed by the Stern-Volmer equation

$$\frac{Fo}{F} = 1 + Ksv\ [Q]$$

Where F_0 and F are the fluorescence intensities in the absence and presence of quencher respectively, Ksv the stern-volmer quenching constant and [Q] is the concentration of the quencher.

Determination of binding constant [K] and number of binding sites (N)

When small molecules binds independently to set of equivalent sites on a macromolecules, the equilibrium between free and bound molecules is given by the following equation [17,18].

$$Log\frac{(F0 - F)}{F} = LogK + n\ Log\ [Q]$$

Where K and n are the binding constant and number of binding sites respectively thus a plot of Log (F_o-F)/F versus [Q] can be used to determine K as well as n.

UV spectra of cystatin in the presence of Amytriptyline

The UV measurement of brain cystatin in the presence and absence of antidepressant was made in the range of 200-300 nm and the inhibitor (Cystatin) concentration was fixed at 1μM while the drug concentration was varied to different extent. Absorption spectra were recorded on a double beam Shimadzu UV-vis spectrophotometer UV-1700 using a cuvette of 1 cm path length.

Results

Interaction of Amytriptyline with Cystatin

For macromolecules, the fluorescence measurements can give some information about the binding of small molecules with proteins, such as the binding constants, binding sites and binding affinity. Fluorescence intensity of a compound may get affected by interactions of protein with drugs.

Fluorescence Spectra of cystatin with Amytriptyline

The fluorescence spectra of cystatin (1μM) in the presence of different concentrations of amytriptyline were recorded in the range of 300-400 nm upon excitation at 280 nm. Amytriptyline caused quenching of the intrinsic fluorescence of cystatin (Figure 4) with no shift in wavelength; however with the increase in Amytriptyline concentration, fluorescence intensity was further decreased (Table 1).

The maximum decrease in fluorescence intensity (48%) occurred at 8 μM concentration. These results indicated that there were interactions between Amytriptyline and brain cystatin and the binding reactions resulted in non-fluorescent complex. Fluorescence quenching data was analyzed by the Stern-Volmer equation as given in the methods section data is presented in Table 2.

Cystatin (1 μM) was incubated with various concentration of Amytriptyline varying from 2 μM to 8 μM for 30 min. The fluorescence was recorded in wavelength region 300-400 nm, exciting wavelength was 280 nm. The slits were set at 10 nm for excitation and emission. The path length of the sample was 1 cm.

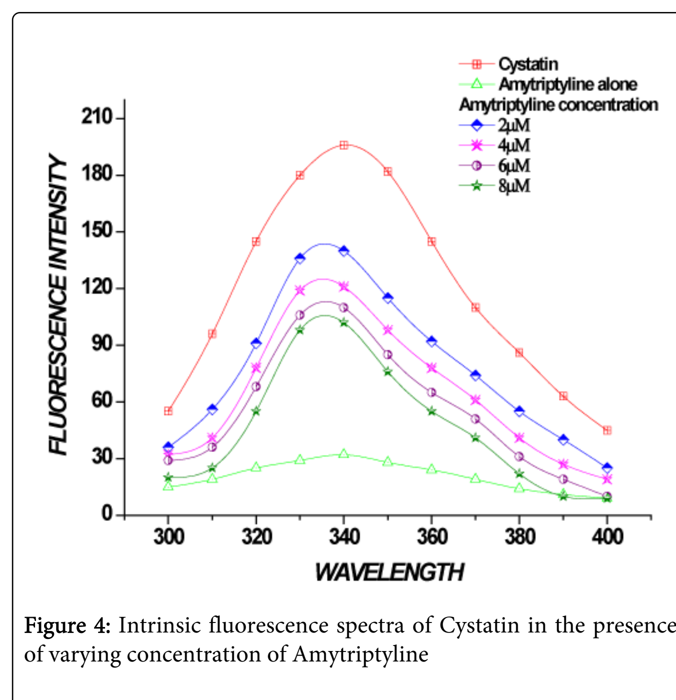

Figure 4: Intrinsic fluorescence spectra of Cystatin in the presence of varying concentration of Amytriptyline

Cystatin alone fluorescence	2 μM	4 μM	6 μM	8 μM
100%	29%	38%	44%	48%

Table 1: Percent decrease in Tryptophan fluorescence of BC in the presence of Amytriptyline.

