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Full length research paper

A comparative study of cyclo-oxygenase inhibitors on immune response with special reference to Cox-2 inhibitors

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The object of this study is to observe the *in vivo* effect of cyclo- oxygenase-2 (Cox-2) inhibitors on the humoral response after immunization of rabbits with *Salmonella typhi* antigen. Albino rabbits of either sex were divided into five groups of six animals each and were administered aspirin (100 mg/kg, (BD), p.o), celecoxib (30 mg/kg O.D, p.o), indomethacin (12.5 mg/kg BD, p.o) and etoricoxib (17 mg/kg O.D, p.o) for seven days starting one day prior to immunization with *S. typhi* antigen (0.5 ml in each thighs), respectively. The antibody titres were measured weekly for a month using Widal agglutination test. The antibody titres in the first week were augmented in all the groups. The response was more marked in treated group as compared to control group. Later on antibody titre fell markedly in the treated groups. Selective Cox-2 inhibitors administration caused higher antibody suppression in comparison to non-selective Cox inhibitors treatment. These findings support that NSAIDs and the new Cox-2-selective drugs have an unsuspected target, the B cell, and attenuate antibody production.

Key words: Non-steroidal anti-inflammatory drugs (NSAIDS), cost, immunomodulators, antibody production.

INTRODUCTION

Prostaglandins (PGE_1 and PGE_2) are critical mediators of inflammation that affect both humoral and cell-mediated immune response. Prostaglandins, which play a role in immuno- regulating activity, have been shown to promote antibody formation to sheep red blood cells in mice (Ishizuka et al., 1974). PGE_2 enhances antibody production and promotes type 2 immune responses (Harris et al., 2002; He et al., 2002). PGE_2 has been shown to directly promote immunoglobulin (Ig) class in B cells acting through the EP_2 and EP $_4$ PGE_2 receptors (Fedyk and Phipps, 1996). Activated T cells express cyclo-oxygenase-2 (Cox-2) (Fedyk et al., 1996; Iniguez et al.,, 1999), an inducible enzyme that catalyzes a series of reactions to generate PGs, led us to hypothesize that human B cells express Cox-2 and therefore synthesize PGs upon activation. Indeed, this hypothesis is supported by previous findings that proinflammatory signals increased Cox-2 expression and PG production in B cells. Cox-2 is the predominant isoform contributing to high levels of PGE_2 found in chronic inflammatory conditions (Fung and Kirschenbaum, 1999).

Disturbances in immune function found in several human conditions and diseases have been linked to changes in PGE mediated immunoregulation. Earlier studies have shown that either increased production of PGE or increased sensitivity to PGE results in depressed cellular immunity. Conversely, drugs which inhibit PGE production act as stimulants of cellular immune function *in vitro* and *in vivo* (Goodwin and Ceuppens, 1983).

But recently, it has been shown that PGE_2 enhances antibody production and promotes type 2 immune responses (Harri et al., 2002), whereas PGE_1 is effective in inhibiting the antibody synthesis by B cells precommitted to IgM class anti-double stranded DNA (dsDNA) antibody production, but the production of immunoglobulin G (IgG) class anti- dsDNA antibody by memory B cells present in young and aged mice is resistant to the inhibitory effects of PGE_1 (Yoshikawa et al., 1993), thus PGE_1 inhibits IgM but have no effect on IgG. PGE_2 suppressed all B-cell functions except for IgG

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Table 1. Effect of NSAIDS on antibody titres.

GROUP	7th Day	14th Day	21st Day	28th Day
Control (Normal saline)	58.3± 20.4	466.6 ± 163.3	333.3 ± 242.2	233.3 ± 81.6
Aspirin (100 mg/kg p.o)	133.3± 132.2	183.3 ± 40.3[a]	125.0 ± 61.2[b]	58.3 ± 20.4[a]
Indomethacin (12.5 mg/kg p.o)	350.0± 100.0	125.0 ± 50.0[b]	87.5 ± 25.0[b]	31.25 ± 12.5[b]
Celecoxib (30 mg/kg p.o)	383.3 ± 240.1[a,c]	183.3 ± 40.3[a]	100 ± 54.8[b]	29.2 ± 24.6[a]
Etoricoxib (17 mg/kg p.o)	550.0 ± 300.0[b,c]	62.5 ± 25.0[b,c,d]	100.0 ± 70.7[b]	37.5 ± 25.0[b]

Kruskal Wallis Test P value: 0.002, 0.000, 0.018 and 0.001. [a]P < 0.005 in comparison to control; [b]P < 0.05 in comparison to control; [c]P < 0.005 in comparison to aspirin; [d]P < 0.005 in comparison to celecoxib.

synthesis (Yoshikawa et al., 1993). Therefore, drugs inhibiting PGE_2 production will have opposite effect on B-cell. The synthesis of IgM was increased and that of IgG was decreased (Yamamot, 1996).

