Effect of dietary iron overload in rat brain: Oxidative stress, neurotransmitter level and serum metal ion in relation to neurodegenerative disorders

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Excess iron causes cell injury by reacting with superoxide anions (O2·−) and hydrogen peroxide (H2O2) and producing hydroxyl radical (OH·) and reactive oxygen species (ROS). In the present study, albino rats were fed with biscuits enriched with ferrous sulphate (0.3% w/w) for 10 weeks to have overload iron conditions and observed a significant decrease in serum chromium, brain serotonin and dopamine, while iron and zinc increased significantly in serum. Increasing iron level might be responsible for accelerated dopamine oxidation with subsequent quinone formation.

Keywords Chromium, Dopamine, Ferrous sulphate, Iron, Serotonin, Zinc

Iron plays a vital role in brain, where it is required to sustain the brain’s high respiratory activity and for myelination. It is also essential for production of several neurotransmitters, such as serotonin, dopamine, norepinephrine, and γ-aminobutyric acid1. In spite of its requirement in the body, high level of iron is toxic due to oxidative metabolism, which generates large amount of reactive oxygen species (ROS). Most of the proteins involved in maintenance of iron metabolism are expressed in brain, suggesting that brain cells follow similar homeostatic mechanism as do all other cells in the body2. In normal aging, brain accumulates iron that suggests more in flow of iron into brain than out flow. This increased level of iron can disrupt the brain’s iron homeostatic mechanism. Excess of iron in brain has been implicated in the pathogenesis of a number of human age-related neurodegenerative disorders including Alzheimer’s disease5 and Parkinson’s disease6. It is generally acknowledged that excess iron catalyzes the formation of ROS that causes oxidative damage and affect brain. However, iron accumulation in brain tissues has not been widely considered a primary cause of neurodegeneration.

It has been reported that Fe2+ can increase oxidation of monoamines such as serotonin and dopamine5. Therefore, the formed oxygen free radical products can undergo covalent binding with free sulphydryl group. The latter is the component of proteins such as actin and "serotonin binding proteins" which are present in soluble brain extract5. Similar oxidative mechanisms as seen in in vitro studies can be applied in cell culture studies6. Thus, iron can increase the cytotoxicity of dopamine by increasing in its oxidation rate without intervention of monoamine oxidase B enzyme. These observations are relevant to the mechanism by which dopaminergic neurones are destroyed in neurodegenerative disorders such as Parkinson’s disease5. Furthermore the role of iron in cerebral ischaemia is also very important where it seems to be associated with higher oxidative stress, excitotoxicity and inflame-matory responses6.

Zinc as an essential mineral is found in almost every cell that participates in various activities of approximately 300 enzymes7. It supports healthy immune system, normal growth and development8-12.

Chromium as a rare body mineral can play a significant role in human nutrition and metabolism. It is implicated in glucose regulation within the circulating blood. Insulin has a significant role in regulation of glucose movement in and out of cells and chromium acts as cofactor with insulin13-15. Whether these minerals (chromium and zinc) are affected by excess of iron need further studies.

Therefore, the present work was undertaken to illustrate the effect of iron fortified diet on brain neurotransmitters, mainly dopamine and serotonin. In addition, we have also measured effect on serum level of zinc and chromium. We have also observed pathological changes in brain tissue and their possible correlation with studied biochemical parameters were also discussed.

Twenty male Wistar rats (140–150 g) were selected for the present study. Rats fed with basic diet containing barley and carrots and allowed to free access of tap water and kept under constant environmental conditions at room temperature.

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Animals were divided into two groups, Group I (Gr I) received packed biscuits (50–60 g/day) enriched with ferrous sulphate (0.3%, w/w) daily for 10 weeks modified dose\textsuperscript{16} and obtained from Sd Fine-Chem Ltd.,(India). Iron fortified biscuits were fed to produce in vivo excess iron condition in rats. Group II received packed biscuits (free of iron; 50–60 g/day) for 10 weeks. Both the groups also received extra basic diet of barley and fresh vegetables.

\textit{Blood collection and biochemical analysis} – After completing the diet regimen, the rats were fasted over night, collected blood in coated glass tubes\textsuperscript{17} via retro–orbital bleeding and centrifuged at 1000 x g for 15 min at 4°C. The plasma (supernatant) was collected and stored at–70°C for estimation of Fe\textsuperscript{2+}, Zn\textsuperscript{2+} and Cr\textsuperscript{3+}. Whole brain tissues were removed quickly on ice and homogenized. Lipid peroxidation value (MDA), serotonin and dopamine contents were assayed in the brain homogenate.

Serotonin (5-HT) and dopamine (DA) were determined fluorometrically\textsuperscript{18}. peroxidation (MDA) was determined spectrophotometrically as described by\textsuperscript{19, 20}. Fe, Zn and Cr were determined by atomic absorption photometer\textsuperscript{21}.

