In Vitro Stability of Ibuprofen Medoxomil Prodrug

Israel Nisan[1], Bianca C.Neves[2]

[1] *Clinical Microbiology, The Hebrew University, Faculty of Medicine, POB 12272, Jerusalem 9112, Israel*
[2] *Departamento de Microbiologia, Instituto de Ciencias Biomedicas, Universidade de Sao Paulo Av. Prof. Lineu Prestes,1374 Sao Paulo-SP-Cep 05508-900, Brazil*

ABSTRACT

Synthesis of medoxomil prodrug of ibuprofen (IM) was described. Its stabilities at different pH environments encountered in the gastrointestinal tract and in rat plasma in vitro were tested. Results demonstrated that esterase play an important role in the activation of IM. It can pass through the stomach without degradation, and with very slow degradation in intestinal tract, which indicated that IM may be absorbed with prototype in intestinal tract. While, after absorption, IM can be hydrolized very quickly by esterase in plasma. These results indicate that IM has the characteristics of prodrug.

Key words: ibuprofen, medoxomil, prodrug, stability evaluation, *in vitro*.

INTRODUCTION

Ibuprofen, 2-(4-iso-butylphenyl)-propanoic acid, is a widely used non-steroidal anti-inflammatory drug (NSAID), acts by inhibiting cyclooxygenase (COX)-1 and COX-2, and effective against rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, etc [1,2]. It is poorly soluble in water [3], usually administered orally and rapidly absorbed in intestinal tract. However, the half-life of ibuprofen is less than 2 hours, which means that multiple doses are required to maintain effective drug concentration during the treatment period [4].

4, 5-dimethyl-1, 3-dioxolen-2-one (medoxomil) is a compound of carbonic acid lactone with allyl group, always reacting with the carboxylic groups of drugs to synthesize medoxomil prodrugs, which are hydrolyzed *in vivo* by serum paraoxonase (PON1) locating in both the intestine and plasma[5] (Figure 1). In the past few decades, on the purpose of improving oral bioavailability, reducing irritation, and prolonging action time, medoxomil had been commonly used to modify drugs, and many of them have been brought to market (Figure 2).
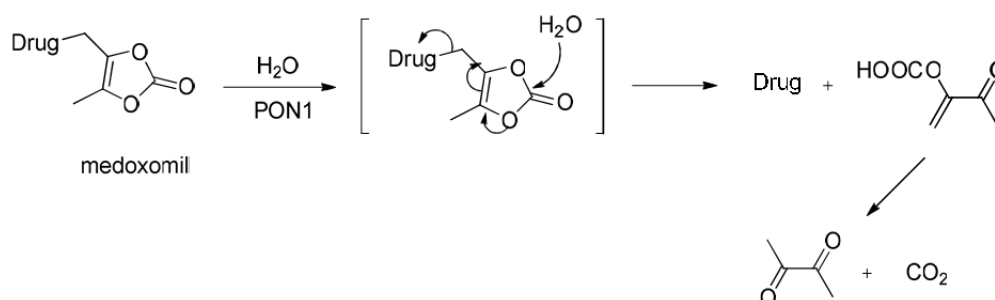

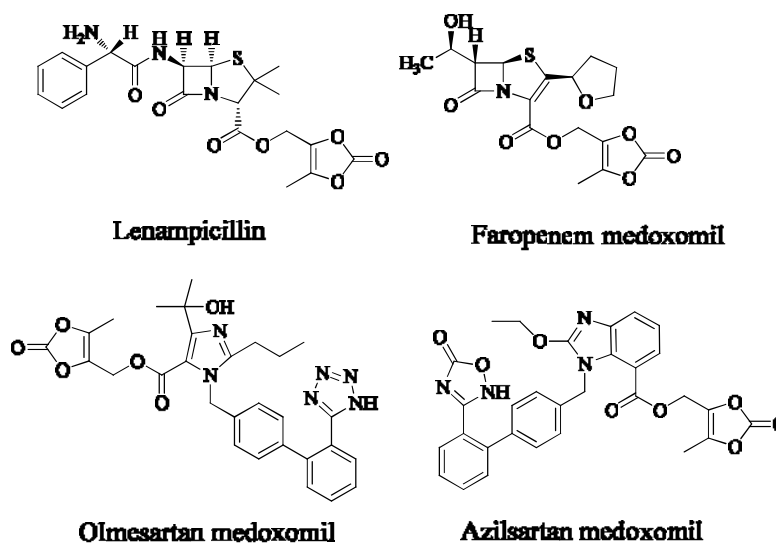**Figure 1 The enzymatic hydrolytic action of medoxomil prodrugs**