METHODOLOGY

The study was approved by institutional animal ethics committee. The study was conducted on healthy adult albino rabbits of either sex weighing 1000 to 1500 g. All rabbits were first screened for the presence of any *Salmonella typhi* 'O' anti-bodies. Only those rabbits which showed no sign of infection by negative Widal test or zero antibody titre were included in the study.

All rabbits were kept in specific cages in an isolated room of animal house under good hygienic conditions. Maximum precaution was taken to prevent any infection through food or water during the period of experiment. Food and tap water was given along with the diet which consisted of Gram and green vegetables. Grams were thoroughly washed and soaked in water for 24 h before administration. Similarly, fresh green vegetables were also washed thoroughly. All rabbits were observed during the times of experiments for any sign of infection.

The rabbits were divided into five groups of 6 animals each. One group serving as control was given normal saline (1 ml/kg p.o), while the other groups as test were administered aspirin (100 mg/kg, BD, p.o), celecoxib (30 mg/kg O.D, p.o), indomethacin (12.5 mg/kg BD, p.o), etoricoxib (17 mg/kg O.D, p.o) for seven days starting one day prior to immunization.

All animals were immunized by *S. typhi* 'O' antigen obtained from the Department of Microbiology of our Medical College. One ml of antigen contained 1×10^6 bacteria of which 0.5 ml was injected intramuscularly in each gluteal region once only.

Blood samples (2 ml each) were withdrawn from marginal ear vein on 1st (before inoculation), 7th, 21st and 28th days of immunization, and were titrated for antibody level against *S. Typhi* 'O' antigen by modified Widal test.

Statistical analysis

The data was compared by Kruskal-Wallis test followed by Mann Whitney U test for comparison between individual samples. Two tailed P value < 0.05 was considered as significant and P value < 0.005 was considered as highly significant.

RESULTS

The antibody titres in all the groups were found raised in the first week, but more marked in treated groups as compared to control (P < 0.005) (Figure 1). Later on antibody titres were found significantly low in the treated groups at 7th, 14th, 21st, and 28th day in comparison to control (P < 0.005). Amongst the treated group, selective Cox-2 inhibitors (etoricoxib and celecoxib) especially etoricoxib administration caused significantly higher antibody suppression in comparison to non-selective Cox inhibitor aspirin. (Table 1) (Figure 2).

However, the suppression by selective Cox-2 inhibitors was not significant in comparison to indomethacin at Day 21 and 28. (Table 1) (Figure 3). Among the selective Cox-2 inhibitors, etoricoxib is more potent in suppressing antibody production as compared to celecoxib, the suppression being significantly higher at day 14 (Table 1) (Figure 4).

DISCUSSION

Since NSAIDs have varying inhibitory effect on Cox-1 and Cox-2, it was considered worthwhile to perform comparative study of various commonly used NSAIDs, including specific Cox-2 inhibitors on immune response in animal model to make rational use of these agents in various conditions.

Our study shows that NSAIDS enhance antibody production after a week of immunization, whereas during the second and third week, antibody titres are markedly low as compared to control. This also becomes evident from earlier studies which have shown that PGE_2 suppressed all B-cell functions except for IgG synthesis (Yamamoto et al.,, 1996). Therefore drugs inhibiting prostaglandin E_2 (PGE) will have opposite effect, increasing IgM antibody production and decreasing IgG production. The results, also demonstrate the role of PGE_2 in the regulation of humoral immune responses (Goodwin and Ceuppens, 1983; Yoshikawa et al., 1993; Yamamoto et al., 1996; Betz and Fox, 1991). In the earlier studies, it has been proved that of all the arachidonic acid metabolites, only prostaglandin E (PGE) has been shown to have a clear role in the regulation of cellular and humoral immune responses. Disturbances in immune function found in several human conditions and diseases have been linked to changes in PGE mediated immunoregulation. A major role of PGE_2 in the pathogenesis of osteoarthritis has been already established

