

EFFECTS OF CELECOXIB AND ROFECOXIB ON NEUTROPHILES AND LYMPHOCYTE COUNTS IN RATS (COMPARATIVE STUDY).

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الخلاصة

مضادات الالتهابات- الغير ستيرويدية كانت وما تزال من الأدوية الشائعة الاستعمال على نطاق واسع جدا لعلاج الالتهابات، الآلام، وان آثارها الجانبية على عدد كريات الدم البيض لا تزال تؤخذ بنظر الاعتبار من قبل الأطباء . خصوصا عند المرضى اللذين يعانون من التهابات المفاصل المزمن. وقد أثبتت الأبحاث وجود نوعين من هذا الأنزيم السايكلووكسجيناز 1 السايكلووكسجيناز 2 وكذلك أن لسايكلووكسجيناز 2 هو المسئول عن خفض الألم والالتهابات عند المرضى لذلك فان اكتشاف النوع الجديد المسمى (المثبط لإنزيم السايكلووكسجيناز) قد رفع من مستوى أمن هذه الأدوية وهناك العديد من هذه الأدوية الجديدة مثل السليكوكسب الروفيكوكسب.

هذه الدراسة تهدف إلى تحديد أيهما أفضل السليكوكسب أم الروفيكوكسب من ناحية الآثار الجانبية وخصوصا التأثير على عدد كريات الدم البيض باستخدام الفحوصات الكيميائية .

استخدمت في هذه الدراسة جردان مختبريه من نوع (Sprague_Dawley) (عدد=30).

وقد قسمت الجردان إلى ثلاثة مجاميع ، كانت المجموعة الأولى مجموعة سيطرة أولى بينما أعطيت المجموعة الثانية السليكوكسب 15 ملغم / كغم والثالثة أعطيت الروفيكوكسب 10 ملغم/كغم وفي بداية ونهاية الدراسة قمنا بحساب عدد كريات الدم البيض العام واللمفية والمتعادلة بعد الفترة العلاجية المحددة وظهرت لنا النتائج والتي تمت مقارنتها مع مجموعة السيطرة الأولى وظهر لنا ما يلي:.

1-سبب السليكوكسب (15 ملغم /كغم/ يوم)الروفيكوكسب (10 ملغم/ كغم /يوم) انخفاض معنوي في عدد كريات الدم البيض في الدم بنوعها اللمفية والمتعادلة.

2-حقق الروفيكوكسب أوطأ نسبة من مقارنة بالسليكوكسب (15 ملغم/كغم/يوم) اعتمادا على هذه النتائج نستطيع أن نقترح أن الجيل الجديد من أدوية مضادات الالتهابات الغير ستيرويدية الروفيكوكسب أظهرت نسبة اقل من خفض كريات الدم البيض يكلا نوعها اللمفية والمتعادلة هذه النتائج يجب أن تؤخذ بنظر الاعتبار عند التقييم السريري للمرضى الذين يستعملون عقارات (مضادات الالتهاب الغير ستيرويدية) لفترات طويلة خصوصا المرضى المصابين بداء السكري، المرضى ضعيفي المناعة وغيرها من حالات نقص أو ضعف المناعة.

Abstract

Non steroidal anti-inflammatory drugs NSAIDs one of the most commonly used drugs all over the world specially for treatment of chronic disease their effect on immune system should be considered in selection of member of non steroidal anti inflammatory drug The objective of this study is to determine whether Celecoxib and Rofecoxib are associated with a lower incidence leucopenia

Thirty (30) Sprague-Dawely male rats were used during this study . The animals were divided in to three groups : group 1 – Control , group 2 received Celecoxib and group 3 received Rofecoxib for 1 month duration .Samples of blood before and after treatment were taken for determination of total W.B.C , neutrophiles and lymphocyte counts. The results were as fallow: 1-Celecoxib 15 mg/kg/day and Rofecoxib 10 mg/kg/day caused significant reduction in total W.B.C , Neutrophiles and Lymphocyte counts .

