

The influence of running exercise training on pharmacokinetics of meloxicam in ratsKaung-Jen Chien^{1,2,#}, Chi-Ting Horng^{2,3,#}, How-Ran Chao⁴, Wan-Ping Lee⁵, Hui-YunWang², Fu-An Chen^{2,*}¹ Department of Pediatrics, Veteran General Hospital-Kaohsiung, Kaohsiung, Taiwan.² Department of Pharmacy & Graduate Institute of Pharmaceutical Technology, Tajen University, Pingtung, Taiwan³ Department of Ophthalmology, Kaohsiung Armed Force General Hospital, Kaohsiung, Taiwan.⁴ Department of Environmental Science and Engineering, National Pingtung University of Science and Technology, Neipu, Pingtung, Taiwan.⁵ Department of Nursing, Tajen University, Pingtung, Taiwan.

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Abstract: Meloxicam (MXM) is a relatively new and a COX-2 preferential NSAID used for inflammation relief of sport injury with less gastrointestinal side effects such as peptic ulcers. The present study is to investigate the influence of running exercise training on pharmacokinetics of MXM in rats. In this study, animals (male SD rats) were divided into three groups: (1) sedentary group, (2) 4 weeks exercise group and (3) 8 weeks exercise group. Progressive training was adopted on a rodent treadmill machine. After single dose administration of MXM, blood samples were taken at different time points. Plasma was subjected to liquid-liquid extraction and further analyzed by a high-performance liquid chromatography (HPLC) method. Chromatographic separation was performed on a Cosmosil 5C18-AR-II reverse-phase HPLC column (150 × 4.6mm i.d., 5 μm) with a mobile phase of 20mM potassium dihydrogen phosphate-acetonitrile (60:40, v/v, pH 3.5) and UV detection at a wavelength of 355 nm. The CYP2C9 is one of most important enzyme affecting the metabolism of non-steroidal anti-inflammatory drugs in the liver. This study also determined CYP2C9 activity to explore the running exercise training on the correlation of liver enzymes and blood levels of MXM. The results revealed that long term running exercise can increase Tmax and decrease Cmax and the area under curve (AUC), which could be associated with the liver microsomal CYP2C9 activity.

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1. Introduction

Sports activities at any level have an inherent risk of injury which decreases the athlete's exercise capacity and can further influences athletic performance (1). Mazieres et al. suggest that nonsteroidal anti-inflammatory drugs (NSAIDs) are useful in benign ankle sprain, without revealing unexpected adverse events (2). The effect of NSAIDs on delayed-onset muscle soreness (DOMS) is also been approved by Almekinders (3).

NSAIDs work in part by blocking certain enzyme pathways called the cyclooxygenase (COX) pathways, thereby reducing the production of prostaglandins, thus achieve analgesic, antipyretic and anti-inflammatory effect (4). There are two main COX pathways that NSAIDs target, COX-1 and COX-2. These drugs such as diclofenac, ibuprofen, naproxen and meloxicam can be applied to the pain caused by sports injuries (1,5). Long-term use of non-selective NSAIDs such as diclofenac and ibuprofen will affect

the synthesis of prostaglandins, which can induce side effects including hypertension, altered renal function, gastrointestinal disturbance (including peptic ulceration), and the recently discovered increased rates of myocardial infarction with non-selective NSAIDs (6,7).

Meloxicam (MXM) is an enolic acid that belongs to oxicam class of NSAIDs and is a potent COX-2-selective inhibitor. MXM has a ten-fold selectivity in inhibiting Cox-2 over Cox-1 in vitro. The specificity for COX-2 is believed to make MXM less likely to cause gastrointestinal mucosal injury compared to standard NSAIDs. Therefore, it has been widely used. Exercise may affect biological factors such as metabolism resulting in influence on the pharmacokinetics of drugs (8). There is limited research on the effect of different sport cycle on pharmacokinetics of MXM. The purpose of this study is the influence of running exercise training of rats on pharmacokinetics of MXM and the correlation

between different sport cycle and the activity of liver metabolic enzyme, CYP2C9.

2. Material and Methods

Material

Meloxicam (MXM) and piroxicam (PXM) and were purchased from Sigma Chemicals (St. Louis, MO, USA). LC-grade acetonitrile was obtained from Tedia (Fairfield, OH, USA). Ortho-phosphoric acid was purchased from Merck (Darmstadt, Germany). The Cosmosil C18-AR-II reverse-phase high-performance liquid chromatographic (HPLC) column (150 × 4.6 mm i.d., 5 µm) was obtained from Nakalai Tesque (Kyoto, Japan). Bradford reagent for total protein assay was from Sigma (St. Louis, USA). Commercial diagnostic kits for rat cytochrome P450 2C9 (CYP2C9) enzyme were purchased from Cusabio (Newark, DE, USA). All other chemicals were of analytical reagent grade.

