SUMMARY AND CONCLUSIONS
In the modern era of Science and Technology where man is successful in many fields, his knowledge of function and diseases of nervous systems is still scarce. Numerous investigators have shown alterations in the levels of neurotransmitters, their receptors and cellular signaling playing an important role in functioning of brain like Parkinson's disease, Huntington's, chorea, Alzheimer's disease, epilepsy, schizophrenia. Neuroscientists have attempted to study these signaling molecules/pathways under clinical conditions using body fluids and post-mortem brain samples from disease affected individuals. The data obtained from post mortem brain samples is not reliable due to difficulties associated with proper isolation and preservation of tissue. Moreover, the choice of age, sex and stage of disease is not possible.

Many techniques have been developed to quantitate the neurotransmitters receptors like PET, SPECT and NMR. But there are some limitations including high cost of operations as well as expertise for their operation and interpretation of data. Thus it has become very important to develop peripheral markers/biomarkers for various diseases to detect early pathological effects, while the disease is still in reversible phase prior to the irreversible damage so that the neuronal tissue can be salvaged.
Circulating blood components like platelets and lymphocytes have been used to study the uptake and release of certain neurotransmitters and receptors. Because of their easy availability and sharing of some functions similar to those of CNS neurons. Alterations in the binding of $^3$H-spiiperone to lymphocytes in Parkinsonism and Wilson disease and that $^3$H-dihydroalprenolol in depressed patients have reported. Several similarities between platelets and neuron with respect to morphology, biochemistry and pharmacology have further strengthened their usefulness as peripheral model. As the changes are specific and parallel with those observed in the brain, the assay of platelet receptors appears to be a useful tool for these studies. Such studies have not only helped in understanding the disease process but also in monitoring the disease condition during the course of treatment and follow up. Although platelets appear to be fascinating model to study the functions of brain, the mechanism by which dopamine and serotonin receptors are modulated is not yet clearly understood. The response of neurotoxicant and specificity to lymphocytes is also not clear. Changes in the biochemical parameters in platelet and lymphocyte such as neurotransmitter receptors and enzymes have been reported in disease condition or on exposure to toxicants but their validation is required to establish the use of platelets and lymphocytes as peripheral model.

Present study was aimed to investigate the characterization receptor of platelet DA-D4 and, study the effect of selected neurotoxicants namely cadmium, fenvalerate, methyl parathion) on lymphocytes and brain on biochemical parameters and membrane function parameters. Status of platelet serotonin receptors were studied in clinical cases of in drug abuse.

**Identification and Characterisation of DA-D4 in human platelets**

Involvement of D4 receptors in pathophysiology of schizophrenia has been speculated due to its involvement in mediating the action of atypical neuroleptics. Studies on the polymorphism of D4 receptor gene have suggested that any alterations in this gene may contribute in increasing the risk of developing schizophrenia. Hence the candidate attempted to identify and characterize D4
receptor binding sites in human platelets using $^3$H-Clozapine, a selective agonist
and also to compare the binding characteristics with those present in the CNS.
$^3$H-Clozapine binding to human platelet has been found to be saturable, time,
temperature and protein dependent. Scatchard analysis indicated that DA-D4
receptors in human platelets exhibit a single binding site with dissociation
constant (Kd) 1.29 nM. The maximum binding site was found to be 236 pmoles
bound /gm protein for human. Competitive binding studies using various
dopamine agonists and antagonists in displacing bound $^3$H-Clozapine were
performed. The order of potency of these displacers in human platelets was
U-101,958>L-50,667>Clozapine>Butadramol>Haloperidol>Ketanserin>Dopamine
> R-(+)-7-OH- DPAT>SCH-23390.

Effect of Cadmium, Fenvalerate and Methyl Parathion on selected
biochemical parameters in brain and lymphocyte

Cadmium, a well known neurotoxicant cause oxidative stress through free
radicals over production. There is an urgent need to validate peripheral test
system which could be utilized to monitor such neurotoxicological disorders.
Since blood lymphocytes reflect changes of the CNS and are proposed as a
peripheral model, the present study was aimed to investigate whether neurotoxic
effects of cadmium are reflected in lymphocytes. Exposure of cadmium (0.4
mg/kg b.wt., i.p.) in adult rats for 30 days caused a significant increases in
membrane fluidity as evidenced by decreased fluorescence polarisation
(lymphocytes 24% and Olfactory lobe 22%). Intracellular calcium (lymphocyte
144% and Olfactory lobe 163%) and Lipid peroxidation (lymphocyte 63% and
Olfactory lobe 65%) were increased in comparison to controls. A significant
decrease was observed in the reduced glutathione content (lymphocyte 38% and
Olfactory lobe 40%) following exposure to cadmium.

