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Effect of Diclofenac Sodium on the Role of Brain Systemic Enzymes in the Induced Arthritogenesis

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ABSTRACT

Effect of diclofenac sodium on systemic enzymes activity and their role on pro-inflammatory situation in brain environment was studied to understand corresponding drug induced changes. Male Wistar rats *Rattus norvegicus* were assigned to three groups like Control, FCA+FIA and DS. The activities of ACP, ALP, LDH, AST and ALT were estimated from brain tissue. The result shows reduced ACP activity in FCA+FIA and DS group and one fold increased ALP activity in FCA+FIA group and reduction in the DS group. The LDH and AST in DS group shows reduced activity and also in the FCA+FIA group, but ALT activity was found to be marginally elevated in DS group when compared to FCA+FIA. Reduced ACP activity in DS and FCA+FIA correlated to dynamic inflammatory relevant functional metabolism. Significantly decreased ACP, ALP, LDH, AST and ALT in DS treatment are mainly attributed to inflammatory changes and active bone lesion/turnover. The decreased enzymes activity in the DS acknowledged to the fundamental alteration in mitochondrial function.

Keywords: Arthritogenesis, ACP, ALP, LDH, AST, ALT, FCA, FIA

Abbreviations

FCA: Freund's Complete Adjuvant; FIA: Freund's Incomplete Adjuvant; DS: Diclofenac sodium treatment; ACP: Acid phosphatase; ALP: Alkaline Phosphatases; LDH: Lactate Dehydrogenases; AST: Glutamate-Oxaloacetate transaminase; ALT: Glutamate-Pyruvate Transaminase; NSAID: Nonsteroidal Anti-Inflammatory Drugs

INTRODUCTION

Diclofenac sodium (DS) shows neurotoxic effects through various metabolic pathways which leads to region-specific oxidative damages and more specific endangered drug induced toxicity in the central nervous system (CNS) [1,2]. Free radicals released from different regions of CNS can induce mitochondrial damage, suppressed ATP production that resulted in mitochondrial permeability to cytochrome-C release and followed by caspases involved apoptosis in the surrounding environment [3]. DS toxicity can induce cyclooxygenase (COX) activity, prostaglandin synthesis, increased lipid oxidation by decreasing glutathione production, increasing free radical formation induced oxidative stress, massive genomic DNA fragmentation and apoptosis. DS increased LDH, AST and ALT enzymes activities noted in this present study. This study engaged to understand ACP, ALP, LDH, AST and ALTs participation in the cascading events behind the DS toxic manifestation since the brain region is highly privileged.

MATERIALS AND METHODS

Animals

This present study used eighteen male Wistar rats *Rattus norvegicus* weighing between 180-220 g procured from King Institute, Chennai, India and followed the India Animal Ethical committee animal use protocol. All animals were housed in polypropylene cages in a temperature controlled room at 24 ± 4°C. The animals were fed with pelleted rat feed with free access to water throughout the experiment. They were acclimatized at least 3 weeks before starting the experiments. All studies were carried out using 6 rats in each group, assigned to three groups (Control, Freund's Complete Adjuvant+Freund's Incomplete Adjuvant (FCA+FIA), Diclofenac Sodium treatment (DS). All studies were carried out using 6 rats in each group. This work has got clearance from the Institutional Animal Ethical Committee (IAEC).

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Drug administration

Control rats were injected with 0.2 ml of deionized water at right leg footpad. In FCA rats, on day '0', 0.2ml of FCA was injected in the right leg footpad and 0.2 ml of FIA was injected with FCA rats' right leg footpad on day '7' and this subsequent dosage was taken as booster dosage. DS rats are treated with 10µl Diclofenac Sodium/day (50µl/5 days)/intramuscularly on days '0', '5', '10' and '15' in FCA+FIA rats.

