

## The Effect of Selectivity of Inhibitors to Cox-2 Enzyme on Hepatobiliary and Platelet Function in Patients with Osteoarthritis

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### Summary:

**Background:** The development of non steroidal anti-inflammatory drugs (NSAIDs) was based principally on inhibiting cyclooxygenases (COX) activity. However, the identification of two COX- isoforms (i.e., COX-1 and COX-2) with different physiological effects has led to the development of COX-2 specific NSAIDs, with fewer adverse effects than traditional NSAIDs. Therefore, They are expected to produce anti-inflammatory activity with minimal adverse effects on GI mucosa, as well as, other structures and cells such as platelets. The aim of this study is to evaluate the effect of selectivity of COX-2 inhibitors on many organs and systems function such as the hepatobiliary system, platelets function, as well as, serum uric acid levels.

**Patients and methods:** Thirty six patients with osteoarthritis participated in this study. Twenty – four of them were treated with 400 mg celecoxib/day. The remainder received 15 mg meloxicam daily for 3 months .In addition to twelve apparently healthy subjects as a control . Measurement of serum alanine transaminase , serum alkaline phosphatase activity ,total serum protein, and serum albumin to evaluate hepatobiliary system . In addition to the estimation of bleeding time to evaluate the effect of selectivity of inhibitors to COX-2 on platelets function.

**Results:** The results showed minor variations in their effects on liver function tests. However, meloxicam, the relatively selective COX-2 inhibitor affects bleeding time more than does celecoxib, the purely COX-2 selective. Whereas, celecoxib elevated serum levels of uric acid more than meloxicam.

**Conclusion:** We could conclude that selectivity to COX-2 enzyme has different odds of risks on platelets function, such effects that could add more risk factors to patients due to their pharmacological action.

**Key words:** Hepatobiliary, COX-2 inhibitors, Platelet function.

### Introduction:

Selective cyclooxygenase (Cox)-2 inhibitors that are widespread in clinical use , were developed to avoid side effects of conventional NSAIDs, including gastrointestinal and renal toxicity (1). These agents offer potentially significant advantages because of their relative lack of gastrointestinal irritation (2). Because of this, it is likely that these medications will be frequently used in the management of dental and other medical conditions (3). Traditional nonsteroidal antiinflammatory drugs (NSAIDs) inhibit both isoforms of the enzyme cyclooxygenase (Cox). The first, Cox-1, is constitutively expressed in most cells throughout the body, and its inhibition has been associated with gastrointestinal bleeding and ulceration (2). In contrast, Cox-2 expression is induced in the presence of inflammation and its inhibition results in the therapeutic effects of NSAIDs.

Thus, the development of selective Cox-2 inhibitors brought about a new way to produce potent antiinflammatory actions with a decreased risk of significant gastrointestinal adverse effects (4). Celecoxib is a NSAID reported to be a selective inhibitor of cyclo-oxygenase-2 (Cox-2). It is used in the treatment of rheumatoid arthritis and osteoarthritis and as adjunctive treatment of adenomatous colorectal polyps (5). Meloxicam selectivity for Cox-2 is dose dependent and is reduced at higher doses. Therefore meloxicam has been labeled a "preferential" inhibitor instead of a "selective" inhibitor of Cox-2(6).The enzyme Cox-2 is not found in platelets, but in endothelial cells induces prostacyclins synthesis, which prevents platelet aggregation and promotes vasodilatation (7). Thus Cox-2 inhibitors block endothelial prostacyclin synthesis, which leads to platelet aggregation and vasoconstriction (8). Unlike the traditional NSAIDs , celecoxib inhibited Cox-1 derived thromboxine A2 ( TXA2) coincident with its impact on prostacycline (PGI2) , the

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cardiovascular effects of TXA<sub>2</sub> would be expected to be exaggerated (9). PGI<sub>2</sub> was shown to be in endothelial cells to stimulate substantially thrombomodulin. Thus removal of this natural constraint to thrombin activation would interact with the augmented platelet activation to promote assembly of the prothrombinase complex and consequent thrombosis, perhaps particularly in the microvasculature (10).

