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The effect of β-cyclodextrin complexation on the bioavailability and hepatotoxicity of clotrimazole

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Clotrimazole, a poorly water-soluble antimycotic agent, is a promising therapeutic agent for various diseases including cancer and sickle cell anemia. The oral bioavailability and hepatic toxicity of clotrimazole were compared with its β-cyclodextrin inclusion form which was prepared by the spray-drying method. The inclusion complex gave significantly higher initial plasma concentrations, C_{max} and AUC than did clotrimazole alone, indicating that the drug from the inclusion compound could be more easily absorbed in rats. Furthermore, mice treated with the inclusion compound showed significantly higher GOT/GPT values compared to clotrimazole alone. The inclusion compound also induced hypertrophy of hepatic cells by fat accumulation and disappearance of hepatic sinusoids, indications of pathological changes of liver, suggesting that the inclusion compound could induce more severe tissue damage in the liver than clotrimazole alone. Thus, hepatotoxicity of clotrimazole seems to be correlated with the enhanced oral bioavailability by inclusion complexation. Our results suggest that, in the development of a novel oral product, appearance or enhancement of hepatic toxicity must be considered along with oral bioavailability.

1. Introduction

Clotrimazole [bis-phenyl-2-(chloro-phenyl)-1-imidazolylmethane], a lipophilic imidazole derivative, is an antimycotic agent with a broad spectrum (Pedersen et al. 1998). Furthermore, it is a promising agent for various diseases including cancer and sickle cell anemia, and rheumatoid arthritis (Wojtulewski et al. 1980) and show an anti-inflammatory effect (Ning et al. 2005). However, clotrimazole when administered orally, exhibits large differences in bioavailability due to its low aqueous solubility (solubility = 0.49 mg/L) (Pedersen et al. 1998, 1993) and slow dissolution in water (Pedersen 1993). Furthermore, oral delivery of clotrimazole induces severe hepatic toxicity (Coe et al. 2006; Miranda et al. 1998; Stuchal et al. 2006).

Recently, to provide better bioavailability of clotrimazole, a rectal formulation has been developed in a form of suppository with P 188 and propylene glycol as delivery vehicles (Yong et al. 2006). Moreover, this rectal delivery has shown to alleviate the hepatic toxicity of clotrimazole. To provide better bioavailability of clotrimazole via another oral product, we have also attempted a new oral formulation of clotrimazole by the inclusion with β-cyclodextrin. In the development of a novel oral product containing clotrimazole, any effect of the formulation on the clotrimazole-induced liver toxicity must be considered.

Thus, in this study, the inclusion compound of clotrimazole with β-cyclodextrin prepared by the spray-drying method was compared with clotrimazole alone in the aspects of the hepatic toxicity of clotrimazole in relation to the pharmacokinetics after oral administration. The GOT/GPT (glutamic oxaloacetic transaminase/glutamic pyruvic transaminase) levels and liver histology were evaluated for signs of toxicity.

Cyclodextrin complexation has been extensively applied to enhance the solubility, dissolution rate and bioavailability of slightly water-soluble drugs (Bekers et al. 1991; Jarvinen et al. 1995; Nakai, et al. 1984). To prepare the inclusion complexes, spray drying has been extensively applied since it has many advantages of a good yield in a short operating time and is suitable for extension to manufacturing scale (Nakai et al. 1984; Pedersen 1997).

2. Investigations, results and discussion

The pharmacokinetic parameters of clotrimazole were determined after oral administration of clotrimazole powder and inclusion compound. The clotrimazole-β-cyclodextrin (1 : 2) inclusion complex was prepared by spray-drying 0.2 g clotrimazole and 1.6 g β-cyclodextrin. Fig. 1 shows the change of mean plasma concentration of clotrimazole after oral administration of the preparations in rats. The total plasma concentrations of clotrimazole obtained from the inclusion compound were higher than those from clotrimazole alone. In particular, the initial plasma concentra-