Biochemical parameters estimation

All group rats were kept to 21 days under constant observation. They are animals mildly anesthetized using chloroform to collect brain tissue. The Capac estimated by Tenniswood et al. [4] and the ALP was done by Tietz et al. [5]. The LDH, AST and ALT activities were estimated by [6-8].

Statistical analysis

All data were analyzed with one-way ANOVA and the difference between test groups and the control was considered significant when $P<0.01$.

RESULTS AND DISCUSSION

Present study observed reduced ACP activity in DS treated and elevated ALP activity in FCA+FIA group with more than one fold increase. While the DS rats show reduced ALP activity compared to control and FCA+FIA. Reduced LDH activity was noted in FCA+FIA and DS groups. In DS treatment, the LDH activity found two fold decreases to control rats **(Table 1)**. **Table 2** shows reduced AST and ALT activity in FCA+FIA and DT. The DS treated rats showed marginally increased activity to that of FCA+FIA rats but still found below control rats.

Table 1. Effect of FCA+FIA and DT treatment on ACP, ALP and LDH enzymes activity.

	Control	FCA+FIA	DS
Brain ACP activity (µg/PNPP/protein/100 mg of wet tissue)			
Mean	0.278 ± 0.050	0.266 ± 0.055	0.200 ± 0.082
Brain ALP activity (µg/PNPP/protein/100 mg of wet tissue)			
Mean	1.507 ± 0.367	3.743 ± 0.645	0.544 ± 0.420 ***
Brain LDH activity (µg/protein/100 mg of wet tissue)			
Mean	41.383 ± 7.536	37.994 ± 7.787	15.573 ± 2.962 ***

FCA - Freund's Complete Adjuvant, FIA - Freund's Incomplete Adjuvant, DS - Diclofenac Sodium treatment, values are means ± sd, n=6. Significance P>0.001

Table 2. Effect of FCA+FIA and DT treatment on AST and ALT enzymes activity.

	Control	FCA+FIA	DS
Brain AST activity (Units/protein/100 mg of wet tissue)			
Mean	131.25 ± 27.92	102.21 ± 10.25	116.73 ± 14.90***
Brain ALT activity (Units/protein/100 mg of wet tissue)			
Mean	162.00 ± 26.84	69.75 ± 6.82	94.50 ± 11.73 ***

FCA - Freund's Complete Adjuvant, FIA - Freund's Incomplete Adjuvant,

*DS - Diclofenac Sodium treatment. Values are means ± sd, n=6. Significance P>0.001**

ACP

Acid phosphatases (ACP) activity is much important for cerebellar development and also they participate in neurodegenerative disorders. Activated lysosomes show their content to outflow to excrete exocytosis and increased functional ability to fuse with cell membranes to produce different activities like axon growth, neuronal migration, vesicular traffic [9], MHC II molecules up-regulation and also involved in neuronal death [10,11]. Glial cells are noted that, it leaks lysosomal acid phosphatase through its intact cell membranes at low osmatic pressure. The secreted ACPs were found to hydrolyze phosphomono ester metabolites and release inorganic phosphates (Pi), which are used to synthesize ATPs, phospholipids, proteins and participated in signaling processes. Squirrel monkey brain study documented that, ACP was more concentrated in different

