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ORIGINAL ARTICLE

The Role of Ketoprofen and Diclofenac in Dexamethasone-Immunosuppressed Mice

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ABSTRACT

Objectives*: Non steroidal anti-inflammatory drugs (NSAIDs) are commonly used in market to treat inflammatory diseases by inhibiting cyclooxygenase (COX), the rate-limiting enzyme in prostaglandins (PGs) synthesis. There are constitutive expression of COX-1 in most cells and inducible expression of COX-2 at inflammatory sites. In recent years NSAIDs like ketoprofen and diclofenac begin to show an immunomodulatory activity predicting its promising use in the treatment of autoimmune disorders. In the present study, we investigate the role of both ketoprofen and diclofenac in dexamethasone-immunosuppressed rats according to some haematological parametes like white blood cells (WBCs) count and neutrophil lymphocyte count ratio (NLCR).* ***Methodology:****Four groups of experimental rats were used in this study, G1 was used as control, G2 was immunosuppressed by dexamethasone for 3 days, G3 and G4 were immunosuppressed by dexamethasone for 3 days followed by administration of ketoprofen and diclofenac potassium respectively for 18 days. WBCs and NLCR were used as indicators of immune system activity.* ***Results:*** *Ketoprofen was proved to have an immunosuppressive role and diclofenac potassium was suggested to cause immunomodulation.* ***Conclusion:*** *Based on our findings NLCR was suggested to be predictor of immune system activity and ketoprofen and diclofenac as commonly used NSAIDs may have an important role in the treatment of autoimmune disorders in the future.*

INTRODUCTION

The immune system is a system of biological structures and processes within an organism that protects against disease. Disorders of the immune system can result in autoimmune diseases, inflammatory diseases and cancer [1,2].

Immunodeficiency occurs when the immune system is less active than normal, resulting in recurring and life-threatening infections. In humans, immunodeficiency can either be the result of a genetic disease such as severely combined immunodeficiency, acquired conditions such as HIV/AIDS, or the use of immunosuppressive medication. Immunosuppressive drugs comprise a large number of drugs that by different mechanisms of action can modulate the immune system[3,4].

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Glucocorticoids are the most commonly used drugs, and are widely used for the management of inflammatory diseases. These drugs inhibit various immune functions by affecting gene transcription events[5,6].

They mediate their actions by binding to intracellular receptors, resulting in altered protein-protein interactions and consequently regulation of gene expressions [7]. They are used to control the inflammatory response in many human diseases. Inhibition of phospholipases1[8] and inhibition of transcription of various cytokines2 have been reported to be major mechanisms for this anti-inflammatory action[9]. Dexamethasone is used to treat many inflammatory and autoimmune conditions, such as rheumatoid arthritis and bronchospasm [10].

NSAIDs inhibit COX, the rate-limiting enzyme for the synthesis of prostaglandins (PGs)[11,12]. Two COX iso forms have been identified in eukaryotic cells, COX-1 and COX-2. COX-1 is constitutively expressed in most cells, while COX-2 is inducibly expressed in a more limited array of cells at inflammatory sites[13,14]. There is

growing evidence showing that NSAIDs may have immunomodulatory activities not apparently related to the inhibition of prostaglandin synthesis. NSAIDs were shown to inhibit T cell proliferation, expression of activation-related molecules such as CD25 and CD71, and the production of cytokines such as IL-2, IFN-γ and TNFα[15,16]. NSAIDs may differentially exert immunomodulatory effects on activated macrophages and lymphocytes at clinically available doses, regulating immune responses such as TNF-α release and nitric oxide (NO) production, cell-cell adhesion, phagocytic uptake, and lymphocyte proliferation [17].

In support of the immunomodulatory activity of NSAIDs on lymphocytes, it has been shown that COX-1 is constitutively expressed on T cells, whereas the expression of COX-2 is inducibly up-regulated in T cells upon stimulation [16, 18].

Ketoprofen belongs to propionic acid class of NSAIDs with analgesic, anti-inflammatory and antipyretic effects [19]. It is most often prescribed for muscle pain, tissue injury, joints pain and for laminitis[20]. Ketoprofen acts by inhibiting the body's production of PGs which are lipid derived autacoids that modulate many physiological systems including the central nervous system, cardiovascular, gastrointestinal, genitourinary, endocrine, respiratory, and immune systems [21].

