INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

EFFECTS OF INTRAMUSCULAR DICLOFENAC USE ON LIPID PEROXIDATION AND SKELETAL MUSCLE HISTOLOGY IN BALB-C MICE

Sushma Sharma and Archana Thakur *
Department of Biosciences, Himachal Pradesh University, Summer Hill, Shimla, India
*Corresponding Author Email: archanathakur@live.in

Article Received on: 13/12/16 Revised on: 23/12/16 Approved for publication: 12/01/17

DOI: 10.7897/2230-8407.08014

ABSTRACT

Diclofenac is a nonsteroidal anti-inflammatory drug that is widely used for the treatment of musculoskeletal complaints, osteoarthritis, rheumatoid arthritis, ankylosing spondylitis and acute muscle pain conditions. There is considerable interest in the toxicity of diclofenac because of its clinical uses. In the present study, the sub-chronic administration of diclofenac (10 mg/kg body weight; 30 days) resulted in various changes in activity of SOD enzyme (a marker of oxidative stress) and lipid peroxidation levels of mice. Changes in the activity of enzyme represent adaptive responses in muscle after diclofenac treatment. Results show that diclofenac is a strong inducer of oxidative stress. Increase in the formation of thiobarbituric acid reactive species (TBARS) and SOD activity is observed which indicates a link between oxidative stress and muscular toxicity. Maximum increase is seen in drug treated mice at 30 days' stage of investigation.

Key words: Diclofenac, SOD, lipid peroxidation

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed categories of drugs worldwide in the treatment of pain and in the treatment of pain and inflammation in many conditions. Diclofenac sodium is a phenylacetate nonsteroidal anti-inflammatory agent[1]. Diclofenac sodium interferes with the action of cyclooxygenase (COX), which is an enzyme that converts arachidonic acid into prostaglandins, thromboxanes and prostacyclins [2,3]. Thus diclofenac inhibits prostaglandin biosynthesis, also reduces leukotriene formation, both of which contribute to its anti-inflammatory activity [4]. The inhibitory action of diclofenac sodium is the main mechanism responsible for both the efficiency and the adverse side effects of diclofenac.

MATERIALS AND METHODS

Chemicals

Diclofenac sodium was purchased from Sigma Aldrich Co., USA. All other chemicals used were of analytical grade.

Experimental Design

Healthy male mice of Balb-c strain (8-10 weeks old), weighing approximately 25-30g were procured from the Central Research Institute (CRI) Kasauli, Himachal Pradesh, India. Mice were housed in standard cages and provided with standard feed (Hindustan Levers Ltd. India) and water *ad libitum.* They were maintained by providing 16 hr day light at 24º±2º in the animal house of the Department of Bioscience under appropriate conditions as approved by Institutional Animal Ethics Committee of Himachal Pradesh University (IAEC approval no: IAEC/BIO/8-2009)

Normal healthy looking mice showing no sign of morbidity were divided into following groups:

a) Mice in the first group (Group I) comprised of age matched control mice.

b) Mice in the second group (Group II) were subjected to intramuscular administration of diclofenac (10mg/kg body wt.) for 10, 20 and 30 days.

All the mice were weighed at the start of the experiment as well as at the end of each investigation stage. The animals were sacrificed by cervical dislocation and collection of lattissimus dorsi muscle was made immediately after the scarification. The excised muscle was weighed and employed for histological and biochemical studies.

Histological studies

Histological studies were carried out using the standard technique of hematoxylin and eosin staining. Muscle tissues were fixed in aqueous Bouin's fixative and dehydrated by passing ascending grades of alcohol through them, clearing them in xylene, and finally embedding them in paraffin wax (mp 58–60ºC). Transverse sections of 5-µm thickness were cut on a rotary microtome. These sections were stained with haematoxylin and eosin in alcohol, dehydrated in alcohol, cleared in xylene, mounted in DPX, and examined microscopically.

Biochemical assay

The protein concentration of the samples was determined using bovine serum albumin as a standard [5].