Pharmacokinetic study

Male Sprague-Dawley (SD) rats (5 weeks old) were bought from BioLASCO Taiwan Co. Ltd. Rats were maintained under standard laboratory conditions (12 h light/dark cycle, temperature (22 ± 2) °C). Rats were procured 1 week before the experiments to allow them to acclimatize to the laboratory environment. Standard chow and water were available *ad libitum*. This study was approved by the appropriate animal care and use committee of Tajen University with approval No. IACUC-97-020.

In this study, rats were divided into three groups: not exercise group (CTRL), 4 weeks (Ex-4W) and 8 weeks (Ex-8W) of treadmill training group. Training model adopted progressive mode in rodents treadmill motivated training (9). The training programs were seen in Table 1. Motivation was provided by an electric shock zone at the rear of each compartment. After training those rats was feed MXM by gastric gavage and blood collection at different time and the MXM blood concentration was measured by a high performance liquid chromatography (HPLC) method.

High performance liquid chromatography method using UV detection (HPLC-UV) for the determination of MXM in rat plasma was applied with modification (10). The chromatographic separation of MXM was carried out using a reverse phase Cosmosil C18-AR-II (150 mm x 4.6mm, particle size 5µm) with a mobile phase of 20mM potassium dihydrogen phosphate-acetonitrile (60:40, v/v, pH 3.5) and UV detection at a wavelength of 355 nm. The flow rate of mobile phase was 1.2 mL/min and the temperature of incubator was 30 °C.

Before HPLC analysis 20 µl of the internal standard products (Piroxicam) and 20 µl 5 M HCl solutions add into 100 µl of plasma with Vortex, then, adding 1 mL ether with Vortex. After centrifuging, the supernatant solution was taken and dried with nitrogen. The residue added 200 µl mobile phase back solution with vortex, then injected 50 µl to HPLC analysis. The pharmacokinetic parameters were analyzed using a pharmacokinetic software WinNonlin (Pharsight, Mountain View, CA, USA).

Table 1. Running exercise training program of rats on the treadmill.

Weeks	Exercise time (min)	Speed (m/min)				
		Day 1	Day 2	Day 3	Day 4	Day 5
1	30	8	8	8	8	8
2	45	10	12	12	12	12
3	45	15	15	18	18	18
4	60	21	21	24	24	24
5	60	24	24	24	24	24
6	60	24	24	24	24	24
7	60	24	24	24	24	24
8	60	24	24	24	24	24

Analysis of the activity of rat liver microsomal CYP2C9 enzyme

The preparation of rat liver microsomes was referred to Guengerich (11). After scarifying the rats, the rat was dissected and the liver was cleared by injecting 60 mLs of ice-cold saline into the hepatic portal vein and shear breaking inferior vena cava for excess blood discharge. Livers were dissected out rapidly and placed on ice cold saline and washed with ice-cold saline. The weight of the livers was recorded. The washed livers were blotted dry with filter paper and broken up by mincing. The liver mince was placed on 3mL/g liver homogenizing solution (1.15% KCl, 50 mM Tris-HCl, 1 mM EDTA, 20 µM butylated hydroxytoluene (BHT), 0.1mM phenylmethyl sulfonyl fluoride (PMSF), pH 7.4) and was homogenized 5 times by the Teflon pestle-glass homogenizer. The homogenate was centrifuged at 10,000 g for 20 minutes and the resulting supernatant then centrifuged at 100,000 g for 60 min to yield supernatant. The microsome pellet was resuspended in washing buffer solution (0.1 M potassium pyrophosphate, 1 mM EDTA, 20 µM butylated hydroxytoluene (BHT), 0.1mM phenylmethyl sulfonyl fluoride (PMSF), pH 7.4) by 4 times of homogenization and centrifuged at 100,000 g for 60 min. The washed microsome pellets were finally resuspended in 0.25 mL/g liver 0.25 M sucrose solution at 4 times of homogenization. The

sample of rat liver microsomes was aliquoted and all samples placed at -80°C (12).