#### **Patients and Methods:**

Open label study was performed on 36 patients with osteoarthritis (OA) with age ranged between 30 & 60 years (44±8.5) at Al-Basrha General Hospital under the supervision of a senior physician from November 2006 to March 2007. Twelve of the selected patients treated with meloxicam 15mg per day to be taken at night for 3 months. The other 24 osteoarthritis patients treated with celecoxib 400mg per day to be taken in two subdivided doses at morning and at night for 3 months. Besides, twelve apparently healthy individuals were considered as a control group. Fasting blood specimens were obtained before & after three months of starting tested therapy. Serum alanine transaminase-ALT, was measured by monitoring the concentration of pyruvate hydrazone formed after the addition of 2, 4-dinitrophenylhydrazine (11). Serum alkaline phosphatase (ALP) was measured in an alkaline medium (PH=10) by measuring the liberated phenol to get the activity of this enzyme (12). Total serum proteins measured by (Biuret Method) (13). Serum albumin was measured utilizing bromocresol green (BCG) (14). Bleeding time was estimated by template method (15). Uric acid is oxidized by uricase to allantoin and hydrogen peroxide. The liberated hydrogen peroxide then reacts with 4-aminophenazone in the presence of peroxidase measurable colored complex (16). All patients participating in the study had good information on the drug they used, and the aim of prescribing it by the specialist physician where they accepted the plan of treatment. Data were expressed as mean ± standard error of the mean, and the results compared by student T- test.

#### **Results:**

In figure (1), the data show that pretreatment values of serum ALT were non significantly different from that of normal group. Patients treated with either meloxicam or celecoxib for 3 months showed a significant increase ( $p < 0.05$ ) in serum ALT values (percentage increase was + 50.23% & +32.93%, respectively) as compared to pretreatment values of patients. By the data showed in figure (2), the pretreatment values of serum ALP were not significantly altered in patients, neither before treatment nor after that. The values of serum albumin of patients with osteoarthritis were non significantly different from that of normal group. Patients treated with

meloxicam or celecoxib for 3 months showed no significant change in serum albumin values, as shown in table (1). In table (2) the pretreatment values of total serum protein were not altered from that of normal group. After treatment, non of the tested group of patients exhibited significant change ( $p > 0.05$ ) in total serum protein values. The data showed that pretreatment values of bleeding time were no significantly different ( $p > 0.05$ ) from that of normal group. Whereas, patients treated with meloxicam for 3 months showed a significant increase in bleeding time values from that of normal group, and from that of pretreatment values of patients (percentage increase from pretreatment was + 28%). But, those patients treated with celecoxib for 3 months showed no significant change ( $p < 0.05$ ) in their bleeding time values as compared to both normal group, and pretreatment results of patients, percentage increase from pretreatment was +7.46%, figure (3). In figure (4), the data showed that pretreatment patient's values of serum uric acid were non significantly changed from that of normal group. Meanwhile, patients treated with meloxicam for 3 months showed no significant change ( $p > 0.05$ ) in serum uric acid values from that of normal group, and from that of pretreatment group of patients (percentage decrease from pretreatment was - 2.85%). These are results were obtained for treated with celecoxib for 3 months.

#### **Discussion:**

From the above results, data analysis indicated that both the selective Cox-2 inhibitor celecoxib and the preferentially selective or relatively selective Cox-2 inhibitor meloxicam could affect hepatic function as indicated by elevating serum ALT activity. However, the percentage increase that resulted by the use of meloxicam was greater than that resulted by the use of celecoxib. This can be attributed to the fact that Cox-1 enzyme is expressed in many tissues all over the body; one of them is the liver while Cox-2 enzyme has lower range of expression in tissues, and one of them is the liver. I.e. the effects of celecoxib on liver will be to lower extent than does the meloxicam. Such effect on serum ALT could reflect the hepatic cells integrity, where majority of ALT present in these cells (17). Whereas, the term "serum ALP" is applied to a group of enzymes that catalyze hydrolysis of phosphate esters at an alkaline pH. The enzymes are widely distributed and may originate from bone, liver, intestine, kidney, or placenta and it's more indicative for biliary disorders than to hepatocytes (17). In our study, celecoxib increased serum ALP activity to more extent than meloxicam; and this may indicate that celecoxib could cause cholestasis more probably than meloxicam. Physicians should be aware to that, despite a better safety profile for gastrointestinal side effects than conventional non-steroidal anti-inflammatory drugs, celecoxib may