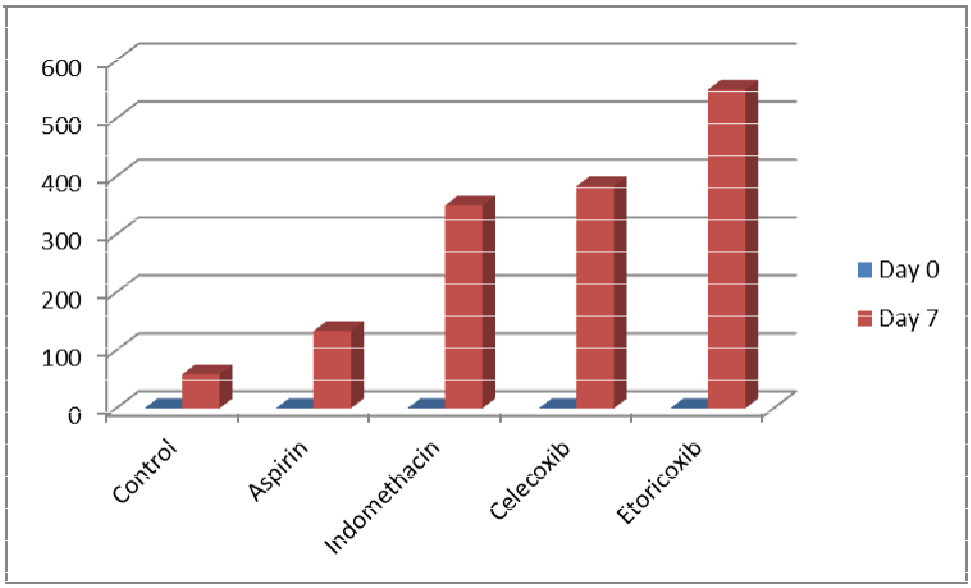

Figure 1. Effect of Nsaids on Antibody Titres after S.typhi 'O' Antigen inoculation in rabbits at Day 7.

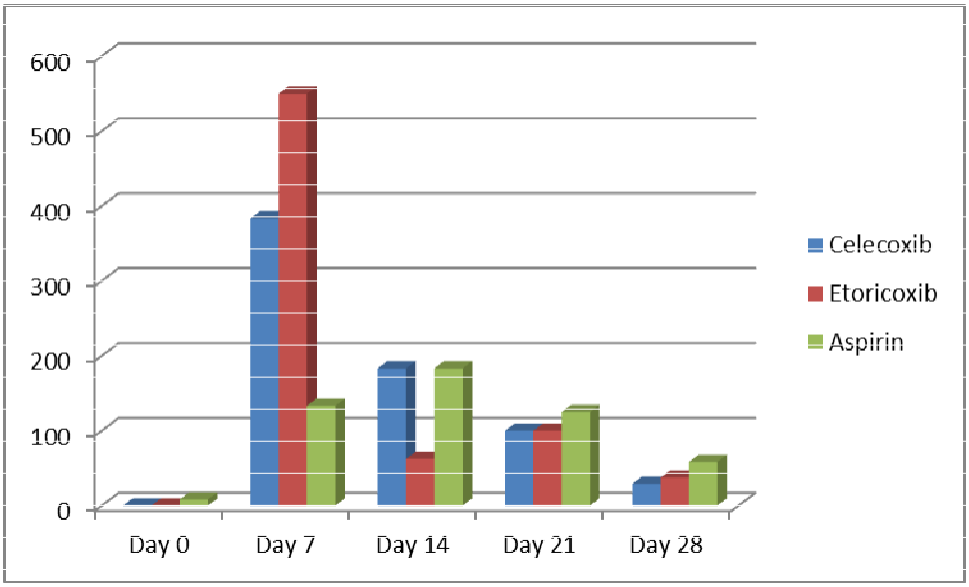

Figure 2. Effect of Selective and Non-selective Cox inhibitors on Antibody Titres after S.typhi 'O' Antigen inoculation.

which shows that chondrocytes isolated from patients with osteoarthritis produce 50-fold more PGE_2 than chondrocytes from patients without osteoarthritis (Amin et al.,, 1997; Robinson et al.,, 1975; Inoue et al.,, 2001). Interestingly, elevated Cox-2 levels have been reported in autoimmune diseases, such as systemic lupus erythematosus, where chronic inflammation persists at multiple sites in the body. This explains the clinical utility of highly selective Cox-2 inhibitors, such as celecoxib (Celebrex) and etoricoxib to reduce the pain associated with inflammation.