Measurement of Malondialdehyde

Muscle lipid peroxidation product such as malondialdehyde (MDA) was determined by the method of Dhindsa et al [6]. Tissue was homogenized in 2 ml of 0.1% TCA was then centrifuged at 6000 rpm for 15 minutes. To the supernatant, 2 ml of 0.5% TBA prepared in 10% TCA was added. The test tubes were then cooled in ice-cold water bath and then centrifuged again. Absorbance of the supernatant was recorded in a HITACHI Spectrophotometer (VSU-2 model 150). MDA contents formed were calculated in n moles/ml.

Measurement of Superoxide Dismutase Activity

Assays of superoxide dismutase (SOD) activity in tissue was determined by the method of Misra and Fridovich [7] by monitoring an autoxidation of epinephrine. The optical absorbance was measured at wave length 490 nm against blank reagent.

Statistical analysis

The results were obtained as mean ± SEM. The differences between control and treated groups of mice were obtained significant at $*p < 0.05$ by using student's 't' test [8].

RESULTS

Body and organ weight changes

Body Weight: At the beginning of the experiment, the average body weight of the animals was 24.33 ± 0.71g. A slight increase in the body weight of control mice is observed throughout the period of study. The body weight of diclofenac treated mice decreased to 21.33 ± 0.7 g after 30 days.

Organ Weight: Figure 2 shows the effect of diclofenac on lattissimus dorsi muscle.

Histopathological study

Control (Group I): Normal muscle section reveals circular, oval or polygonal cells with sub sarcolemmal disposition of nuclei (Figure a).

Treated (Group II): At 10 Days stage clumping of nuclei is observed which form a long streak in the interfibrillar spaces (Figure b). At 20 days' stage, large interfascicular spaces alongwith splitting of fibers are depicted (Figure c). At 30 days' stage diclofenac sodium treated muscle shows variable shapes like polygonal and elongated fibers. Muscle hypertrophy with degenerating connective tissue is observed (Figure d).

Biochemical study

Intramuscular administration of diclofenac sodium induced a significant ($*p < 0.05$) increase of lipid peroxidation in mice skeletal muscle after 10, 20 and 30 days' stages. Muscle from control mice at 10, 20, 30 days' stages maintained MDA level of 24.9 ± 0.76 n moles/g, 25 ± 0.47 n moles/g and 26.63 ± 1.55 n moles/g of fresh tissue weight respectively (Figure 4). A significant ($*p < 0.05$) percentage increase in the formation of thiobarbituric acid reactive species (TBARS) is observed in the skeletal muscle. This percentage increase is maximum in drug treated mice at 30 days' stage of investigation. The activity of antioxidant enzyme SOD in muscle homogenate of mice was increased by 31.25%, 60.27% and 98.61% respectively (Figure 5). These results demonstrated that the diclofenac toxicity is due to lipid peroxidation and impairment of the antioxidant systems in muscle.

Table 1: Body weight and brain weight of normal mice and treated mice with diclofenac sodium after 10 to 30 days' period