still be associated with severe hepatotoxicity. Celecoxib should be stopped if the results of liver function tests are abnormal (18). Serum proteins (total serum proteins and albumin) did not altered significantly after the use of both celecoxib and meloxicam, and this may indicate that both albumin and total proteins are affected only after chronic liver injury reflecting synthetic capability of the liver (17). In general, albumin is a good marker of severity of chronic liver disease, but levels may be affected by chronic renal insufficiency, urinary protein losses, or gastrointestinal losses (19). When meloxicam was used for 3 months a significant increase in bleeding time values when compared to baseline values, while celecoxib had no effect on bleeding time. This indicates that meloxicam could affect platelet function more than celecoxib. We can attribute this to the fact that Cox-1 enzyme is expressed in platelets while Cox-2 is not, and since meloxicam affects both Cox-1 & Cox-2 it affects platelet function, in contrast to the selective Cox-2 inhibitor celecoxib. Recent studies have investigated that since COX-2 is not expressed in platelets; selective COX-2 inhibitors are unlikely to interfere with platelet aggregation and should, therefore, induce fewer hemorrhagic adverse events than nonselective NSAIDs, especially in the GI tract (20). Finally, celecoxib elevated serum levels of uric acid in patients with osteoarthritis. Although this elevation was statistically non-significant when compared to baseline values of the patients, but it was statistically significant as compared to that of meloxicam treated group of patients. So celecoxib elevates serum levels of uric acid more than meloxicam does and this property may be attributed to the effects of celecoxib on renal system (i.e. nephrotoxicity) which is mostly related to elderly patients (21).

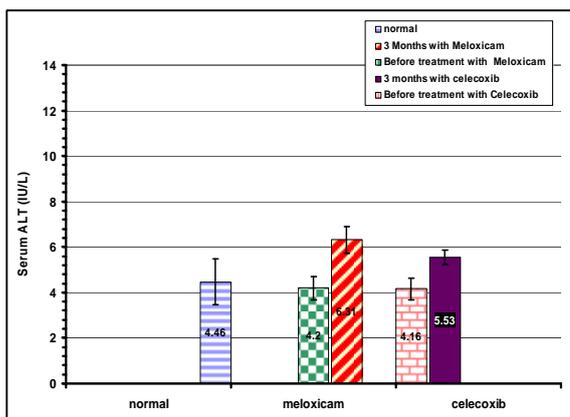


Figure (1) Histogram showing effect of treatment with Celecoxib and Meloxicam For three months, on serum ALT (IU/liter), in patients with osteoarthritis as compared with Pretreatment & normal individual groups that not received any NSAID. a significantly different ( $p < 0.05$ ) as compared with pretreatment values.

**Conclusion:**

We could conclude that selectivity to COX-2 enzyme has different odds of risks on platelets function, such effects that could add more risk factors to patients due to their pharmacological action.

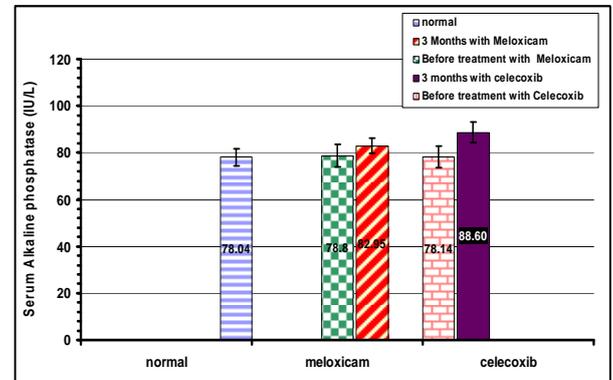


Figure (2) Histogram showing effect of treatment with Celecoxib and Meloxicam For three months, on serum alkaline phosphatase (IU/liter), in patients with osteoarthritis as compared with Pretreatment & normal individual groups that not received any NSAID.