regions namely, in nuclei of basalis Meynert, diagonalis band of Broca, magnocellular hypothalamic, corpus mammillaris, thalamus, motor neurons of cranial nerve nuclei, giant pyramidal cells of cerebral cortex and in the Purkinje cells of cerebellar cortex [12]. Lipid phosphatases are involved in the nerve cells proliferation, survival, apoptosis, migration and also in the development and maintenance of the central nervous system [5]. The protein phosphatases are found to participate in the N-methyl-D-aspartate receptors (NMDARs) mediated excitotoxicity-induced neuronal death [13]. In the brain region, the ACP activity is attributed to more related static cell metabolism than to dynamic activity [12]. DS induced toxicity shows reduced ACP and elevated ALP, AST, ALT activities accompanied with increased TNF-α, uric acid levels and TNF-α gene expressions. This has been attributed to active bone lesion [14-16]. Similarly, DS caused imbalanced antioxidant system, lipid peroxidation, pro-inflammatory responses were also noted. The elevated ALP, AST, ALT, LDH activities are attributed to oxidative stress induced tissue damage or death [17]. The oral DS administration showed increased uric acid, ALT and decreased total protein may be associated with the reactive metabolites production via hepatic cytochrome P450 [18,19]. Although, the breakdown of the antioxidant system may be the result of pro-inflammatory responses. In this present study, reduced ACP activity in DS treatment and marginally altered in FCA+FIA group has been noted **(Table 1)**. This may be taken as, no vertical imbalance in the anti-oxidative or lipid peroxidation systems and the reduced activity in DS treatment below Control rats may be taken as corresponding toxicity in the brain oxidative metabolism and may be attributed to tissue damage followed by inflammation induced dynamic functional metabolism.

ALP

ALP is the enzyme that primarily participates in liver, kidneys and brain endothelial cell activities. They also play more important and fundamental roles in neurogenesis, plasticity development, neuronal tissues cortical functions and purinergic signalling mechanism during inflammation [20,21]. The ALP purinergic signalling activity has been found to accomplish with ATP-nucleotide receptors complex involved inflammatory activity after an acute stress event. The elevated ALP activity is implicated in neuronal death through increased tau dephosphorylation, high-sensitivity C-Reactive Protein (hs-CRP) and disease severity prediction dementia patients [22-24]. In concurrence to the above, three times greater ALP activity was noted to higher oxidative stress, ischaemia patients mortality and vascular smooth muscle calcification followed by arteriosclerosis, ischemic cerebral stroke, leukodystrophy, and intracranial hemorrhage [25,26]. In contrast, extracellular ATP degradation can reduce inflammatory signals and can induce adenine receptors involved anti-inflammation responses [27,28]. Similarly, ALP treated lippopolysaccharides showed reduced capacity to induce inflammation through T Cell Receptor through lipid Ahydrolysis, which is central in TLR4 recognition [29]. In addition, elevated ALT, AST, ALP, urea and creatinine levels found reduced by DS administration added with cytochrome p-450 inhibitors to the culture [30]. In this present study, more than one fold increased ALP activity in the FCA+FIA rats and nearly two fold decreased activity in DS treatment compared to control rats is observed. The increased ALP activity in the FCA+FIA group may result from the central nervous system injury by increased activity of dephosphorylation. The decreased activity with DS treatment can be taken as oxidative stress. Since, variable metabolic pathways profoundly can lead to region-specific accumulation of oxidative damage in different regions of the CNS and followed by specific brain region to become more vulnerable to drug toxicity.

LDH

LDH gets encoded by two distinct genes, LDH-A and B. Increased LDH-A gene is more associated to HIF-1 direct transcriptional regulation and c-myc expression. DS treatment is found to inhibit COX-2 activity by c-myc expression followed by decrease in glucose transporter 1(GLUT-1) gene expression, and lactate secretion [31,32]. DS inhibited STAT-3 phosphorylation, c-myc gene expression, G2/M phasecell cycle arrest, reduced lactate levels in cell culture and inhibited LDH activity was also documented by Verena et al. [33] and Desmet et al. [34]. Similarly, DS administration inhibited Stat3 gene expression, IL-10 transactivation and IL-12 gene expression blockade are noted [35,36] DS administration induced Cox-2 upregulation and PGE2 synthesis favoured IL12 production from dentritic cells has also been documented [37]. DS toxicity from different experiments are documented for long decades of study. The DS administration is found to increase TNF-α cytotoxicity in HepG2 and Hepa1c1c7 cells and TNF-α release from activated Kupffer cells [38,39]. The TNF-α is found to involve in both pro-inflammatory and anti-inflammatory conditions. The DS associated reduced inflammation is attributed to NFκB pathway and followed by the transcriptional factors gene expression activated [40]. In this present study the LDH activity found reduced with DS treatment and unaltered in FCA+FIA group. The reduced LDH activity in DS is positively implicated upon its role on the anti-inflammatory activity as discussed.