Determination of ΔG^0 of interaction between Amytriptyline and Cystatin

The forces of interaction between drugs and biomolecules include hydrophobic force, electrostatic interactions, vander Waals

interactions and hydrogen bonds. In order to identify the interacting forces between amytriptyline with cystatin, the thermodynamic parameters i.e. free change (ΔG^0) of the interactions were calculated from the following equations (Table2).

$$\Delta G^o = -RT\,Ink\,(KJ/mol)$$

The value obtained for cystatin – amytriptyline interaction was -36.97 kJ/mol indicating the reaction to be spontaneous.

DRUG PARAMETER	K_{SV} (Stern-volmer Constant) Mol^{-1}	K (Binding constant) Mol^{-1}	n (number of binding sites)	ΔG^0 (Free energy change) KJ/mol
Amytriptyline	0.115 x 10^6	3.018 x 10^6	0.89	-36.966

Table 2: Interaction of Amytriptyline with cystatin (1μM) data calculated using stern volmer equation

UV-vis absorption spectra of Amytriptyline cystatin complex

The interaction between Amytriptyline-cystatin was studied from UV-VIS absorption spectral data as given in method section (Figure 5). Cystatin concentrations were fixed at 1μM while the Amtriptyline concentration was varied from 2 μM-8 μM.

The UV-vis absorption spectra were computed at all the antidepressant concentrations. However, no profound changes were noted at 2-8 μM Amytriptyline. The spectra obtained for BC at 2 μM Amytriptyline shows two distinct positive peaks at 210 nm and 240 nm, the gross conformation of Cystatin at all concentrations of Amytriptyline was found to be unaffected. The UV absorption intensity of Cystatin increased with the variation of Amytriptyline concentration. The Addition of drug did not result in the shift of λ_{max} towards longer wavelength. However there was quenching in fluorescence. This evidence clearly indicated the interaction and some complex formation between Amytriptyline and Brain Cystatin. [19,20].

Brain Cystatin concentrations was fixed at 1μM while the Amytriptyline concentration was varied from 2 μM-10 μM. Absorption spectra of native BC and in presence of Amytriptyline were recorded in the range of 200-300 nm ,a cuvette of 1 cm path length for 30minin the final reaction volume of 1 ml in 0.05 M sodium phosphate buffer pH 7.5.

Inhibitory activity of Cystatin in the presence of Amytriptyline

Effect of Amytriptyline on Cystatin function was assessed by monitoring changes in its anti-proteolytic activity by caseinolytic assay of papain [16]. 1μM of Cystatin was incubated with increasing concentration of Amytriptyline (2-8μM). The results obtained are summarized in (Table 3). When Cystatin incubated with increasing concentration of Amytriptyline, it resulted in rapid decline of anti-proteolytic activity showing 58% loss at 6 μM concentration of Amytriptyline with half of its inactivation taking place at a concentration of 8 μM. The data obtained also indicated that inactivation of Cystatin by Amytriptyline is concentration dependent.

Changes in the inhibitory activity of cystatin (1 μM) after incubation with increasing concentration of Amytriptyline (2-8 μM) for 30 min in the final reaction volume of 1 ml in 0.05 M sodium phosphate buffer pH 7.5. Cystatin inhibitory activity without amytriptyline was taken as 100%.

All data are expressed as mean ± S.E for four different sets of experiments spastically significance was conducted employing ONE WAY ANOVA a probability level of 0.05 was selected showing results are significant.

Changes in the inhibitory activity of cystatin (1 μM) after incubation with increasing concentration of Amytriptyline (2-8 μM) for 30min in the final reaction volume of 1 ml in 0.05M sodium phosphate buffer pH 7.5. Cystatin inhibitory activity without amytriptyline was taken as 100%.