2-Rofecoxib 10 mg/kg/day caused the less reduction in total W.B.C. count, neutrophiles and lymphocyte counts as compared with Celecoxib 15 mg.kg.day/ day

Introduction

Non-Steroidal Anti-inflammatory Drugs(NSAIDs): is one of the commonly used drugs all over the world because it deal with the most common symptoms of the disease that is the pain by its variant degrees. The other criteria for the non-steroidal anti-inflammatory drugs that its long duration of use by the patient because they are almost always used for treatment of chronic disease like rheumatoid arthritis and other chronic inflammatory condition .Non-Steroidal Anti-inflammatory Drugs is the name given to a class of drugs whose members are structurally heterogeneous but posses a single common mode of action which is to block the prostaglandins G/H synthase .This effect of NSAIDs is termed as anti-eicosanoid ,anti-prostanoid effect. Cyclo-oxygenases (now termed prostaglandin G/H synthase) .This enzyme changes the linear fatty acids in to the cyclic structure of the prostaglandins .NSAIDs exert their anti-inflammatory effects by inhibiting prostaglandins G/H

synthase (cyclo-oxygenase) .The latter is found in at least two forms .Cyclo-oxygenase –1(Cox-1) which is non-inducible and present in many tissues including platelets, stomach and kidneys .Cyclo-oxygenase –2(Cox-2) which is induced at sites of inflammation by cytokinases and endotoxine.(1)

Lipoxygenase : which metabolites arachidonic acid in to straight –chain hydroperoxy acids and then to leukotriens which cause increased vascular permeability ,vasoconstriction and broncho constriction , as well as ,chemo tactic activity for leukocytes. Inhibitors of lipoxygenase, and leukotriens receptor antagonists, are being evaluated and are likely to find uses in inflammatory and allergic conditions. (1)

white blood cells: are normally 4000-11000 per micro liter
Neutrophiles: seek out, ingest, and kill bacteria and have been called the body first line of defense against bacterial infection .the average half life of neutrophiles in the circulation is 6 hours. to maintain normal blood level , it is therefore necessary to produce over 100 billion neutrophiles per day.
lymphocyte:

after birth , some lymphocyte are formed in the bone marrow , but most are formed in the bone marrow, thymus and spleen from precursor cells that originally came from the bone marrow. lymphocytes enter the blood stream for the most part via the lymphatic . it has been calculated that in human 3.5×10^{10} lymphocyte per day enter the circulation via the thoracic duct alone, however this count include the cells that enter the lymphatic and thus traverse the thoracic duct more than once .the lymphocyte are the key of the immune system which has the ability to produce antibodies against millions of different foreign agent that may invade the body.

Aims of the study:This study is an attempt to re-evaluate two new members of NSAIDs Rofecoxib and Celecoxib ,which are selective Cox-2 inhibitors ,in regard to their effects on neutrophiles and lymphocyte count to select the most appropriate on for immune- compromised patient .

Materials and Methods:

materials

Animals

thirty healthy adult males Sprague- Dawley rats with weight ranging between 200-230 gm, and aged between 18-20 weeks were obtained from the National Center for Drug Control and Research/ Baghdad. The rats were kept in cages, each cage contained five animals. The animals were left to acclimatize to the animal house condition for one week and were fed with standard rodent chew diet and tap water.

Drugs used in experiment:

1-Rofecoxib tablets: selective Cox-2 inhibitors NSAIDs obtained as Dioxx[®], each tablet contains Rofecoxib 25 mg, Batch No. 2-03 manufactured by Dafar drugs Company.

2-Celecoxib capsules : selective Cox-2 inhibitor NSAIDs obtained as Celebrex, 200 mg /cap. Batch No. O11-534620 manufactured by G .D. Searle and CO. USA.

Experimental animals

Animals were randomly divided in to following groups

1-Group 1: Control group contained 10 animals , all the animals of this group were kept on standard rodent chew diet and tap water through out the duration of experiment .

2-Group 2: Celecoxib group, contained 10 animals ,all the animals of this group were given Celecoxib 15 mg/kg once daily for one month..

3-Group 3 : Rofecoxib group, contained 10 animals ,all the animals of this group were given Rofecoxib 10 mg/kg once daily for one month.