AST and ALT

AST and ALT enzymes activity has important roles in brain region glucose metabolism, neurotransmitters production, synapses maintenance, amyloid-β and tau accumulation.The reduced ALT and AST activity is implicated to reduced pyruvate production, altered gluconeogenesis and CSF glutamate levels [41]. The increased ALT activity is related to glucose metabolism, especially in the bilateral frontal,

parietal and temporal lobes. In contrast, the higher ALT activity is implicated to reduce glucose metabolism in the bilateral frontal, parietal and temporal lobes [42] and but which is associated to elevated alkaline phosphatase activity. This study also found that, the increased AST to ALT ratio is associated to reduced CSF amyloid-βaccumulation, higher CSF p-tau and t-tau deposition. In addition, AST reactionare found to induce α-ketoglutarate, aspartate and the altered ammonia generation in the neuronal mitochondria environment. Those metabolites are largely utilized by astrocytes during normal and pathogenic situations. Neuron lack beta-oxidation enzymes and profoundly uses amino acids, ketone bodies, citric acid cycle intermediates, pyruvate and lactate for energy purpose. The large change which takes places in amino acids, glutamine, glutamate, aspartate, alanine levels in the inflammatory brain must be considered to be the driving force for the inflammatory progression. These precursors are found to be taken by aminotransferases immediately [43]. In this present study is decreased AST and ALT in FCA+FIA was observed and elevated activity was seen in DT treatment. The elevated AST and ALT in DS treatment is positively correlated to the glutame levels in the brain region and may be associated with the increased glucose metabolism especially in the bilateral frontal, parietal, and temporal lobes [44]. The decreased levels of ALT found with FCA+FIA treatment may implicate greater amyloid-β deposition and stress environment in the brain region. Similarly, the reduced ALT and the elevated alkaline phosphatase activity in the FCA+FIA group were also attributed to the brain inflammatory situation persist in the present investigation [45,46].

CONCLUSION

In this present study, reduced ACP activity in DS treatment and unaltered in FCA+FIA group is taken as no vertical imbalance in the anti-oxidative and lipid peroxidation system. The reduced ACP activity in DS group below Control rats may implicate upon corresponding toxicity in the brain oxidative metabolism and can be attributed to be active bone lesion/turnover. The increased ALP activity in the FCA+FIA group may result from the central nervous system injury. The decreased activity with DS treatment can be taken as oxidative stress. Since, variable metabolic pathways will lead to region-specific accumulation of oxidative damage in different regions of the CNS and can cause specific brain regions to become more vulnerable to drug toxicity. In this present study the LDH activity was found reduced with diclofenac treatment and was unaltered in FCA+FIA group. The reduced LDH activity in diclofenac is positively implicated upon its role on the anti-inflammatory activity as discussed. The elevated AST and ALT in DS treatment is positively correlated to the glutamate levels in the brain region and may be associated with the increased glucose metabolism especially in the bilateral frontal, parietal, and temporal lobes. The decreased levels of ALT found with FCA+FIA treatment may implicate upon greater amyloid-β deposition and stress situation in the brain region. Similarly, the reduced ALT and the elevated alkaline phosphatase activity in the FCA+FIA group is also attributed to the brain inflammatory situation persist in the present investigation.

AUTHOR CONTRIBUTION STATEMENT

SS conceived, designed research, conducted experiments, analyzed data, wrote the manuscript and Institute central Laboratory has contributed reagents and analytical tools. The author has read and approved the manuscript.

COMPLIANCE WITH ETHICAL STANDARDS

All applicable international, national, and institutional guidelines for the care and use of animals were followed.

CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

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