\textit{Statistical analysis}—Data collected were analyzed by one-way ANOVA utilizing computerized statistical program (InStat).

In the iron treated rats, brain serotonin, dopamine and serum chromium decreased significantly compared to controls, whereas brain MDA, serum iron and zinc increased significantly (Table 1).

\textit{Histopathology changes}—Brain overload with iron showed meningeal haemorrhage, congestion and edema (Fig. 1). Choroid plexus of ventricles got dilated and haemorrhagic focal areas of encephalomalacia with congested cerebral blood vessels were observed. Further, cerebral cortex had degenerated neurons, satellitosis and neuronophagia.

The present study demonstrated that intake of iron fortified diet significantly decreased brain serotonin and dopamine. In addition, there was a significant increase MDA level indicating an increased oxidative stress. Increased iron is known to induce lipid peroxidation (oxidative stress)\textsuperscript{5}. Thus, elevated MDA contents observed in the present study were the consequence of excess iron in brain.

Jimenez \textit{et al.}\textsuperscript{8} have reported that serotonin binding proteins (SBP) located in brain extract are involved in storage, protection and/or transport of serotonin as well as catecholamines. Such binding is increased by Fe\textsuperscript{2+}, but not by Fe\textsuperscript{3+}. It is believed that Fe\textsuperscript{2+} binds first to SH\textsuperscript{−} group of SBP. Monoamines also form coordination bonds with trapped iron leading to potential change in SBP functions. These findings show iron-induced oxidative stress adversely influencing neurotransmitters which may lead to neurodegeneration\textsuperscript{22}.

Others have demonstrated that direct injection of ferrous ammonium sulfate into cerebral spinal fluid of experimental rats induced 2-fold increase in iron content of brain cortex synaptosomes\textsuperscript{23}. This may demonstrate the iron potential to cross an additional obstacle (blood-brain-barrier) to enter brain cells. However the exact mechanism of iron intake and export from the brain is not fully understood\textsuperscript{2}.

Generally metals (manganese, copper) can oxidize monoamines either directly or through oxygen free radicals produced by iron. Many studies have proposed that iron induces lipid peroxidation\textsuperscript{24} and demonstrated in confirmation that Fe\textsuperscript{2+} behaves like oxidants (sodium peroxide) and superoxide radicals.

Dopamine biosynthesis may be also affected due to its exposure to mild oxidizing conditions leading to its partial oxidation. Dopamine-quinones covalently modify cysteinyi residues in tryptophan hydroxylase

\begin{table}[h]
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\textbf{Parameters} & \textbf{Control group} & \textbf{Treated group} \\
\hline
Brain & & \\
5-HT (μg/g) & 25.32 ± 1.85 & 13.24 ± 0.83* \\
DOPA (ng/g) & 557.1 ± 17.7 & 206.4 ± 21.6* \\
MDA (n mol/l) & 12.21 ± 0.39 & 22.05 ± 0.69* \\
Serum & & \\
Iron (μg/dl) & 31.25 ± 4.09 & 75.12 ± 2.90* \\
Zinc (μg/dl) & 23.12 ± 4.21 & 85.6 ± 8.31* \\
Chromium (μg/l) & 16.25 ± 3.26 & 2.0 ± 0.37* \\
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\end{tabular}
\caption{Brain serotonin, dopamine, peroxidation value (MDA), serum iron, zinc and chromium of rats fed on the test diet for 10 weeks. [Values are mean ± SE for 10 rats]}
\end{table}
(TPH; the rate-limiting enzyme in serotonin), leading to loss of its catalytic activity.

However, serotonin and melatonin can inhibit reactive oxygen species (ROS) production, MDA, carbamyl ion and mitochondria oxidation of thiols in addition to degradation of 2-deoxyribose. This may conclude the protective role of serotonin on iron mediated neuronal damage.

Accordingly disturbances in neurotransmitters levels like serotonin and dopamine and their oxidation metabolites may be associated with neurodegenerative diseases. Thus, the obtained changes results from metabolites may be associated with neurodegenerative disorders such as Parkinson’s and Alzheimer’s diseases. Iron levels increase with the severity of neuropathological changes in Parkinson's disease (PD), presumably due to increased transport through the blood-brain barrier in late stages of parkinsonism.

Abnormal amounts of iron in the brain have been demonstrated in a number of age-related neurodegenerative disorders including Alzheimer Disease (AD) and Parkinson Disease (PD). Histopathological changes observed in the brain indicated that iron overload was associated with neurodegenerative changes.

Serum zinc demonstrated significant increase, the reverse was true for chromium. It seemed that dietary supplementation of iron must be taken with caution.

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