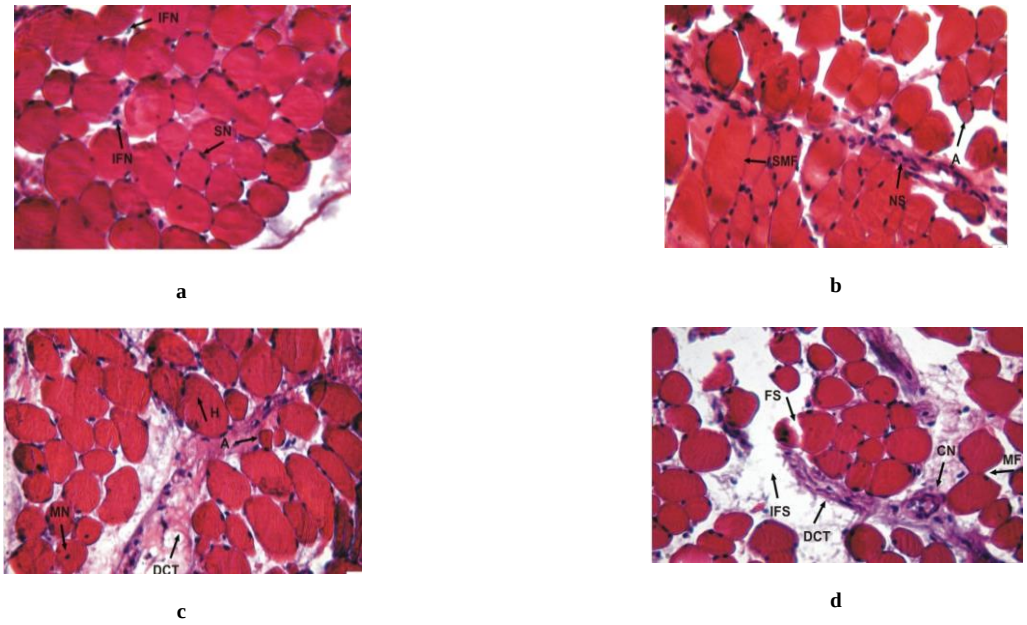
	Days		
	10	**20**	**30**
Body weight (g) Normal	24.33±0.71	24.66±0.54	25±0.46
Body weight (g) Treated	21.00±1.24	19.67±1.27*	21.33±0.72*
% decrease	-13.69	-20.24	-14.68
Muscle weight (mg) Normal	56.73±0.67	58.90±0.94	58.40±1.67
Muscle weight(mg) Treated	52.90±1.84	48.86±1.53*	54.06±2.08*
% decrease	-6.75	-17.05	-7.44

Values are mean ± SEM; n = 3 ($*p < 0.05$).

Table 2: Lipid peroxidation and SOD activity in normal mice and diclofenac sodium treated mice after 10 to 30 days' period

	Days		
	10	**20**	**30**
Lipid Peroxidation (n mole MDA /g of tissue) Normal	24.90±0.76	25.00±0.47	26.63±1.55
Lipid Peroxidation (n mole MDA /g of tissue) Treated	49.18±0.47*	60.23±0.93*	99.43±3.11*
% increase	97.51	140.92	273.37
SOD (units/mg tissues) Normal	7.23±0.4	7.25±0.13	7.20±0.12
SOD (units/mg tissues) Treated	9.49±0.12*	11.62±0.25*	14.30±0.11*
% increase	31.25	60.27	98.61

Values are mean ± SEM; n = 3 ($*p < 0.05$)



a b c d

Figure 1: Fig.a: T.S. of haematoxylin-eosin stained normal anterior latissimus dorsi (ALD) muscle of mice at 10 days' stage showing cells with circular, oval shapes. Sarcolemmal disposition of nuclei can be identified (SN). Many interfibrillar nuclei can be observed (IFN) (x 400). Fig.b: T.S. of anterior latissimus dorsi muscle of diclofenac treated mice at 10 days' stage showing long streaks of nuclei (NS) in the interfibrillar spaces. Muscle atrophy (A) and spindle shaped muscle fibers are also observed (SMF) (x 400). Fig.c: T.S. of anterior latissimus dorsi muscle of diclofenac treated mice at 20 days' stage revealing atrophied (A) fibers. Degenerating connective tissue (DCT) is also noticed. Migration of nuclei from subsarcolemmal position to center of fiber is observed (MN). Hypertrophied (H) and elongated fibers are also seen (x 400). Fig.d: T.S. of anterior latissimus dorsi muscle of diclofenac treated mice at 30 days' stage exhibiting clumping of nuclei in the interfascicular space (IFS). Degenerating connective tissue (DCT) and merging of fibers is observed (MF). Large interfascicular spaces are depicted (IFS). Splitting of fiber is seen (FS) (x 400).