The doses of the drugs used above are the typical doses in rats for assessment of gastric damage induced by NSAIDs)2) All the drugs were diluted in normal saline and were administrated as liquid solutions through stomach tube according to .animals body weight .

Methods

Blood samples

Before drug administration, 1 ml of blood was taken from each rat for measurement total W.B.C., neutrophiles and lymphocyte counts. The blood was withdrawn from the caudal artery at the ventral aspect of the tail at the junction with the body.

After one month of drug administration the blood was taken from each rat for measurement of, total W.B.C., neutrophiles and lymphocyte Counts. These blood samples were obtained directly from the heart after labratomy.

Measurement of total W.B.C., neutrophiles and lymphocyte counts.

Make a 1 in 20 dilution of blood by adding 0.1 ml of blood to 0.38 ml of diluting fluid in a 75 X 10-mm glass or plastic tube. The method of counting is according to Daci and Lewis,2001.(3)

Calculation: -

Count (Per liter)= No. of cells counted X dilution X106
Volume counted (ml)

Results

W.B.C Count:

Total W.B.C counts:

The results of total W.B.C counts revealed that Celecoxib 15 mg/ kg /day and Rofecoxib 10 mg/kg/day for 1-month treatment caused significant reduction in total W.B.C count ($p < 0.05$) as compared with control group (control-1) (Table 1). Rofecoxib 10 mg/ kg /day for 1-month treatment caused less reduction in total W.B.C counts as compared with Celecoxib 15 mg /kg/day for the same period of treatment (Table 3).

There were significant no differences in the percentage of reduction in total W.B.C counts between Celecoxib and Rofecoxib (Table 2).

Table (1): Effects of different NSAIDs on total WBC

Groups	W.B.C.c ounts		Reduction %	P- value
	before treatment	after treatment		
Control	8480.00±409.5	8360.00±408.8	-1.41	P>0.05
Celecoxib 15 mg/kg/day	4860.00±255.6	4450.00±108.7	-8.43	P<0.05
Rofecoxib 10 mg/kg/day	4940.00±233.4	4620.00±226.4	-6.47	P<0.05

counts

Table(2):Comparison of mean differences from baseline in total WBC counts in animals treated with Celecoxib 15 mg/kg/day and Rofecoxib 10 mg/kg/day for 1-month.

Groups	Celecoxib	Rofecoxib	Control
Control	404.32*	270.8*	
Rofecoxib	35.6		

Neutrophiles counts:

Results of neutrophiles count revealed that Celecoxib 15 mg /kg /day and Rofecoxib 10 mg/ kg/day for 1-month treatment caused significant reduction in number of neutrophiles (p< 0.05) as compared with control (1) group (Table 3).

Rofecoxib 10 mg/ kg /day for 1 month treatment caused less reduction in neutrophiles counts as compared with Celecoxib 15 mg/ kg /day for the same duration of treatment (Table 4)

Table(3): Effects of different types of Cox-2 inhibitors on neutrophiles counts.

Groups	Neutrophiles counts		Reduction %	P-value
	before treatment	after treatment		
Control	2180.00±26.4	2070.00±188.0	-5.04	P>0.05
Celecoxib 15 mg/kg/day	1820.00±71.1	1520.00±38.8	-16.48	P<0.05
Rofecoxib 10 mg/kg/day	1880.00±129.7	1640.00±90.9	-12.76	P<0.05

Table(4): Comparison of mean differences in neutrophiles counts after 1-month treatment with Rofecoxib 10 mg/kg/day, Celecoxib 15mg/kg/day and in the control group.

Groups	Celecoxib	Rofecoxib	Control
Control	607.09*	660.91*	
Rofecoxib	206.18		

Lymphocyte counts:

Result of lymphocyte count revealed that during the same period of treatment (1-month) Celecoxib 15 mg/ kg /day and Rofecoxib 10 mg /kg /day caused significant reduction in lymphocytes counts (p< 0.05) as compared with control group (Table 5). Also it was found that Rofecoxib 10 mg /kg/day for 1 month treatment caused less reduction in lymphocytes counts (P<0.018) as compared with Celecoxib 15 mg /kg /day (P<0.025) for the same period of treatment (Table 5).