The measurement of protein in liver microsomes referred to the method of Bradford (13). Bovine serum albumin (BSA) at the concentration of 0, 31.25, 62.5, 125, 250, 500 $\mu\text{g}/\text{mL}$ were used as the standard to prepare the calibration curve. Liver microsome suspension was diluted 100 times using 0.25 M sucrose. 10 μL diluted liver microsomes were pipetted into 96 well microplate and 200 μL coomassie brilliant blue G250 solution (0.01% coomassie brilliant blue G250, 5% ethanol, 8.5% phosphoric acid) was added to each well. All samples were measured at 595 nm on a 96 well microplate reader. The content of protein in liver microsomes was obtained from the constructed calibration curve. Enzyme activities for CYP2C in rat liver microsomes was determined using the rat cytochrome P450 2C9 (CYP2C9) ELISA kit (Cusabio, Newark, DE, USA) according to an adaptation of the protocol provided by the manufacturer.

Statistical analysis

All the experimental values are presented in the means \pm standard deviation (SD). Statistical comparisons were made by one-way ANOVA and subsequently applying Duncan test was performed using a SPSS statistic software, version 10.0 (Illinois, USA). Statistical significance was defined as $p < 0.05$.

3. Results and discussion

The aim of the study was to investigate the influence of running exercise training on pharmacokinetics of meloxicam (MXM) in rats. In order to measure the concentration of MXM in rat plasma, a HPLC method was established and validated. The chromatograms of MXM and its internal standard (piroxicam, PXM) was shown in Fig. 1. The validation parameters of HPLC such as linearity (>0.999), precision (both intraday and interday variability was less than 8.5%) and accuracy (98.5 - 104.6%) were satisfied the criteria of USP standards. In this study, animals (male SD rats) were divided into three groups: sedentary group (CTRL), 4 (Ex-4W) and 8 weeks (Ex-8W) exercise group. Progressive training was adopted in a rodent treadmill machine. After single dose administration of MXM, blood samples were taken at different time points. Plasma was subjected to liquid-liquid extraction and further analyzed by the validated high-performance liquid chromatography (HPLC) method. The pharmacokinetic parameters such as T_{max} , C_{max} and the area under curve (AUC) were analyzed

using WinNonlin pharmacokinetic software. According to Fig. 2, the T_{max} of Ex-8W groups was significantly higher than that of CTRL group ($p < 0.05$). As shown in Fig. 3 and Fig. 4, the C_{max} and the area under curve (AUC) of Ex-8W group were significantly lower than that of CTRL group ($p < 0.05$). The results indicate that long term running exercise can affected the pharmacokinetic parameters of MXM in rats.

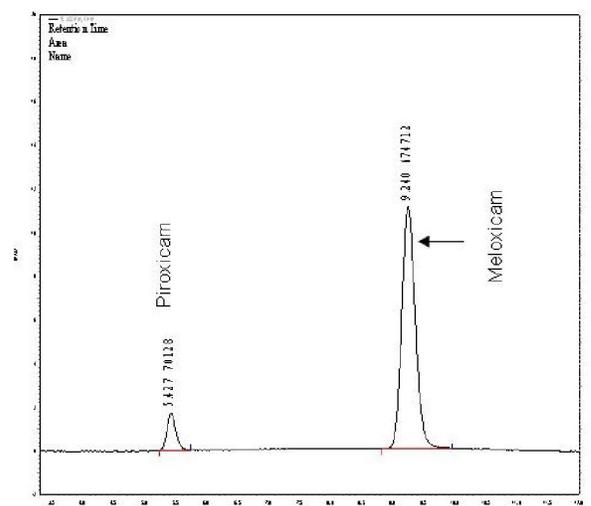


Fig. 1. HPLC chromatograms of piroxicam (internal standard) and meloxicam authentic standards.

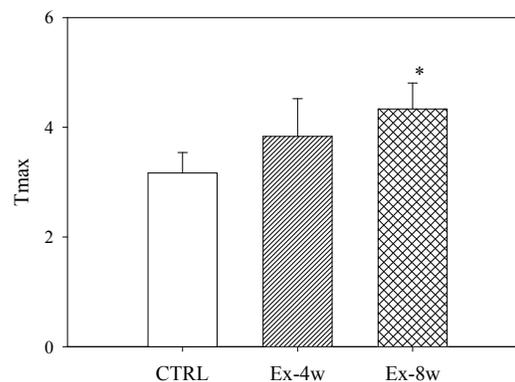


Fig. 2. Effect of running exercise training on pharmacokinetic parameter (T_{max}) of meloxicam. CTRL: not exercise group; Ex-4W: exercise training for 4 weeks; Ex-8W: exercise training for 8 weeks. Values (mean \pm SD) were obtained for each group of 6 animals. * $p < 0.05$ compared to the values of CTRL rats.