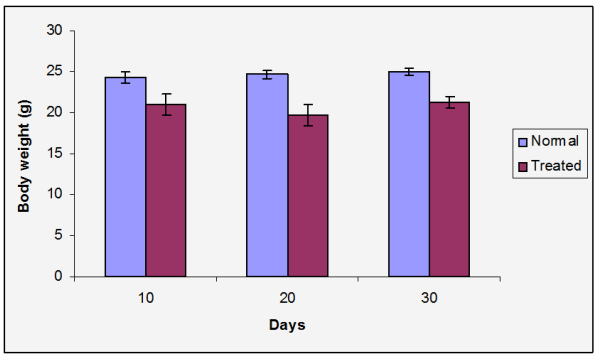


Figure 2: Changes in body weight (g) of normal and diclofenac sodium treated mice after 10-30 days' period. Values are mean ± SEM; n = 3 (*p < 0.05)

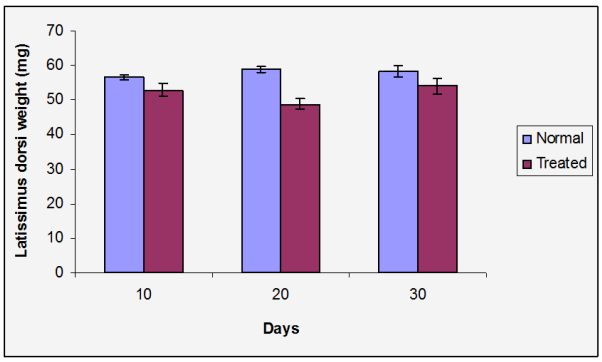


Figure 3: Changes in anterior latissimus dorsi weight (mg) of normal and diclofenac sodium treated mice after 10-30 days' period. Values are mean ± SEM; n = 3 (*p < 0.05)

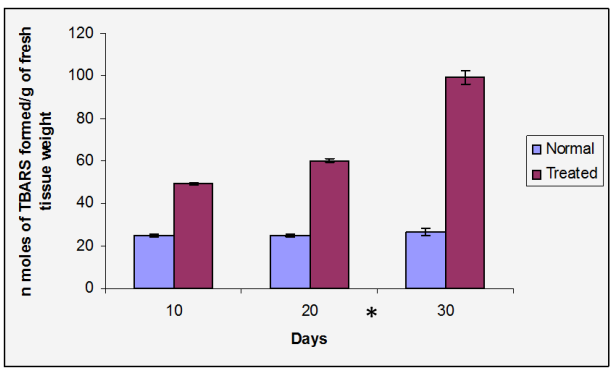


Figure 4: Lipid peroxide (n moles of TBARS formed/g of fresh tissue weight) in anterior latissimus dorsi of normal and diclofenac treated mice after 10-30 days' period. Values are mean ± SEM; n = 3 (*p < 0.05)

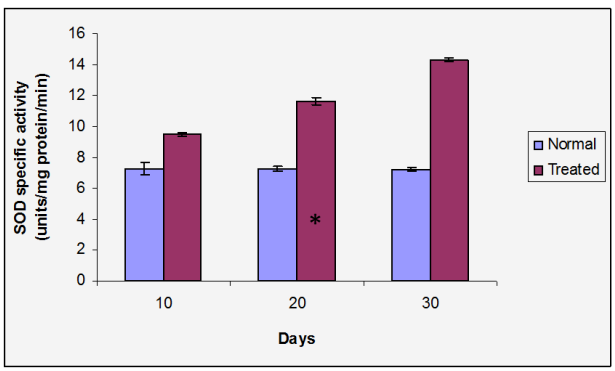


Figure 5: Superoxide dismutase specific activity (units/mg protein/min) of normal and diclofenac sodium treated anterior latissimus dorsi muscle after 10-30 days' period. Values are mean ± SEM; n = 3 (*p < 0.05)