Lenampicillin

Faropenem medoxomil

Olmesartan medoxomil

Azilsartan medoxomil

Figure 2 The represatatives of medoxomil prodrugs on marketing

In the present investigation, in order to prolong the half life of ibuprofen, reduce the frequency of administration, ibuprofen medoxomil (IM, Figure 3) were designed and synthesized, its stability in aqueous solutions and rat plasma *in vitro* were also evaluated.
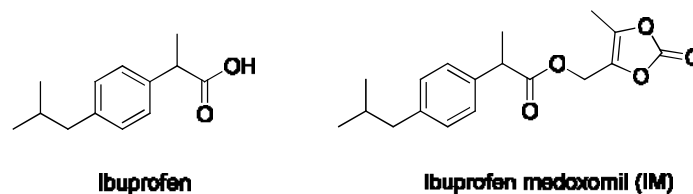

Ibuprofen

Ibuprofen medoxomil (IM)

Figure 3 The chemical structures of ibuprofen and its medoxomil prodrug

EXPERIMENTAL SECTION

1. General

Ibuprofen was purchased from Boylechem Co., Ltd (Shanghai, China), and 4-chloromethyl-5-methyl-1,3-dioxol-2-one from Energy Chemical (Shanghai, China). Other commercial reagents and solvents were used without further purification. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a Bruker Advance 400 nuclear magnetic resonance spectrometer. Compound purity was assessed by HPLC analysis on an Agilent 1200 HPLC system and shown to be > 95%. Drug concentrations in the tests and MS spectra were determined by LC-MS/MS using an Agilent 1100 Series HPLC system and a QTRAP 2000TM mass spectrometer.

2. Synthesis of IM

Ibuprofen (2.1 g, 10 mmol) was dissolved in DMF (20 mL) at room temperature. Sodium carbonate (0.8 g, 7 mmol) and 4-chloromethyl-5-methyl-1,3-dioxol-2-one (1.5 g, 10 mmol) were added, and the mixture was stirred for 12 h at room temperature. Reaction completion was monitored by TLC. Then the reaction mixture was transferred to a mixture of ethyl acetate (50 mL), water (100 mL), and sodium thiosulfate (0.5 g) under vigorous stirring. The organic phase was separated, washed with brine (200 mL), and filtered. The solvent was evaporated completely in vacuo, and the crude product was purified by column chromatography (silica gel, ethyl acetate-petroleum ether 1:2) to afford desired product IM as a light yellow oil (2.0g, 64%); MS (+ESI) m/z = 318.1 (M+H)$^+$; ^{1}H-NMR (CDCl$_3$) δ 0.87 (d, 6H, 2CH$_3$), 1.44 (m, 3H, -CH$_3$), 1.82 (m, 1H, -CH), 2.03 (s, 3H, -CH$_3$), 2,42 (m, 2H, -CH$_2$), 3,69 (m, 1H, -CH), 4,76 (m, 2H, -CH$_2$), 7.06 (d, J = 7.8, 2H, Ar-H), 7.14 (d, J = 7.8, 2H, Ar-H). ^{13}C-NMR (CDCl$_3$) δ 8.60, 17.95, 21.89 (2C), 29.71, 44.38, 44.54, 53.58, 126.67 (2C), 128.97 (2C), 133.14, 136.76, 139.53, 140.26, 151.54, 173.55.

3. Stability evaluation in aqueous solution

The aqueous solution of IM was studied at pH 1.2, 5.5, and 7.4 at 37℃ using HCl and phosphate buffer. 45 μL of IM solution (100 μg/mL, in water-methol) were added to the buffer solution. The solutions were placed into a thermostatically controlled water bath at 37℃. The samples were withdrawn at appropriate time interval (0, 2, 5, 10, 15,20, 30, 60, 90, 120, 150, 180, 210, 240 min) and assayed for IM by LC-MS/MS [6, 7].

4. Stability evaluation in rat blood

45 µL of IM solution (300 µg/mL, in water-methol) were added to the fresh rat plasma. The solutions were placed into a thermostatically controlled water bath at 37℃. The samples were withdrawn at appropriate time interval (0, 2, 5, 10, 15, 20, 30, 60 min) and assayed for IM by LC-MS/MS.

5. Stability evaluation in rat blood with esterase inhibitor

55 µL of DDV (dimethyl-2,2-dichlorovinyl phosphate, 25 mg/mL) solution was added to the fresh rat plasma, then 45 µL of IM solution (300 µg/mL, in water-methol) added. The solutions were placed into a ice-bath. The samples were withdrawn at appropriate time interval (0, 2, 5, 10, 15, 20, 30, 60 min) and assayed for IM by LC-MS/MS.

RESULTS AND DISCUSSION

IM was synthesized as shown in Scheme 1. Ibuprofen **1** with 4-chloromethyl-5-methyl-1, 3-dioxolen-2-one afforded **2**, KI was needed to exchange chloride to accelerate reaction[8, 9].
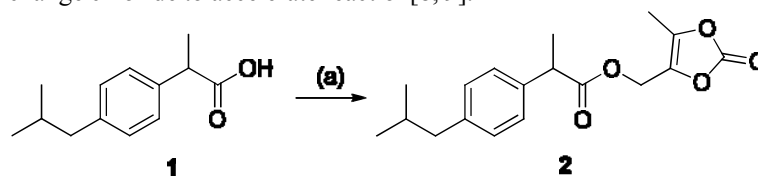
Scheme 1 (a) Reagents and conditions: 4-chloromethyl-5-methyl-1, 3-dioxolen-2-one, K₂CO₃, KI, DMF, r.t., 12 h

