Research Article

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

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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. Diclofenac sodium interferes with the action of cyclooxygenase (COX), which is an enzyme that converts arachidonic acid into prostaglandins, thromboxanes and prostacyclins. Thus diclofenac inhibits prostaglandin biosynthesis, also reduces leukotriene formation, both of which contribute to its anti-inflammatory activity. 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 25-30 g 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.
Measurement of Malondialdehyde

Muscle lipid peroxidation product such as malondialdehyde (MDA) was determined by the method of Dhinda et al. Tissue was homogenized in 2 ml of 0.1% TCA and 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 measured 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 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.

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. 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 demonstrate 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

<table>
<thead>
<tr>
<th>Days</th>
<th>Body weight (g) Normal</th>
<th>Body weight (g) Treated</th>
<th>% decrease</th>
<th>Muscle weight (mg) Normal</th>
<th>Muscle weight (mg) Treated</th>
<th>% decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>24.33±0.71</td>
<td>21.00±1.24</td>
<td>-13.69</td>
<td>56.73±0.67</td>
<td>52.90±1.84</td>
<td>-6.75</td>
</tr>
<tr>
<td>20</td>
<td>24.66±0.54</td>
<td>19.67±1.27*</td>
<td>-20.24</td>
<td>58.90±0.94</td>
<td>48.86±1.53*</td>
<td>-17.05</td>
</tr>
<tr>
<td>30</td>
<td>25±0.46</td>
<td>21.34±0.72*</td>
<td>-14.68</td>
<td>58.40±1.67</td>
<td>54.06±2.08*</td>
<td>-7.44</td>
</tr>
</tbody>
</table>

% decrease: - = decrease in weight; *p < 0.05.

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

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

% increase: * = increase; *p < 0.05.
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 ± SE (x 400) M; 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)
NSAIDs are a disparate group of weakly acidic, highly protein bound compounds having the common pharmacological property of inhibiting prostaglandin biosynthesis16. 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 weight12. 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 the superoxide anion to 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 species15. 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 thiorbarbituric 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 skeletonmuscular 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

19. Ismail MF, Shohda A, Maraghy EL, Sadik NAH. Study of the immunomodulatory and anti-inflammatory effects of...


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

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