DISCUSSION

NSAIDs are a disparate group of weakly acidic, highly protein bound compounds having the common pharmacological property of inhibiting prostaglandin biosynthesis[9]**.** The present study has convincingly demonstrated that diclofenac sodium treatment to normal mice results in loss of body weight and muscle weight. Many past studies have also confirmed diclofenac induced body weight loss in rats [10,11]. Many other NSAIDs also suppress the weight gain of mice resulting decrease in body weight[12]. Other mechanisms which cause reduction in muscle mass and muscle strength include physical inactivity, oxidative stress and chronic inflammation. Most of the non-steroidal anti-inflammatory drug commonly used by elderly, accelerate sarcopenia [13]. Studies conducted in young, healthy persons demonstrated that NSAIDs tend to blunt the protein synthesis response that naturally occurs after eccentric exercise via the inhibition of a prostaglandin signal [14].

SOD is an endogenously produced intracellular enzyme which catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide which are produced by polymorphonuclear leukocytes when they ingest bacteria or immune complexes. Diclofenac sodium acts as a mitochondrial toxin and significantly increases the intracellular reactive oxygen species[15]. Increase in SOD activity is an indicator of the oxidative stress in tissues of drug treated mice and is required for protection against free radicals. This antioxidant enzyme acts directly or indirectly to remove reactive oxygen species and thus leading to elevation in SOD level. Recently [16] also showed that total antioxidant substances and superoxide dismutase are increased in diclofenac treated mice.

A significant increase in thiobarbituric acid species (TBARS) is observed in brain of diclofenac sodium treated mice at all stages of investigation. NSAIDs cause mitochondrial injury by dissipating the mitochondrial transmembrane potential and inducing mitochondrial permeability transition pore, which liberates cytochrome C. This enzyme generates reactive oxygen species which trigger the cellular lipid peroxidation, resulting in cellular apoptosis [17]. Earlier findings have also suggested that NSAIDs raise the MDA level with respect to the control value representing their significant lipid peroxidation activity [18]. A significant increase in the MDA level after diclofenac treatment is observed by Ismail et al [19]. Our findings are in accordance with many previous studies which have clearly demonstrated that acute NSAID overdose increases the lipid peroxidation in renal tissue [20].

CONCLUSION

The present study has clearly demonstrated altered structure of skeletomuscular tissue due to diclofenac sodium treatment. After so many years of use, the effects of diclofenac sodium are well known, but some of the unwanted effects are now emerging or being defined. Therefore, a safe alternative to the diclofenac sodium is needed which can be used for long term treatment.