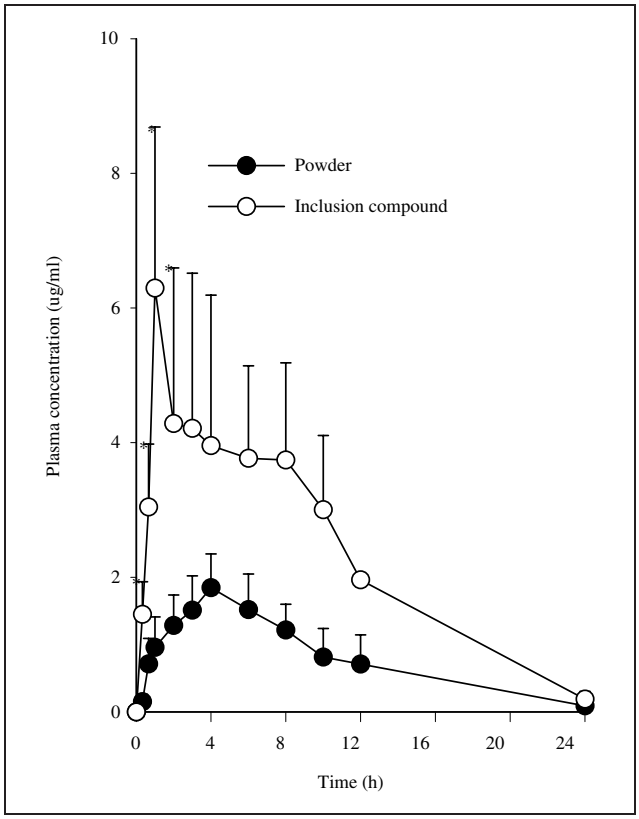

Fig. 1: Plasma concentration-time profiles of clotrimazole. After oral administration of clotrimazole alone (closed circle) and inclusion compound (open circle) to rats, 0.25 ml of blood was collected from the right femoral artery at predetermined time, and plasma was used for HPLC analysis. Each value represents the mean ± S.D. (n = 6). *P < 0.05 compared with clotrimazole powder

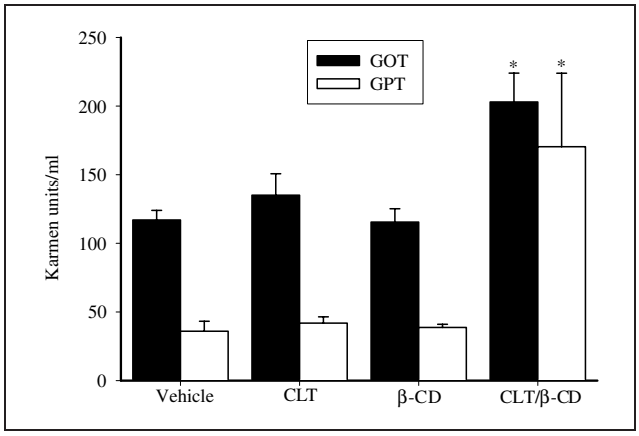

Fig. 2: The effects of clotrimazole in powder itself and inclusion compound on the serum levels of GOT and GPT in mice. The glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) activities were measured in the serum by using Serum Transaminase assay kit (Asan Pharm, Seoul, Korea). Blood was collected from drug treated mice by cardiac drainage, and serum was obtained by centrifugation at 3000 × g for 20 min

tions of clotrimazole from inclusion compound, until 2 h, were significantly higher than those of clotrimazole powder ($P < 0.05$). Our results suggest that the higher initial plasma concentrations of clotrimazole after administration as inclusion compound in rats were due to the increased dissolution rate of the drug (Kimura et al. 1997; Stella and Rajewski 1997).

The pharmacokinetic parameters are shown in the Table. The inclusion compound gave significantly shorter T_{max}, higher AUC, and C_{max} of clotrimazole than did clotrimazole alone ($P < 0.05$). In particular, the AUC of clotrimazole from the inclusion compound was about 3-fold higher than that from clotrimazole alone, indicating that the enhanced oral bioavailability of clotrimazole in the inclusion compound resulted from the marked increase in the absorption rate of clotrimazole due to the increased dissolution rate of clotrimazole from the inclusion compound

Table: Pharmacokinetic parameters of clotrimazole after oral administration of clotrimazole powder and inclusion complex at a dose of 80 mg/kg to rats