In order to test the stability of IM at different pH environments encountered in the gastrointestinal tract, the aqueous stability of which was determined in PBS buffer at 1.2, 5.5, and 7.4 at 37℃. The estimated half-lives ($t_{1/2}$) of IM in these buffer were calculated from linear regression plots of IM concentrations versus time and as shown in Table 1. In PBS buffer at 1.2, the simulated gastric juice, half-life of IM was 154.45 h, which is the longest half-life in all PBS buffer. In PBS buffer at 5.5, half-life of IM was 29.43 h, and in PBS buffer at 7.4 buffer, the simulated intestinal juice, half-life of IM was 3.15 h. (Figure 4) The results showed IM was very stable in acidic condition encountered in the stomach and alkaline condition encountered in the intestine, which indicated that IM can pass through the stomach without degradation, and with very slow degradation in intestinal tract.

The stability of IM was determined in rat plasma, and rat plasma with DDV *in vitro* to obtain the contribution of enzymatic hydrolysis (Figure 5). The estimated half-lives of IM in these plasma were calculated from linear regression plots of IM concentrations versus time and summarized in Table 1. In rat blood, half-life of IM was found to be 9.03 min. While in rat blood with DDV, half-life of IM was 79.96 h. The results showed that IM were rapidly metabolized to ibuprofen by esterase in plasma.
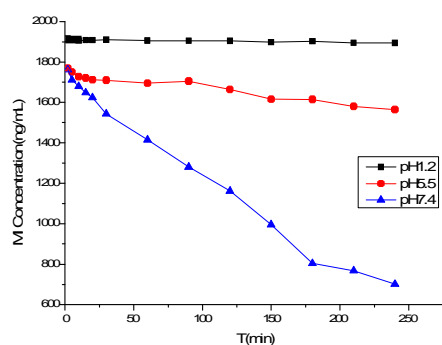
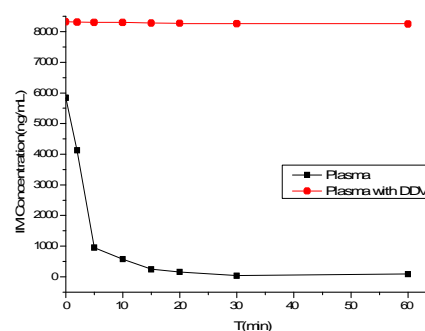**Figure 4 Degradation curve of IM at different pH environments**

Figure 5 Degradation curve of IM in rat blood and blood with DDV

Table 1 half life ($t_{1/2}$) of IM in different pH buffer and rat blood

$t_{1/2}$	pH = 1.2	pH = 5.5	pH = 7.4	Rat blood	Blood with DDV
	154.45 h	29.43 h	3.15 h	9.03 min	79.96 h

CONCLUSION

Novel medoxomil prodrug of ibuprofen was designed and synthesized. Its *in vitro* stabilities in simulated gastrointestinal tract juice were examined, and half-lives of IM were calculated. In addition, in order to obtain the contribution of enzymatic hydrolysis, the *in vitro* stability of IM was determined in both rat plasma and plasma with esterase inhibitor. The newly synthesized ibuprofen medoxomil prodrug IM showed high stability in gastrointestinal tract juice and rapid hydrolysis in rat plasma, which demonstrated esterase play a key role in the activation of IM. These primary *in vitro* results indicate that IM has the characteristics of prodrug, whose pharmacokinetic evaluation *in vivo* is ongoing.

REFERENCES

[1] DM Dirig; PC Isakson; TL Yaksh, *J Pharmacol Exp Ther.*, **1998**, 285(3): 1031-8.

[2] JF Mann; M Goerig; K Brune; FC Luft, *Clin Nephrol.*, **1993**, 39(1): 1-6.

[3] M Babazadeh , *Int J Pharm.*, **2006**, 316(1-2): 68-73.

[4] A Nayak; A Jain, *Sci Pharm.*, **2011**, 79(2): 359-73.

[5] T Ishizuka ; I Fujimori ; A Nishida ; H Sakurai ; Y Yoshigae ; K Nakahara ; A Kurihara ; T Ikeda ; T Izumi , *Drug Metab Dispos.*, **2012**, 40(2): 374-80.

[6] V Ioffe ; T Kalendarev ; I Rubinstein ; G Zupkovitz , *J Pharm Biomed Anal.*, **2002**, 30(3):391-403.

[7] NR Chatterjee ; AA Kulkarni ; SP Ghulekar , *Eur J Med Chem.*, **2008**, 43(12): 2819-23.

[8] BP Bandgar; RJ Sarangdhar ; S Viswakarma ; FA   Ahamed , *J Med Chem.*, **2011**, 54(5): 1191-201.

[9] BP Bandgar ; RJ Sarangdhar ; F Khan ; J Mookkan ; P Shetty ; Singh G, *J Med Chem.*, **2011**, 54(16): 5915-26.