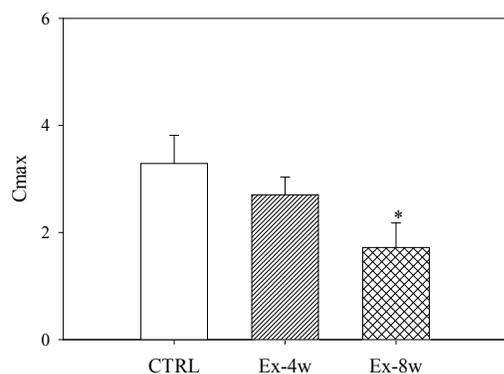


Fig. 3. Effect of running exercise training on pharmacokinetic parameter (Cmax) of meloxicam. CTRL: not exercise group; Ex-4W: exercise training for 4 weeks; Ex-8W: exercise training for 8 weeks. Values (mean±SD) were obtained for each group of 6 animals. * $p < 0.05$ compared to the values of CTRL rats.

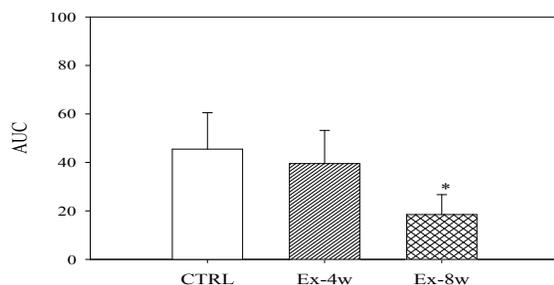


Fig. 4. Effect of running exercise training on pharmacokinetic parameter (AUC) of meloxicam. CTRL: not exercise group; Ex-4W: exercise training for 4 weeks; Ex-8W: exercise training for 8 weeks. Values (mean±SD) were obtained for each group of 6 animals. * $p < 0.05$ compared to the values of CTRL rats.

Metabolisms can change the medicine's distribution and the speed at which it leaves the body (14). MXM is metabolized extensively in the liver into four pharmacologically inactive metabolites that are excreted in both the urine and feces (15). The liver CYP2C9 enzyme plays important role on the metabolism of non-steroidal anti-inflammatory drug in the liver (16). This study also determined rat liver microsomal CYP2C9 activity to explore the running exercise training on the correlation of liver enzymes and blood levels of MXM. As shown in Fig. 5, the rat liver microsomal CYP2C9 enzyme activity

of Ex-8W groups were significantly higher than that of the CTRL group ($p < 0.05$).

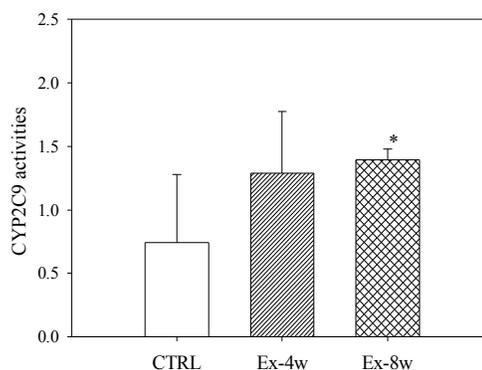


Fig. 5. Effect of running exercise training on liver microsomal CYP2C9 activity. CTRL: not exercise group; Ex-4W: exercise training for 4 weeks; Ex-8W: exercise training for 8 weeks. Values (mean±SD) were obtained for each group of 6 animals. * $p < 0.05$ compared to the values of CTRL rats.

The effects of exercise on pharmacokinetic changes were different for different medicines (8,17,18). Khazaemia et al. indicates that exercise will increase digoxin binding in working skeletal muscle with a concomitant decrease in the serum digoxin concentration (8). Panton et al. indicates that the plasma protein binding of propranolol is not changed after 16 weeks of moderate intensity exercise training (19). In case of MXM, the results revealed that long term running exercise can increase Tmax and decrease Cmax and AUC, which could be associated with the liver microsomal CYP2C9 activity. Persky et al. recommended that according to the specificity of the drug, when administration of drugs, the difference of medicinal properties and individual's physical level need to be considered (20). These results can provide the MXM dosage adjustment references for different physical level of the individual including athletes.

In conclusion, we have demonstrated that long term running exercise can affect the pharmacokinetic parameters of MXM such as Tmax, Cmax and AUC, which could be correlated with the liver microsomal CYP2C9 activity. The results provide important information on MXM dose adjustment according to different physical standards in order to achieving the ultimate purpose of the medication safety.

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