



UNIVERSITI PUTRA MALAYSIA

**TOXIC EFFECTS OF NON-STEROIDAL ANTI-INFLAMMATORY
DRUGS PIROXICAM AND MEFENAMIC ACID AND THEIR ROLES AS
CANCER CHEMOPREVENTIVE AGENTS**

FAIZAH SANAT

FPSK (M) 2004 3

**TOXIC EFFECTS OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS
PIROXICAM AND MEFENAMIC ACID AND THEIR ROLES AS CANCER
CHEMOPREVENTIVE AGENTS**

By

FAIZAH SANAT

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfilment of the Requirements for the Degree of Master of Science**

January 2004



To:

My beloved family

My parents, Sanat Md. Nasir and Nahariah Lias. My sister Farhani, this time you're the first. My brothers Mohd. Zahid , Mohd. Sirajuddin and Mohd. Hafizuddin. Welcoming the new member of the family, my sister-in-law, Hasmaniza and not forgetting little Hani Zahira.

Thank you for your love, support, encouragement and above all, patience.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

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Chairman: Associate Professor Muhammad Nazrul Hakim Abdullah, Ph.D.

Faculty: Medicine and Health Sciences

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely used drugs worldwide. More NSAIDs were being produced and manufactured everyday. It was an overwhelming view that the thought of all NSAIDs were important due to their therapeutic actions by inhibiting the production of prostaglandin was challenged by the discovery that they affect a wide variety of cellular processes along the way. The NSAIDs piroxicam and mefenamic acid have dissimilar chemical structures, enolic and carboxylic acid respectively, but with the same mode of action for therapeutic uses. They both inhibit prostaglandin synthesis by inhibiting the cyclooxygenase (COX) pathway as of other conventional NSAIDs. However in some cases, they did differ with each other depending on the gravity of their effects on certain aspects.

Although the toxicity of piroxicam was well known and documented, mefenamic acid is still not the safest drugs of all. Histologically, mefenamic acid showed a marked toxicity to the liver and kidney of rats compared to piroxicam. Morphological changes



such as inflammation and fibrosis of liver were frequently observed in repeated doses of mefenamic acid with elevation of protein plasma alkaline phosphatase (ALP) and alanine transferase (ALT), higher than piroxicam. Piroxicam on the other hand, did cause higher toxicity in the gastrointestinal tract but not significant to mefenamic acid. Nevertheless, both drug showed a significant different ($p < 0.05$) when compared to control in post-treated plasma levels and also the mean lesion scores of samples treated with repeated doses of NSAIDs.

Using liver perfusion technique, freshly isolated rat hepatocytes were obtained for the *in vitro* treatment of NSAIDs. The cell viability test was done by trypan blue exclusion. As a result both piroxicam and mefenamic acid caused reduction in cell viability of hepatocytes up to 50% of cell death at highest concentration. However, mefenamic acid exerted its cytotoxicity even more so than piroxicam in both time- and dose-dependent manner. Meanwhile, the effects of piroxicam and mefenamic acid on Phenobarbital-induced rat hepatocytes were not pronouncedly shown. It was concluded that Phenobarbital-induced rat hepatocytes did not alter the cytotoxicity of both drugs in both time- and dose- dependent fashion.

Both piroxicam and mefenamic acid did significantly reduce the cell viability of cancer cells especially the colon cancer cells. MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) assay was done to determine the cell viability of the cancer cells. Both colon cancer cells used (HCT 116 and Caco 2) showed a significant reduction in cell viability after being treated with both piroxicam and mefenamic acid. It was postulated that this event occurred due to their ability to inhibit the



prostaglandin synthesis which were upregulated in colon adenocarcinomas. That might be the possible reason behind the reduction of colon cancer cells' viability treated with NSAIDs.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**KESAN TOKSIK DADAH ANTI-KERADANGAN BUKAN STEROID
PIROXICAM DAN ASID MEFENAMIK DAN PERANAN MEREKA SEBAGAI
AGEN KIMOPREVENTIF KANSER**