Parameters	Clotrimazole powder	Inclusion compound
AUC (h · μg/ml)	18.90 ± 6.24	56.15 ± 35.70*
T_{max} (h)	4.16 ± 0.98	1.00 ± 0.00*
C_{max} (μg/ml)	1.93 ± 0.46	6.29 ± 3.39*
K_{el} (h^{-1})	0.19 ± 0.10	0.20 ± 0.08
$T_{1/2}$ (h)	4.50 ± 2.47	3.90 ± 1.32

* P<0.05 compared with clotrimazole powder.
** Each value represents the mean ± S.E. (n = 6).

in rats (Becket et al. 1999; Gandhi and Karara 1988; Wong et al. 2001). However, the K_{el} and $t_{1/2}$ values of clotrimazole from the inclusion compound were not significantly different from those of clotrimazole alone. Our results suggested that the inclusion compound would be useful to deliver clotrimazole in a pattern that allows fast absorption in the initial phase. Thus, the oral bioavailability of clotrimazole could be improved markedly by inclusion complexation.

Hepatotoxicity of clotrimazole, β-cyclodextrin and the inclusion compound was evaluated after oral administration in mice. The hepatotoxicity was assessed as the activity of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvic transaminase (GPT). The mice treated with clotrimazole hardly gave higher GOT and GPT compared to control and β-cyclodextrin. However, the mice treated with inclusion compound showed significantly higher GOT/GPT values than those treated with clotrimazole alone (Fig. 2).

In the histological examinations as shown in Fig. 3, there were no remarkable changes associated with tissue damage in the liver tissues treated with clotrimazole or β-cyclodextrin for 24 h compared to normal liver tissue (A, B). In the cytoplasm of liver tissues from the clotrimazole-treated group (C, D), an initial stage of fat accumulation (arrows) was observed. The similar changes were shown in the β-cyclodextrin-treated group (E, F). However, in the inclusion compound-treated group (G, H), there was a severe change of normal hepatic structures. The nucleus has been condensed, which is an evidence of cell necrosis, and the number of Kupffer's cells was markedly decreased. Moreover, the hepatic cells showed hypertrophy by fat accumulation and hepatic sinusoids have been narrowed (arrows). These results suggest that the inclusion compound could induce more severe tissue damage in the liver than clotrimazole (Coe et al. 2006; Miranda et al. 1998; Stuchal et al. 2006).

In conclusion, the inclusion compound gave significantly higher initial plasma concentrations, C_{max} and AUC of clotrimazole than did clotrimazole alone, indicating that the drug could be more easily absorbed from the inclusion compound. However, the inclusion compound induced significantly more severe tissue damage in the liver.

