Comparative study of the effects of Nimesulide, Celecoxib and Indomethacin on hepatic and gastric tissues in rats

Thesis

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ABSTRACT

Nimesulide, a non-steroidal anti-inflammatory drug (NSAID) with preferential COX-2 inhibition, was found to be well-tolerated in patients as it causes less gastro-intestinal side effects compared with other non-selective NSAIDs. Its safety on the liver is controversial.

In the present study, three different NSAIDs were used: a non-selective drug (indomethacin), a partially selective one (nimesulide), and a selective COX-2 inhibitor (celecoxib). Albino rats were used and were divided into two main groups: group I: normal rats that were divided into 4 subgroups, and group II: thioacetamide induced hepatotoxicity group. Pyloric ligation was performed; gastric acidity and mean ulcer score were measured to assess the effect of different drugs on the gastric tissue. Liver function tests and histo-pathological examination of the stomach and the liver were performed.

Nimesulide showed good gastric tolerance. However, it elevated significantly most of the hepatic bio-chemical and histo-pathological parameters in comparison to other drugs in healthy and diseased liver. Celecoxib and indomethacin effects on liver were variable.

Keywords: Nimesulide; Celecoxib; Indomethacin; Thioacetamide; Hepatotoxicity; Gastric Acidity; Mean Ulcer Score; Pyloric Ligation.

CONTENTS

LIST OF ABBREVIATIONS

ALT:	Alanine transferase
ALP:	Alkaline phosphatase
ANOVA:	Analysis of Variance
AST:	Aspartate transferase
ATP:	Adenosine triphosphate
bid:	Twice daily
CHUMP:	Committee for Medicinal Products for Human use
CNS:	Central nervous system
COX:	Cyclooxygenase enzyme
COXIBs:	Cyclooxygenase -2-selective inhibitors
CSF:	Cerebrospinal fluid
CV:	Cardio-vascular
CYP:	Cytochrome P
DILI:	Drug-induced liver injury
DMARD:	Disease modifying anti-rheumatic drug
DNPH:	Dinitrophenylhydrazine
ECL:	Enterochromaffin-like
EMA:	The European Medicines Agency
EU:	European Union
FDA:	Food and Drug Administration
FOI:	Freedom of Information
GI:	Gastro-intestinal
GOT:	Glutamic oxaloacetic transaminase
GPT:	Glutamic pyruvic transaminase
GSH:	Glutathione
H&E:	Hematoxylin and Eosin
HAI:	Histology activity index
IFN:	Interferon
IL:	Interleukin
iNOS:	Inducible nitric oxide synthase

I.P.:	Intraperitoneal
IU:	International Unit
KC:	Kupffer cells
LOX:	Leukotriene oxygenase enzyme
LSD:	Least significant difference
LTs:	Leukotrienes
NAPQI:	N-acetyl-p-benzoquinone imine
NASH:	Non alcoholic steatohepatitis
NK:	Natural killer
NKT:	Natural killer T cells
NO:	Nitric oxide
NSAIDs:	Non-steroidal anti-inflammatory drugs
P-C:	Portal to central
PGI2:	Prostacyclin
PGHS:	Prostaglandin-H2 synthase
PGs:	Prostaglandins
pH:	Power of hydrogen
pKa:	Acid dissociation constant
P-P:	Portal to portal
P.T.:	Portal tract
qid:	4 times daily
ROS:	Reactive oxygen species
SD:	Standard deviation
sPDA:	Symptomatic patent ductus arteriosis
TAA:	Thioacetamide
tds:	Three times daily
TNF-α:	Tumor necrosis factor-alpha
TXA2:	Thromboxane A2
UK:	United Kingdom
ULN:	Upper limit of normal
US:	United States

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INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most widely used medications. The use of NSAIDs plays a fundamental role in controlling inflammation and pain relief **(Hawboldt, 2008)**.

These drugs act on the cyclooxygenase enzyme (COX) that converts arachidonic acid into the endoperoxide precursors of prostaglandins, important mediators of inflammation. Cyclooxygenase has at least 2 isoforms: COX-1 and COX-2. COX-1 isoform tends to be homeostatic in function, while COX-2 is induced in inflammation and tends to facilitate the inflammatory response. Some of these drugs are non-selective, i.e. acting on both COX-1 and COX-2, such as aspirin, indomethacin, and diclofenac; while others are selective, i.e. act only on COX-2 inhibitors, such as celecoxib **(Furst et al., 2007)**.