Oleh

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Dadah anti-keradangan bukan steroid (NSAIDs) adalah kumpulan dadah yang paling meluas penggunaannya serata dunia. Lebih banyak NSAIDs telah dihasilkan dan dikeluarkan setiap hari. Ia adalah satu pandangan yang membingungkan tentang anggapan bahawa semua NSAIDs adalah sangat penting disebabkan oleh tindakan terapeutik mereka iaitu menyekat penghasilan prostaglandin, dicabar oleh penemuan yang mereka juga memberi pelbagai kesan yang meluas terhadap proses-proses sel sepanjang aktiviti itu. NSAIDs piroxicam dan asid mefenamik mempunyai struktur kimia yang berbeza, masing-masing asid enolik dan karboksilik, tetapi dengan gaya tindakan yang sama untuk kegunaan terapeutik. Kedua-duanya menyekat sintesis prostaglandin dengan menghalang laluan siklooksigenes (COX) sepertimana NSAIDs yang lain. Bagaimanapun, mereka memang berbeza di antara satu sama lain bergantung kepada tahap kesan masing-masing dalam sesetengah aspek.



Walaupun ketoksikan piroxicam telah diketahui dan didokumentasikan, asid mefenamik masih juga bukan dadah yang paling selamat. Secara histologinya, asid mefenamik menunjukkan kesan ketoksikan yang ketara terhadap hati dan buah pinggang tikus-tikus berbanding piroxicam. Perubahan morfologi seperti keradangan dan fibrosis pada hati telah dilihat dengan kerap dalam suntikan asid mefenamik secara berulang-kali dengan peningkatan terhadap plasma protein alkalin fosfatase (ALP) dan alanin trasferase (ALT) yang lebih tinggi berbanding piroxicam. Piroxicam pula memberi kesan ketoksikan yang lebih tinggi pada saluran pencernaan tetapi ia tidak signifikan berbanding asid mefenamik. Namun demikian, kedua-duanya menunjukkan perbezaan yang signifikan ($p < 0.05$) apabila dibandingkan dengan kawalan pada tahap plasma selepas rawatan dan juga min skor lesi bagi sampel yang dirawat oleh NSAIDs secara berulang-kali.

Dengan menggunakan teknik perfusi hati, pengasingan sel hepatosit tikus segar dapat dibuat untuk digunakan dalam rawatan *in vitro* oleh NSAIDs. Ujian untuk menguji sel-sel hidup dibuat menggunakan tripan biru. Piroxicam dan asid mefenamik menyebabkan penurunan kepada bilangan sel hepatosit yang hidup sebanyak 50% kematian sel pada kepekatan yang tertinggi. Bagaimanapun, asid mefenamik menunjukkan kesan kesitotoksikan yang lebih tinggi berbanding piroxicam dalam keadaan berkadar langsung dengan masa dan dos. Sementara itu, kesan piroxicam dan asid mefenamik terhadap sel-sel hepatosit yang telah dirawat terlebih dahulu dengan Phenobarbital tidak ditunjukkan dengan ketara. Sebagai konklusi,, sel-sel hepatosit yang telah dirawat dengan Phenobarbital tidak mengubah kesitotoksikan kedua-dua dadah dalam keadaan yang berkadar langsung dengan masa dan dos.

Kedua-dua piroxicam dan asid mefenamik secara signifikan telah menurunkan bilangan sel-sel kanser yang hidup terutama sel-sel kanser bagi kolon. Esei MTT (3-[4, 5-dimetilthiazol-2-yl]-2, 5-difeniltetrazolium bromid) telah dibuat untuk menentukan sel-sel kanser yang hidup. Kedua-dua sel kolon yang digunakan (HCT 116 dan Caco 2) menunjukkan penurunan sel-sel hidup yang signifikan selepas dirawat oleh piroxicam dan asid mefenamik. Ia telah didakwa bahawa kejadian ini berlaku disebabkan oleh kemampuan mereka menyekat sintesis prostaglandin yang terdapat dengan banyaknya dalam adenokarsinoma. Mungkin itu adalah salah satu sebab kepada penurunan bilangan sel-sel kanser yang hidup selepas dirawat oleh NSAIDs.



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I certify that an Examination Committee met on 15th January 2004 to conduct the final examination of Faizah Sanat on her Master of Science thesis entitled “Toxic Effects of Non-Steroidal Anti-Inflammatory Drugs Piroxicam and Mefenamic Acid and their Roles as Cancer Chemopreventive Agents” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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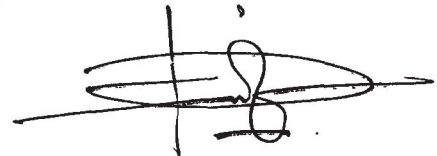
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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



FAIZAH SANAT

Date: 13 APR 2004

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