Conclusions

Our compelling finding of reduced antibody production by specific Cox-2 inhibitors suggests that these agents may be suppressing autoantibody production via direct effects

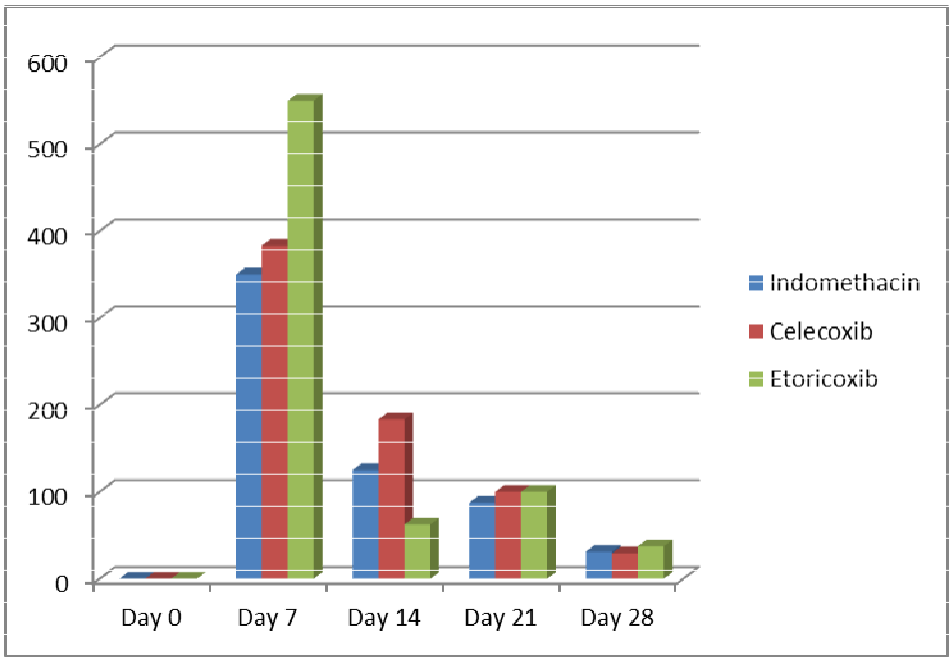

Figure 3. Effect of Indomethacin, Celecoxib and Etoricoxib on Antibody Titres after S.typhi 'O' Antigen inoculation in rabbits

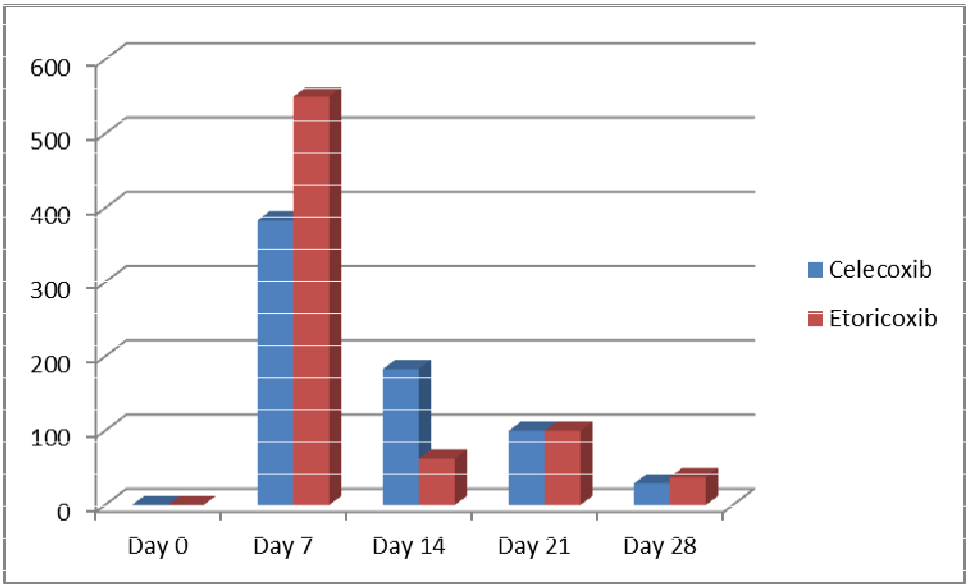

Figure 4. Effect of Selective Cox-2 inhibitors on Antibody Titres after S.typhi 'O' Antigen inoculation in rabbits

on B cells. Thus, it will be important to further evaluate these drugs as potential therapeutic agents to control the abnormal antibody production seen in autoimmune diseases as well as abnormal B lymphocyte proliferation seen in non-Hodgkin lymphoma.

The findings reported herein, also have important implications for the use of Cox-1/Cox-2 inhibitory drugs following vaccinations, where the goal is to promote a humoral immune response. Although, these drugs are commonly used to alleviate the pain associated with injection of vaccine, our findings suggest that there may be an adverse effect on antibody production and/or the

immune response following secondary exposure.

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