It was demonstrated that repeated administration of ketoprofen could further modulate the inflammatory responses (acute-phase proteins) associated with castration[22]. Diclofenac is a non steroidal anti-inflammatory drug belongs to phenyl acetic acid group. Diclofenac sodium has been used in human medicine for many years for the long term symptomatic treatment of rheumatoid arthritis, osteoarthritis, and soft tissue inflammation / injuries. It may also be useful for short term treatment of acute musculo-skeletal injury and dysmenorrheal pain [23, 24].

In the immune system, cytokines and PGs are produced by antigen presenting cells such as macrophages and dendritic cells. Cytokines and PGs regulate immune responses[25]. Produced by many cell types, PGE2 has been shown to affect various aspects of the immune and inflammatory responses by acting on all components of the immune system[26]. Targeted inhibition of cyclo-oxygenase, or more likely specific antagonism of individual prostaglandins or their receptors (or both), may thus provide an immunomodulatory therapeutic target in certain patient populations. Although stratified prospective trials would be needed to verify this potential, these old drugs may yet possess new and unexpected tricks [27].

In recent years, several publications in different fields have focused on changes in the differential blood count during infectious and other diseases. The neutrophil lymphocyte count ratio (NLCR) is proposed as a parameter of systemic inflammation and stress in severely ill patients in intensive care unit [28].

In the present study, we examined the immunomodulatory effects of the therapeutic doses of ketoprofen and diclofenac potassium on the immune system following administration of immunosuppressive drug like dexamethazone.

METHODOLOGY

Experimental animals

Male wistar mice and rats, in-house bred were used for the study. The animals were cared for and used in accordance with the Institute of Laboratory Animal Research (ILAR) guidelines for care use of animals in experimental studies [29]. The animals were acclimatized to laboratory conditions for 10 days, and kept in cages under standard laboratory conditions (light period of 12 hours per day) and temperature of (27±2)^{o}C. They were fed with pelleted feed and had free access to water.

Experimental immunosuppressant

Dexamethasone sodium phosphate injection (Dexamethazone®; Eipico,Egypt) was used to induce immunosuppression. Each millilitre of Dexamethazone® was labelled to contain 4mg of dexamethasone sodium phosphate [30].

Immunomodulatory activity studies

Twenty four male wistar rats were divided into four groups of six animals per group. Group 1 (G1) animals served as the control group, and were administered with food and water only. Group 2 (G2) animals were treated with dexamethasone 5 mg/kg intraperitonially (Amriya Pharmaceutical Industries, Egypt) twice daily for three days. Group 3 (G3) animals were treated with dexamethasone 5 mg/kg intraperitonially, twice daily for three days followed by Ketoprofen (Profenid®;Sanofi Aventis; Egypt) (2.9mg/kg) intraperitonially on days 4 to 21. Group 4 (G4) animals were treated with dexamethasone 5mg/kg intraperitonially, twice daily for three days, followed by Diclofenac potassium (Cataflam®; Novartis Pharma; Egypt) (2mg/kg) intraperitonially on days 4 to 21 [30].

Haematological analysis

On the 22nd day, blood was collected by retro-orbital puncture of each rat into EDTA and lithium-heparinised bottles for haematological analysis. Haematological parameters such as white blood cells count, neutrophils and lymphocytes percentages were parameters analysed using the Sysmex autohaematology analyser [30].

Statistical analysis

GraphPad Prism version 5.0 for Windows was used for all statistical analyses. Data were presented as mean ± SD and analyzed by one-way ANOVA. Analysis at $p \leq 0.05$ showed no statistical significant difference in all analyse.

RESULTS

Table 1: Effect of ketoprofen on dexamethasone-immunosuppressed rat

Parameters	Groups			P-value
	G1	G2	G3	
WBCs (x10^9 / L)	9.13±2.04	15.4±0.75	16.72±5.08	> 0.05
NEU (%)	33.5±3.82	14.0±2.95	26.67±7.36	< 0.0001
LYM (%)	70.3±3.35	36.7±4.51	73.33±7.18	< 0.0001
NLCR	0.476	0.382	0.364	

WBCs: white blood cells, NEU: neutrophils, LYM: lymphocytes, NLCR: neutrophil lymphocyte count ratio, G1: control, G2: dexamethasone-immunosuppressed rats, G3: rats given dexamethasone followed by ketoprofen, values are expressed in mean ±SD, p-value ≤0.05 compared against control group.

Table 2: Effect of diclofenac on dexamethasone-immunosuppressed rat.