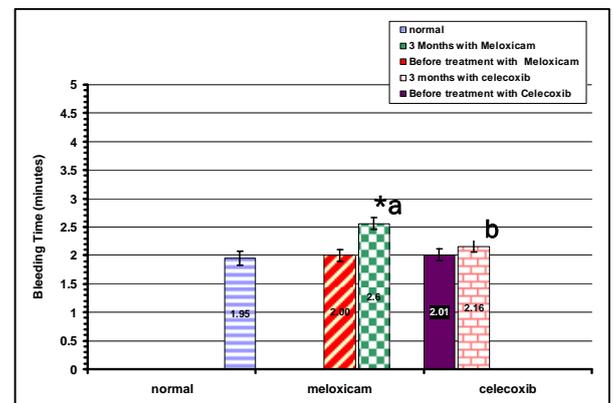


Figure (3) Histogram showing effect of treatment with Celecoxib and Meloxicam For three months, on bleeding time (minutes), in patients with osteoarthritis as compared with Pretreatment & normal individual groups that not received any NSAID.

\* significantly different ( $p < 0.05$ ) as compared with normal values.  
 a significantly different ( $p < 0.05$ ) as compared with pretreatment values.  
 b significantly different ( $p < 0.05$ ) as compare celecoxib with meloxicam groups.

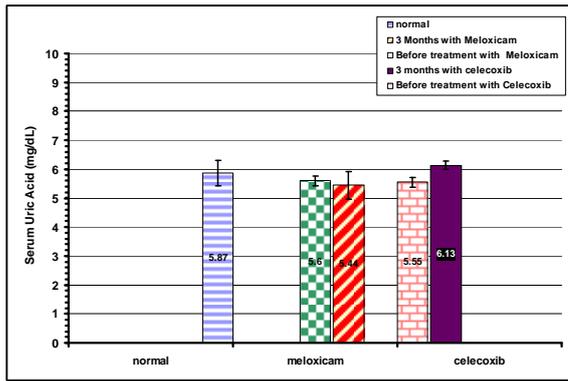


Figure (4) Histogram showing effect of treatment with Celecoxib and Meloxicam For three months, on serum uric acid (mg/dl); in patients with osteoarthritis as compared with Pretreatment & normal individual groups that not received any NSAID.

Table (1) Effect of celecoxib and meloxicam treatment on serum albumin (gm/dl), in patients suffered from osteoarthritis; as compared to pretreatment values and to the normal individuals (that not received any NSAID). Values expressed as mean + standard error of the mean.

Groups	Number of subjects	Serum Albumin (gm/dl)		Δ %Change
		pretreatment	After 3 months treatment	
Normal	12	3.55± 0.18		
Meloxicam	12	3.5± 0.12	3.89±0.17	+ 11.14
Celecoxib	24	3.51 ± 0.12	3.38± 0.06 b	- 3.7

Values are expressed as mean + standard error of mean.

Δ Percentage change was calculated as compared to pretreatment values.

b significantly different (p<0.05) as compare celecoxib with meloxicam groups.

Table (2) Effect of celecoxib and meloxicam treatment on total serum protein (gm/dl), in patients suffered from osteoarthritis; as compared to pretreatment values and to the normal individuals (that not received any NSAID). Values expressed as mean + standard error of the mean.

Groups	Number of subjects	Total Serum Protein (gm/dl)		Δ %Change
		pretreatment	After 3 months treatment	
Normal	12	5.49± 0.18		
Meloxicam	12	5.5± 0.21	5.96±0.18	+ 8.36
Celecoxib	24	5.43 ± 0.21	5.59±0.08	2.94 +

Values are expressed as mean + standard error of mean. Δ Percentage change was calculated as compared to pretreatment values.

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