Fenvalerate which is a type II pyrethroid is of high bioefficacy and its
exposure leads to behavioral and biochemical deficits. Keeping this in view,
studies were carried out to understand its mechanism of neurotoxicity and
investigate whether such changes are reflected in lymphocytes on selected
biochemical parameters to validate usefulness of lymphocytes as a peripheral model. Effect of fenvalerate (10 and 20 mg/kg) was studied on membrane fluidity, intracellular calcium, superoxide dismutase, catalase, reduced glutathione and lipid peroxidation both in lymphocytes and brain of rats after 10 and 21 days of exposure. A decrease in fluorescence polarisation (20% and 25%) and superoxide dismutase (69% and 78%) in lymphocyte was observed likewise decrease in fluorescence polarisation (25% and 33%) and superoxide dismutase (48% and 88%) following exposure for 10 and 21 days at both doses was noted in corpus striatum in comparison to controls. These rats also exhibited an increase in intracellular calcium levels in lymphocyte (18% and 20%), and corpus striatum (32% and 40%), 10 days after exposure at both the doses.

Methyl parathion, an organophosphate pesticide also induces an oxidative stress which leads to generation of free radicals. It is a known neurotoxin and reported to cause inhibition of acetylcholinesterase activity of neurons at cholinergic synapses. In order to investigate whether neurotoxic effects of methyl parathion are also reflected in lymphocytes, its effect were studied on selected biochemical parameters studied in cerebral cortex and lymphocytes. The treated rats exhibited a decrease in ACHE activity (38% in cerebral cortex and 68% in lymphocytes), reduced glutathione (27% and 12%), superoxide dismutase (26% and 18%) and catalase activity were (38% and 44%). However, the levels of MDA increased. Changes in these biochemical parameters were restored to some level to control values at 21 days (i.e. 8 days cessation after 14 doses). Thus, the results suggest that exposure to Methyl parathion may alter the membrane function as evident from both in lymphocyte and brain.

Modulation of Serotonin receptors in Platelets of Drug abuse patients

Drug dependence is a complex phenomenon. Basically it leads to dependence of an individual to a particular drug. Generally the patient over a period of time develop dependence and requires higher doses of drug for its effects. They also exhibit withdrawal symptoms when the drug is withdrawn. The mechanism of development of tolerance and withdrawal system are not well
understood. Thus inspite of several studies in this direction have shown that 5-HT (serotonin) sites are involved in development of dependence. These studies indicate that serotonin sites are important for further research in substance use disorders. Not only in substance use disorders, in various psychiatric disorders like depression, panic disorder, nocturnal enuresis and suicidal cases, involvement of serotonin sites has been suggested. Currently efforts are being made to examine correlation, if any, between serotonergic symptoms and exposure to substance of abuse in exposed individuals. Unlike other substances, limited studies have been conducted on opioid dependence, which involve the serotonin sites. Therefore the candidate has attempted to investigate this by studying the levels of 5-HT receptor in platelets of human taking opioids. Our knowledge of the CNS, to a large extent is derived from work on peripheral tissues, platelet is used as peripheral marker for serotonergic neurons. In this study, [H]-Paroxetine is used as a ligand as compared to [H]-imipramine because of its more affinity to binding sites. The present study showed that the dissociation constant and number of maximum bindings sites showed no significant difference in patients based on the duration of opioid dependence.

Conclusions

The present study indicates the presence of dopamine D4 receptor binding sites in human platelets having similar characteristics with that present in the brain. Thus platelets D4 receptors could possibly serve as peripheral markers in neuropsychiatric disorders like schizophrenia.

The results suggested that cadmium mediates its neurotoxicity through increased oxidative stress and altered membrane functions as indicated by decreased superoxide dismutase activity, catalase activity, increased membrane fluidity, increased intracellular calcium levels and reduced glutathione levels in both lymphocytes and brain.

Fenvalerate, a commonly used pesticide appears to exert its neurotoxic effects by altering membrane functions, which in turn probably, increased
oxidative stress. The changes observed in brain were also exhibited in lymphocytes.

Results of methyl parathion showed a parallel change in oxidative stress has been observed in lymphocyte as compared to brain.

Parallel changes in lymphocytes as compared to brain, strengthened the usefulness of lymphocytes as neuronal model for monitoring the changes induced by exposure to these chemicals.

No significant difference in the affinity (Kd) and number of receptor binding sites (Bmax) of platelets was observed in opioid dependence cases as compared to first degree relatives and healthy controls. Also no change in both Kd and Bmax was observed with respect to duration of dependence. However, a negative correlation between Bmax and total craving score was observed in drug dependence cases suggesting the role of serotonin uptake inhibitors in reducing the craving for opioid. Further studies may be undertaken to understand the possible involvement.
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