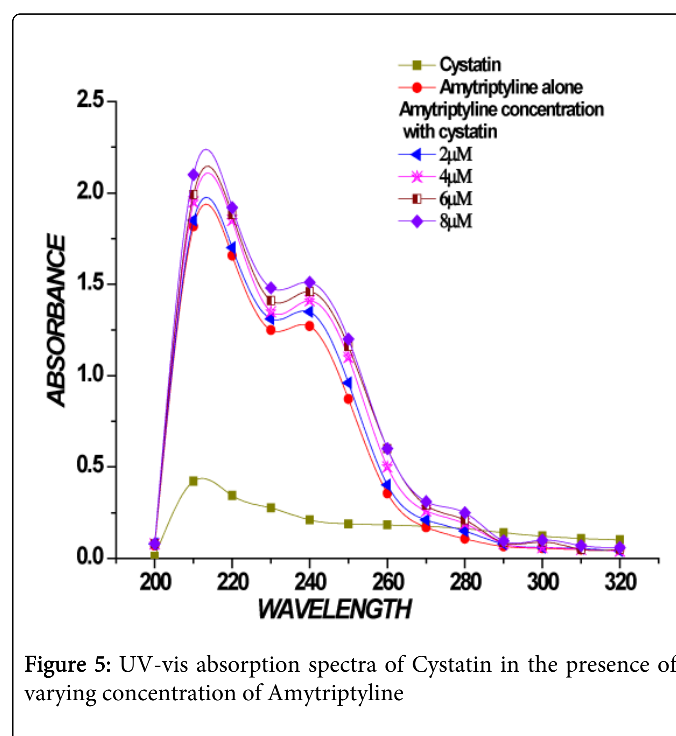

Figure 5: UV-vis absorption spectra of Cystatin in the presence of varying concentration of Amytriptyline

S.NO	Amytriptyline concentration	% Inhibitory Activity Retained
1	Cystatin alone	100
2	Cystatin + 2 μM Amytriptyline	71 ± 0.922
3	Cystatin +4 μM Amytriptyline	57 ± 0.755
4	Cystatin +6 μM Amytriptyline	42 ± 0.623
5	Cystatin +8 μM Amytriptyline	38 ± 0.98

Table 3: Inhibitory activity of Cystatin in the presence and absence of Amytriptyline

Discussion

Antipsychotic and antidepressant drugs are imperative in the treatment of schizophrenia and other brain disorders. These drugs exert their therapeutic effects at least in part through perturbation of the dopamine, noradrenalin and serotonin neurotransmitter systems in the brain, but additional molecular mechanisms of action are likely to contribute to their clinical effects.

The MAOIs monoamine oxidase (Antidepressants) prevent the breakdown of monoamine neurotransmitters and therefore increase the concentration of the neurotransmitters (serotonin and dopamine) leading to depression in the brain.

Amytriptyline is a medication used to treat various forms of depression, pain associated with the nerves (neuropathic pain), and to prevent migraine headaches. It is often used to manage nerve pain resulting from cancer treatment. Amytriptyline acts to block reabsorption of chemicals that transmit nerve messages to the brain.

It produces its effects on the central nervous system via its actions on synaptic transmission. In general, the antidepressants affect the transmitters, like catecholamine's and acetylcholine. They also increase the transmission in the brain and may affect the secretion of anterior pituitary hormones by regulating the hypothalamic secretions [21]. The aim of the present study was to investigate the effect of antidepressants Amytriptyline on structural and functional modification of cystatin. To the best of our knowledge interaction of amitriptyline with cystatin has been done for the first time. However amitriptyline alone [when not bind with cystatin] increases serotonin level and the side effect of amitriptyline is that it binding with cystatin creates proteinase-antiproteinase imbalance. Investigations reveal that amitriptyline binds with cystatin reducing the effective concentration of amitriptyline therefore effectiveness of amitriptyline in depression is undercut by cystatin.

Cystatins are very important in the pathogenesis of depression, although it does not bind with serotonin. The published results have revealed that serotonin unfolds cystatin, which renders cystatin unable to regulate the activity of cathepsins leading to a proteinase - anti proteinase imbalance this may result in many chronic diseases. Several studies have shown that cystatins also bind with antidepressant monoamine oxidase inhibitor (MAOI) [22].

As a result MAOI may not be able to act on monoamine oxidase (MAO) whose function is to degrade serotonin and therefore destruction of serotonin continues, which lead to depression. Thus our study shows the involvement of cystatin in the pathway leading to depression.

Drug–protein interaction studies are used to determine the ability of antidepressants to analyze its interaction to number of binding sites binding of a protein. Drug binding is evaluated at various drug concentrations and the Information derived from binding studies has been used to determine the affinity of amytriptyline for cystatin. However, binding parameters are only the first step in understanding overall pharmacologic effects. In the present study some binding reaction Parameters, including the binding constants and the numbers of the binding sites has been determined.

Intrinsic fluorescence of protein provides considerable information about protein structure, in terms of quenching, enhancement of intensity and spectral shift which are used for elucidation of related structure-dynamics in proteins [23]. In the present study, fluorescence spectroscopic measurements were undertaken to gain insight into the interaction of cystatin with amytriptyline. It was found that in the presence of drug fluorescence intensity decreased indicating a conformational change in the protein (Figure 4).