Parameters	Groups			P-value
	G1	G2	G4	
WBCs (x10^9 / L)	9.13±2.04	15.4±0.75	8.75±3.82	< 0.0001
NEU (%)	33.5±3.82	14.0±2.95	41.8±2.11	< 0.0001
LYM (%)	70.3±3.35	36.7±4.51	57.5±1.71	< 0.0001
NLCR	0.476	0.382	0.728	

WBCs: white blood cells, NEU: neutrophils, LYM: lymphocytes, NLCR: neutrophil lymphocyte count ratio, G1: control, G2: dexamethasone-immunosuppressed rats, G4: rats given dexamethasone followed by diclofenac, values are expressed in mean ±SD, p-value ≤0.05 compared against control group.

Dexamethasone suppressed the immunity of tested rats and this was suggested by the high WBCs count and the very low NLCR of dexamethasone-treated group. Both ketoprofen and diclofenac proved to have an immunosuppressive and immunomodulatory roles respectively in dexamethasone-immunosuppressed rats (Tables 1 and 2).

DISCUSSION

As demonstrated from the present work, immunosuppressive role of dexamethasone is suggested from high WBCs count (15.4±0.75) x 10^9 / L and low NLCR level (0.382). Dexamethasone lowers the activity of immune system resulting in high susceptibility of infection and increase in leukocytes. Ketoprofen is proved to have an immunosuppressive role recording lower NLCR value (0.364) and higher WBCs count (16.7±5.08)x10^9/L compared to group2 (G2). Diclofenac is suggested to have an immunomodulatory role by stimulating the immune system after suppression by dexamethasone showing higher NLCR value (0.728) compared to group2 (G2) and normal WBCs count (8.75±3.82) x 10^9/L.

NLCR is used as an indicator of immune system activity and dexamethasone is administrated to suppress the immunity [30]. The predictive values of blood count alterations during malaria are investigated illustrating the role of NLCR as a better predictor for severe malaria especially in semi-immune patients [31]. NLCR is a better predictor of bacteremia than routine parameters like C-reactive protein (CRP) level[32].

Prolonged administration of high doses of NSAIDs may impair the dendritic cells (DCs) capability to present Ags in association with MHC molecules, as aspirin and ibuprofen inhibit the intracellular processing event of phagocytosed Ags [33].

Chemotactic response of polymorphonuclear cells (PMN) is inhibited by different NSAIDs. Capacity of NSAIDs to block the chemotactic activity of substance P which is present in synovial fluid control their effect on progress of arthritic disease [34].

Ketoprofen is an immunosuppressive agent that may be used in autoimmune disorders as it can suppress the Ab-mediated immune response by suppressing the ability of an individual cell to produce Abs [35]. Ketoprofen has an immunosuppressive effect on cell-mediated immunity supporting its use in allografts or autoimmune diseases[36].

Alterations in level of interlukins, tumor necrosis factor (TNF-α) and interferon gamma (IFN-γ) are observed after administration of a single dose of aspirin in rats [37].

NSAIDs may modulate the activation of microglial cells suggesting they can control brail inflammation. Microglial cells are target for the activity of NSAIDs in the brain as they are macrophages of brain parenchyma that produce PGs (prostaglandins) [38,39].

Significant decrease in serum anti-SRBCs antibody titer compared to control is observed in ketoprofen-treated mice. Mortality ratio is increased significantly. Decrease in the weight of immune organs like spleen and thymus and mild medullary atrophy of thymus are recorded. Ketoprofen, therefore is considered to have an immunosuppressive effect on humoral immunity [40].

Diclofenac may be used as an anti-inflammatory in major surgery as it lowers leucocyte countand CRP concentration and temperature, suggesting its modulatory role [41].

An increase in interlukin-6 levels after 24hr. treatment with diclofenac and indomethacin is observed[42]**.** Antiinflammatory cytokines (IL-10) are improved by diclofenac suggesting the positive contribution of diclofenac in the cytokine production in astrocyte cell culture [43]. Immunomodulatory effects of NSAIDs on activated macrophages and lymphocytes are illustrated and some of these effects may potentiate anti-inflammatory action of NSAIDs[44]. Kv1.3 voltage-dependent potassium channels are targeted by diclofenac resulting in immunomodulation[45]. An inhibitory effect on thymocyte Kv1.3 - channel currents is recorded by diclofenac Na^+, salicylates and indomethacin [46].

Based on all mentioned findings there is an agreement with our study supporting the immunosuppressive role of ketoprofen, predicting the possible immunomodulatory effect of diclofenac and illustrating the promising role of NLCR in predicting infectious disease and indicating immune system activity.

CONCLUSION

Commonly used NSAIDs like ketoprofen and diclofenac could be used in the treatment of autoimmune disorders in the future.

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