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A Comparative Study of Non-Steroidal Anti-Inflammatory Drugs on Immune Response with Special Reference to Cox-2 Inhibitors

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Abstract: This study was conducted to observe the *in vivo* effect of Cox-2 inhibition on immune response. Albino rabbits of either sex were divided into five groups of six animals, each were administered aspirin (100 mg kg^{-1}, B.D., p.o.), celecoxib (30 mg kg^{-1} O.D., p.o.), indomethacin (12.5 mg kg^{-1} B.D., p.o.), etoricoxib (17 mg kg^{-1} O.D., p.o.) for 7 days starting 1 day prior to immunization by *S. typhi* antigen (0.5 mL in each thighs). The antibody titre were measured weekly for 1 month using Widal agglutination test. The antibody titres in the 1st week were raised in all the groups but 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: NSAIDs, cost, immunomodulators, antibody production, Cox-2, India

INTRODUCTION

Prostaglandins 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 Ig class in B cells acting through the EP_2 and EP_4 PGE_2 receptors (Fedyk and Phipps, 1996). The recent finding that activated T cells express cyclooxygenase-2 (Cox-2) (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 increase Cox-2 expression and PG production in B cells. Cox-2 is the predominant isoform contributing to high levels of PGE2 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. Previous 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 Cauppens, 1983). But recently it has been shown that PGE_2 enhances antibody production and promotes type 2 immune responses (Harris *et al*., 2002). Whereas PGE_1 is effective in inhibiting the antibody synthesis by B cells precommitted to IgM class anti-dsDNA antibody production but the production of 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).

PGE_2 suppressed all B cell functions except for IgG synthesis. IgG synthesis rather increased by PGE_2. Therefore, drugs inhibiting PGE_2 production will have opposite effect on B cell. The synthesis of IgM was increased and of IgG was decreased (Yamamoto *et al*., 1996).

MATERIALS AND METHODS

The study was approved by Institutional Animal Ethics Committee. The study was conducted on healthy adult Albino rabbits of either sex weighing 1000-1500 g. All rabbits were 1st screened for the presence of any *S. typhi* O anti-bodies because these antibodies have been found to be naturally present in some of the rabbits due to previous infection. Only those rabbits which

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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 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 six animals each. One group serving as control was given normal saline (1 mL kg^{-1} p.o.) while the other group as test were administered aspirin (100 mg kg^{-1}, B.D., p.o.), celecoxib (30 mg kg^{-1} O.D., p.o.), indomethacin (12.5 mg kg^{-1} B.D., p.o.), etoricoxib (17 mg kg^{-1} O.D., p.o.) for 7 days starting 1 day prior to immunization.

All animals were immunized by *Salmonella typhi* O antigen obtained from the Department of Microbiology of the medical college. About 1 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), 7, 21 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. A 2 tailed $p<0.05$ was considered as significant and $p<0.005$ was considered as highly significant.

RESULTS AND DISCUSSION

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

However, the suppression by selective Cox-2 inhibitors was not significant in comparison to Indomethacin. 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 (Fig. 1 and Table 1).

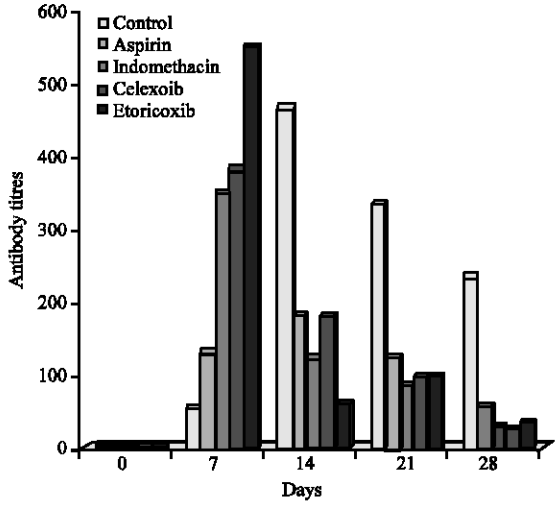

Fig. 1: Effect of NSAIDS on antibody titres after *S. typhi* O antigen inoculation in rabbits

Table 1: Effect of NSAIDs on antibody titres

Groups	7th	14th	21st	28th
	(Days)			
Control (Normal saline)	58.3±20.4	466.6±163.3	333.3±242.2	233.3±81.6
Aspirin (100 mg kg^{-1} 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^{-1} 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^{-1} 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^{-1} p.o.)	550.0±300.0[b, c]	62.5±25.0[b-d]	100.0±70.7[b]	37.5±25.0[b]
Kruskal Wallis-test p-value	0.002	0.000	0.018	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

Since, NSAIDs have varying inhibitory effect on Cox-1 and Cox-2, it was considered worth 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. The study shows that NSAIDS enhances antibody production after a week of immunization whereas during 2nd and 3rd week, antibody titres are markedly low as compared to Control. This also become 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 PGE_2 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 Cauppens, 1983; Yoshikawa *et al*., 1993; Yamamoto *et al*., 1996; Betz and Fox, 1991). In the previous 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 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.

CONCLUSION

The compelling finding of reduced antibody production by specific Cox-2 inhibitors suggests that these agents may be suppressing autoantibody production via direct effects 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, the findings suggest that there may be an adverse effect on antibody production and/or the immune response following secondary exposure.

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