When 1 μM of Cystatin was incubated with 2 μM of amytriptyline for 30 min and the samples were analyzed by fluorescence, the fluorescence emission spectrum showed no shift in λ_{max}, with slight decline in fluorescence intensity as compared to native. The negative value of ΔG° reveals that the interaction process is spontaneous. Our finding is similar to the binding of Congo red with human serum albumin .The value for the number of binding sites (n) equal to 0.89 showing that there is one independent binding site of interaction. The K_{sv} value reported for amytriptyline - cystatin complex was 1.15 x 10^5 Indicating the quenching in fluorescence to be static. These data are in accordance with interactions of lung cystatin with other drugs [24].

Absorption spectral measurements of Cystatin in the presence of amytriptyline provided information related to changes in their structure. The two positive peaks observed at 210 and 240 nm for amytriptyline - cystatin complexes at all concentrations studied, showed contribution from phenylalanine and histidine residues (Figure 5) [25]. When cystatin incubated along with amytriptyline for varying concentration it caused decrease in antiproteolytic activity, there was 58% loss in antiproteolytic activity at 6μM of Amytriptyline concentration. This loss in antiproteolytic activity increased to 62% at 8μM of amitriptyline, (Table-2) showing a concentration dependent effect. These results have shown similarity with other cystatins [26]. The phenomena of binding of drug with cystatin cannot be generalized. The present study shows that cystatin binds with amitriptyline however in other cases antidepressants lead to unfolding of cystatin therefore study of cystatin with more drugs is required to see the effect of other antidepressant drugs on cystatin.

However results prove that cystatin binds with amitriptyline reducing its effective concentration and it also shows the disbalance of crucial regulator cystatin for thiol proteinase activities which is a side effect of the drug.

It is well known that effectiveness of amitriptyline after some time reduced this may be the effect of cystatin interaction with amitriptyline however the complete molecular mechanism of action of psychotropic drugs are still not known and the study needs to be extended with other antipsychotic drugs.

According to our hypothesis the reduced action of amitriptyline may be due to its binding with cystatin, which also reduce the activity of cystatin which rendering it unable to regulate the function of cathepsins leading to a proteinase - anti proteinase imbalance a cause of many chronic diseases [22,27]. The study has sheds some light on cystatin function with regard to amitriptyline action. With regard to the mechanism of action of amitriptyline, other changes may also be considered that take place with chronic antidepressant use to maintain homeostasis, including alterations in the density and functioning of serotonin receptors, transporters, and enzymes [28-31].

However further studies are needed to study the in vivo effect of amitriptyline that is to use a depressed rat model which may be given different doses of amitriptyline and then the activity of cystatin in brain tissues as well as the level of serotonin may be measured. *The information may be used for in-depth knowledge about cystatin function and to understand the mechanism of action of amitriptyline.*

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References

1. Ekiel I, Abrahamson M, Fulton DB, Lindahl P, Storer AC, et al. (1997) NMR structural studies of human cystatin C dimers and monomers. J Mol Biol 271: 266-277.