Nimesulide, a non-steroidal anti-inflammatory drug with preferential COX-2 inhibition **(Altinkaynak et al., 2003),** was found to be as effective as other NSAIDs in relieving pain. It has been shown that nimesulide is well-tolerated in patients who are intolerant to other NSAIDs **(Rainsford, 2006)** as it causes less gastro-intestinal side effects compared to other non-selective NSAIDs **(Borku et al., 2008)**.

Its safety on the liver is controversial; some studies reported cases of nimesulide induced hepatotoxicity that could be fatal **(Tan et al., 2007**; **Page et al., 2009)**, while others confirmed that liver insult induced by nimesulide is minimal **(Traversa et al., 2003)**.

The European Medicines Agency (EMA) has completed a review of the liver related safety of nimesulide and has concluded that the benefits of medicines containing nimesulide still outweigh their risks. However, the agency advised to restrict the use of these medicines in children less than 12 years **(EMA, 2007)**.

AIM OF THE WORK

The aim of this work is to compare the effects and safety of nimesulide, celecoxib, and indomethacin on the liver and gastric tissues in adult rats.

REVIEW OF LITERATURE

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most frequently used classes of medicines in the world and account for nearly 5% of all prescribed medications **(Tomisato et al., 2004)**.

It is expected that the use of NSAIDs will increase because the incidence of rheumatic diseases also is increasing. Their use is more frequent among women and increases with age, as does the incidence of rheumatic diseases. Indeed more than 90% of prescriptions for NSAIDs are made to patients aged more than 65 years **(Sostres et al., 2010)**.

Regarding the pharmacokinetics of NSAIDs, they are weak acids with pKa less than 5. Most of them are rapidly absorbed following oral administration with peak plasma concentration reached within 2-3 hours. Moreover the rate and the extent of NSAIDs absorption are comparable for rectal and oral routes **(Buvanendran and Lipman, 2009)**.

NSAIDs are extensively bound to plasma proteins, usually albumin. Their volume of distribution varies between 0.1 and 0.15 L/Kg. Conditions that alter plasma proteins concentration may result in an increase in the free drug fraction with potential of toxic effects. Most NSAIDs have the potential to displace other highly plasma proteins bound drugs **(Grosser, 2006)**.

NSAIDs constitute a chemically heterogeneous group of drugs that differ in their therapeutic efficacy and toxicity. CYP2C8 (Cytochrome P) and CYP2C9 hepatic microsomal enzymes are the chief enzymes involved in the first steps of NSAIDs metabolism. However, the relative role of CYP2C enzymes in primary metabolism differs among different NSAIDs **(Agúndez et al., 2009)**.

NSAIDs are mostly excreted by the kidney as phase II glucuronides and in a few cases as sulfate conjugates. In addition, small percentages of NSAIDs

are excreted unchanged in urine (Table1). If the drug is excreted unchanged and co-administered with agents that render the urine pH alkaline such as the antacids aluminum hydroxide and milk of magnesia, its rate of excretion is expected to increase **(Mehanna, 2003)**.

Regarding the NSAIDs pharmacodynamics, they inhibit cyclooxygenase (COX), a key enzyme in the biosynthesis of prostaglandins from arachidonic acid. COX exists in at least two isoforms **(Kujubu et al., 1991; Hla and Neilson, 1992)**. These two are COX-1 and COX-2. ‘‘Classical’’ NSAIDs are nonselective inhibitors of both isozymes **(Xie et al., 1992)**.

Table 1. The pharmacokinetic properties of aspirin and some other non-steroidal anti-inflammatory drugs. *Quoted from* **(Furst et al., 2009. Katzung Basic & Clinical Pharmacology).**

Drug	Half-life (hours)	Urinary excretion of unchanged Drug	Recommended Anti-Inflammatory Dosage
Aspirin	0.25	< 2%	1200-1500 mg tds
Celecoxib	11	27%	100-200 mg bid
Diclofenac	1.1	< 1%	50 – 75 mg qid
Diflunisal	13	3 – 9%	500 mg bid
Etodolac	6.5	< 1%	200-300 mg qid
Fenoprofen	2.5	30%	600 mg qid
Flurbiprofen	3.8	< 1%	300 mg tds
Ibuprofen	2	< 1%	600 mg qid
Indomethacin	4 – 5	16%	50-70 mg tds
Ketoprofen	1.8	< 1%	70 mg tds
Ketorolac	4 – 10	58%	10 mg qid
Meloxicam	20	data not found	7.5-15 mg qd
Naproxen	14	< 1%	375 mg bid
Piroxicam	57	4-10%	20 mg qd
Sulindac	8	7%	200 mg bid