Also the results showed a significant differences between Celecoxib, and Rofecoxib (Table 6).

Table(5): Effects of different types of Cox-2 inhibitors on lymphocytes counts.

Groups	Lymphocytes count		Reduction %	P-value
	before treatment	after treatment		
Control	6300.00±321.6	6290.00±364.6	-0.15	P>0.05
Celecoxib 15 mg/kg/day	2940.00±231.9	2530.00±98.9	-13.94	P<0.05
Rofecoxib 10 mg/kg/day	2860.00±165.4	2580.00±181.8	-9.79	P<0.05

Table(6): Comparison of mean differences in lymphocyte counts after 1-month treatment with Celecoxib 15 mg/kg/day, Rofecoxib 10 mg/kg/day.

Groups	Celecoxib	Rofecoxib	control
Control	328.38*	249.36*	
Rofecoxib	90.16		

Discussion :

The selection of rats as animal model in this study is due to the fact that rats are considered as good model for study of gastrointestinal lesions induced by NSAIDs.⁽⁴⁾ Due to their gastrointestinal toxicity, NSAIDs have been widely studied for many years, especially since 1993 when 2 isoforms of cyclo-oxygenase was discovered. The discovery of inducible cyclo-oxygenase enzyme (Cox-2) has given a new way in the development of a safer anti-inflammatory drugs.

The results of animal experiments and early clinical studies with selective Cox-2 inhibitors are quite impressive. This study support that these selective Cox-2 inhibitors will represent an effective

gastrointestinal sparing alternative to classical NSAIDs. They will be beneficial in other clinical situation in which Cox-2 is over expressed, beside their therapeutic indication.⁽⁵⁾

Effects of NSAIDs on total W.B. C counts of rats

In the present study it was found that Celecoxib 15 mg/kg/day and Rofecoxib 10 mg /kg/day caused significant reduction in total W.B.C counts ($P < 0.05$). These finding are similar to the findings of Maria Victoria et al 2000.⁽⁶⁾ who had found a significant decrease in total W.B.C counts after treatment with Celecoxib and Rofecoxib in human being. The proposed explanation for this leucopenia are through immunologically mediated mechanism, either through immune suppression of bone marrow white blood cell precursors or peripheral destruction.⁽⁷⁾

Effects of NSAIDs on neutrophiles and lymphocyte counts In this study it was found that Celecoxib 15 mg/kg/day and Rofecoxib 10 mg /kg/day produce a significant reduction of both, neutrophiles and lymphocyte counts ($P < 0.05$). It was observed that Rofecoxib caused less reduction in total W.B.C, neutrophiles and lymphocyte counts and this can be explained by that the mechanism of reduction of neutrophiles counts by NSAIDs act through a direct toxic effect on the bone marrow, or may be combined by immunological mechanism,⁽⁸⁾ our results are in agreement with that obtained by cryer etal (1998) who found that celecoxib 15 mg/kg/day and rofecoxib 10 mg /kg/day produce a significant reduction of both, neutrophiles and lymphocyte counts.

Conclusion and Recommendation

Our work has shown that:

1-Celecoxib and Rofecoxib in a dose of (15mg/kg/day, 10 mg/kg /day) cause significant reduction in total W.B.C., neutrophiles and lymphocyte counts.

2-Rofecoxib in a dose of 10mg/kg for one month show less reduction in a total W.B.C. count (- 6.25%) as compared by Celecoxib in a dose 15 mg. kg day for one month (-8.45%).

3-Rofecoxib in a dose of 10mg/kg for one month show less reduction in a neutrophiles count(-12.76%) as compared

with Celecoxib 15 mg.kg. day for one month (-16.48). 4-Rofecoxib in a dose of 10 mg.kg.day for one month show less reduction in lymphocyte count (-1.56%) as compared with Celecoxib in a dose of 15 mg.kg.day (- 12.45) for one month .these result deserve clinical evaluation in those with long use of NSAIDs specially those patients with Immune-compromised state like AIDES (HIV virus), Diabetes Mellitus and others .

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