2. Turk V, Stoka V, Turk D (2008) Cystatins: biochemical and structural properties, and medical relevance. Front Biosci 13: 5406-5420.
3. Katzenstein AL, Myers JL (1998) Idiopathic pulmonary fibrosis: clinical relevance of pathologic classification. Am J Respir Crit Care Med 157: 1301-1315.
4. Gupta SK, Shah JC, Hwang SS (1999) Pharmacokinetic and pharmacodynamic characterization of OROS and immediate-release amitriptyline. Br J Clin Pharmacol 48: 71-78.
5. Nelson SR, Mantz ML, Maxwell JA (1971) Use of specific gravity in the measurement of cerebral edema. J Appl Physiol 30: 268-271.
6. Centers for Disease Control and Prevention, Gangadharan D, Smith J, Weyant R (2013) Biosafety Recommendations for Work with Influenza Viruses Containing a Hemagglutinin from the A/goose/Guangdong/1/96 Lineage. MMWR Recomm Rep 62: 1-7.
7. Sneppen K, Zocchi G (2005) Physics in Molecular Biology, Cambridge University Press, New York, NY, USA.
8. Taniguchi K, Ito J, Sasaki M (1981) Partial purification and properties of urinary thiol proteinase inhibitors. J Biochem 89: 179-184.
9. Holzgrabe U, Kapková P, Alptüzün V, Scheiber J, Kugelmann E (2007) Targeting acetylcholinesterase to treat neurodegeneration. Expert Opin Ther Targets 11: 161-179.
10. Takeda K , Wada A , Yamamoto K , Hachiya K , Batra P.P, (1988) "Secondary structure change of myoglobin induced by sodium dodecyl sulfate and its kinetic aspects". J Colloid InterfSci125: 307–313.
11. Stahl S (2008) Stahl's Essential Psychopharmacology: Neuroscientic Basis and Practical Applications. 3rd Edn. Cambridge: Cambridge UniversityPress.
12. Other peripheral mediators: 5-hydroxytryptamine and purines (2001) In: Pharmacology, 4th edition. Rang HP, Dale MM and Ritter JM. Edinburgh, UK: Harcourt Publishers Ltd, 165–176.
13. Dollery Sir Colin (1991) editor. Therapeutic Drugs. Edinburgh: Churchill Livingstone, A95–A100.
14. Amin F, Khan AA, Rizvi SJ, Bano B (2011) Purification and characterization of buffalo brain cystatin. Protein Pept Lett 18: 210-218.
15. Eriksson T, Eriksson TE (1995) Effects of amitriptyline on rat plasma and brain content of monoamine precursors and other large neutral amino acids. J Pharm Pharmacol 47: 1007-1014.
16. Kunitz M (1947) CRYSTALLINE SOYBEAN TRYPSIN INHIBITOR : II. GENERAL PROPERTIES. J Gen Physiol 30: 291-310.
17. Feng XZ, Lin Z, Yang LJ, Wang C, Bai CL (1998) Investigation of the interaction between acridine orange and bovine serum albumin. Talanta 47: 1223-1229.
18. Gao H , Lei L , Liu J , Quin K, Chen X , et al. (2004) The study on the interaction between serum albumin and a new reagent with antitumour activity by spectrophotometric methods. J Photochem Photobiol 167: 213–221.
19. Cui FL, Fan J, Li JP, Hu ZD (2004) Interactions between 1-benzoyl-4-p-chlorophenyl thiosemicarbazide and serum albumin: investigation by fluorescence spectroscopy. Bioorg Med Chem 12: 151-157.
20. Hu YJ, Liu Y, Wang JB, Xiao XH, Qu SS (2004) Study of the interaction between monoammonium glycyrrhizinate and bovine serum albumin. J Pharm Biomed Anal 36: 915-919.
21. Schüle C (2007) Neuroendocrinological mechanisms of actions of antidepressant drugs. J Neuroendocrinol 19: 213-226.
22. Amin F, Naeem A, Bano B (2013) Interaction of Buffalo Brain Cystatin with Serotonin. Curr Nanosci 9: 231-234.
23. Lakowicz JR (2006) Principles of Fluorescence Spectroscopy University of Maryland. Baltimore. USA. 954 : 1255
24. Khan MS, Priyadarshini M, Sumbul S, Bano B (2010) Methotrexate binding causes structural and functional changes in lung cystatin. Acta Biochim Pol 57: 499-503.
25. Donovan JW (1969) Changes in ultraviolet absorption produced by alteration of protein conformation. J Biol Chem 244: 1961-1967.
26. Priyadarshini M, Khan RH, Bano B (2010) Physicochemical properties of thiol proteinase inhibitor isolated from goat pancreas. Biopolymers 93: 708-717.
27. Amin F, Bano B (2013) Studies on interaction of buffalo brain cystatin with donepezil: an Alzheimer's drug. Int J Alzheimers Dis 2013: 842689.
28. Holt A, Baker GB (1996) Inhibition of rat brain monoamine oxidase enzymes by fluoxetine and norfluoxetine. Naunyn Schmiedebergs Arch Pharmacol 354: 17-24.
29. Hyman SE, Nestler EJ (1996) Initiation and adaptation: a paradigm for understanding psychotropic drug action. Am J Psychiatry 153: 151-162.
30. Adell A, Castro E, Celada P, Bortolozzi A, Pazos A, et al. (2005) Strategies for producing faster acting antidepressants. Drug Discov Today 10: 578-585.
31. Kovacevic T , Skelin I , Diksic M (2010) Chronic fluoxetine treatment has a larger effect on the density of a serotonin transporter in the Flinders Sensitive Line(FSL) rat model of depression than in normal rats. Synapse 64: 231–240.