REFERENCES

1. Villarino NF, Vispo TJ, Marcos F, Landoni MF. Inefficacy of topical diclofenac in arthritic horses. American Journal Of Animal And Veterinary Sciences.2006; 1 (1): 8-12.
2. Mitchell JA, Warner TD. Cyclooxygenase-2: pharmacology, physiology, biochemistry and relevance to NSAID therapy. British Journal Of Pharmacology. 1999; 128: 1121-1132.
3. Raksha B, Ganesh B, Aditya S, Shruti B, Mithra NH. Evaluation of the efficiency of intraligamentary diclofenac sodium in reducing postoperative endodontic pain in patients with irreversible pulpitis. Int. Res. J. Pharm. 2013; 4(3): 237-239.
4. Zorica P, Milena P, Milanka J. Pharmacokinetics of diclofenac in pigs after intramuscular administration of a single dose. Acta Veterinaria (Beograd). 2006; 56: 323-331.
5. Lowry OH, Rosenbrough MJ, Farr AL and Randall RJ. Protein measurement with folin phenol reagent. The Journal Of Biological Chemistry.1951; 1993: 265-275.
6. Dhindsa RS, Dhindsa PP, Thorpe, TA. Leaf senescence correlated with increased level of membrane permeability and lipid peroxidation and decrease level of SOD and catalase. Journal Of Experimental Botany. 1981; 32 (126): 93.
7. Misra, HP, Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. The Journal Of Biological Chemistry. 1972; 25: 3170- 3175.
8. Pearson E and Hartley H. Table 12 in Biometrika Tables For Statisticians (Pearson E & Hartley H, eds), Vol.1, Cambridge University Press.1960.
9. Smith WL, Meade EA, deWith DL. Interactions of PGH synthase isozymes-1 and 2 with NSAIDs. Annals of The New York Academy of Sciences. 1994; 744: 50-57.
10. Kim H, Xu M, Lin Y, Cousins JJ, Eckstein RP, Jordan V, Power I, Mather LE. Renal Dysfunction associated with the periopertive use of diclofenac. Anaesthesia & Analgesia. 1999; 89: 999-1005.
11. Besen A, Kose F, Paydas S, Gonlusen G, Inal T, Dogan A, Kibar M, Balal M. The effects of the nonsteroidal anti-inflammatory drug diclofenac sodium on the rat kidney and alteration by furosemide. International Urology and Nephrology. 2008; 41: 919-926.
12. Tugendreich S, Pearson CI, Sagartz J, Jarnagin K, Kolaja K. NSAID induced acute phase response is due to increased intestinal permeability and characterized by early and consistent alterations in hepatic gene expression. Toxicologic Pathology. 2006; 34 (2): 168-179.
13. Schrager M, Bandinelli S, Maggi S, Ferrucci L. Sarcopenia: Twenty Open Questions for a research agenda. Basic Appl Myol. 2003; 13 (4): 203-208.
14. Trappe TA, Fluckey JD, White F, Lambert CP, Evans WJ. Skeletal muscle PGF_2 □ and PGE_2 in response to eccentric resistance exercise: influence of ibuprofen and acetaminophen. The Journal Of Clinical Endocrinology & Metabolism. 2001; 86: 5067-6070.
15. Milusheva E, Baranyi M, Kittel A, Fekete A, Zelles T, Vizi ES, Sperlagh B. Modulation of dopaminergic neurotransmission in rat striatum upon *in vitro* and *in vivo* diclofenac treatment. Journal of Neurochemistry. 2008; 105 (2): 360-368.
16. Curcelli EC, Mullar SS, Novelli Filho JLVB. Beneficial effects of diclofenac therapy on serum lipids, oxidized low density lipoprotein and antioxidant defenses in rats. Life Sciences. 2008; 82 (15-16): 892-898.
17. Nagano Y, Matsui H, Muramatsu M, Shimokawa O, Shibahara T, Yanaka A, Nakahara A, Matsuzakai Y, Tanaka N, Nakamura Y. Rebamipide significantly inhibits indomethacin induced mitrochondrial damage, lipid peroxidation and apoptosis in gastric epithelial RGM-1 cells. Digestive Diseases And Sciences. 2005; 50: S76-S83.
18. Chakraborty S, Roy K, Sengupta C.Exploring effects of different nonsteroidal antiinflammatory drugs on lipid peroxidation. Part II. 4-HNE profile. Acta Poloniac Pharmaceutica Drug Research. 2007; 64 (3): 211-216.
19. Ismail MF, Shohda A, Maraghy EL, Sadik NAH. Study of the immunomodultatory and anti-inflammatory effects of

evening primrose oil in adjusvant arthritis. African Journal Of Biochemistry Research. 2008; 2 (3):74-80.
20. Abdel-Zaher OA, Abdel-Rahman MM, Hafez MM, Omran FM. Role of nitric oxide and reduced glutathione in the protective effects of aminoguanidine, gadolinium chloride and oleanolic acid against APAP-induced hepatic and renal damage. Toxicology. 2007; 243: 124-134.

Cite this article as:

Sushma Sharma and Archana Thakur. Effects of intramuscular diclofenac use on lipid peroxidation and skeletal muscle histology in Balb-C mice. Int. Res. J. Pharm. 2017;8(1):20-24 http://dx.doi.org/10.7897/2230-8407.08014

Source of support: Nil, Conflict of interest: None Declared