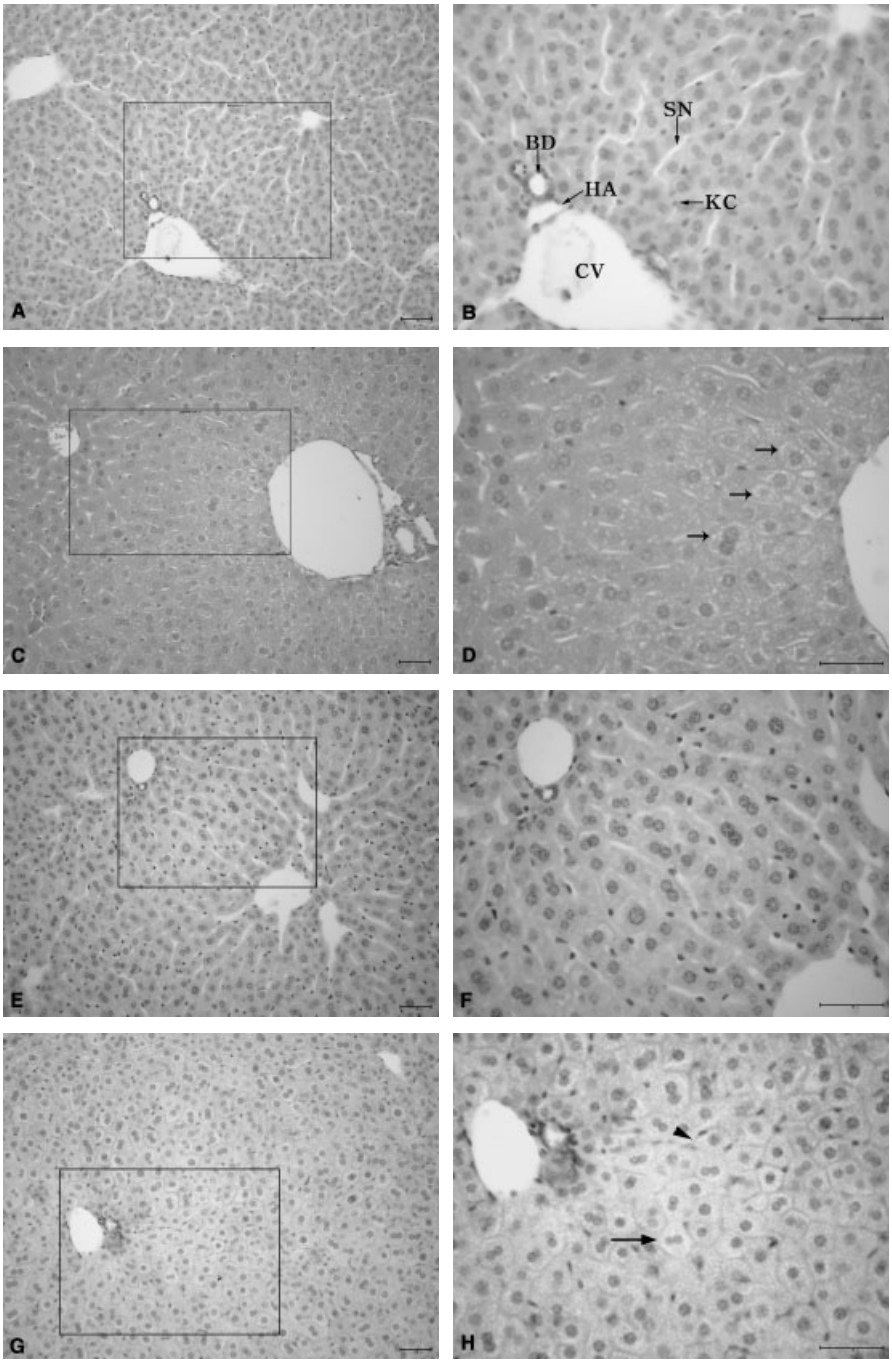

Fig. 3:
Histopathological changes of liver treated with clotrimazole (CLT), β-cyclodextrin (β-CD), and inclusion compound with β-cyclodextrin (CLT/β-CD). The pictures of the right column were high powered copies of rectangles in the pictures of the left column. The pictures (A, B) in the upper panel were from control group. In the groups treated with clotrimazole (C, D) and β-cyclodextrin (E, F), there were no pathological changes as compared to control group. In the mice treated with clotrimazole-β-cyclodextrin (I, H), the hepatic cells were shown hypertrophy by fat accumulation (arrow) and hepatic sinusoids have been narrowed (arrowhead). BD: bile duct, CV: central vein, HA: hepatic arteriole, KC: Kupffer's cell, SN: sinusoid. Bar = 40 μm

3. Experimental

3.1. Materials

Clotrimazole was provided by Boryung Pharmaceutical Co. (Anyang, Korea). β-cyclodextrin was purchased from Aldrich chemical Co. (Milwaukee, WIS, USA). Semipermeable membrane tube (Spectra membrane tubing No. 1) was purchased from Spectrum Medical Industries Inc. (Los Angeles, California, USA). Ethanol was obtained from Duksan Pharmaceutical Co. (Seoul, Korea). All other chemicals were of reagent grade and used without further purification.

3.2. Preparation of inclusion complex

A Büchi 190 nozzle type mini spray dryer (Flawil, Switzerland) was used for preparation of the clotrimazole-β-cyclodextrin inclusion complex. In brief, 0.2 g of clotrimazole and 1.6 g of β-cyclodextrin (molar ratio, 1 : 2) were dissolved in 100 ml ethanol and 100 ml water, respectively, and then mixed. The resulting clear solution was delivered to the nozzle at a flow rate of 5 ml/min using a peristaltic pump and thereafter spray-dried at 120 °C inlet temperatures with a flow rate of 10 ml/min. The residue, clotrimazole-β-cyclodextrin inclusion complex was collected (Choi et al. 2001; Lee et al. 1999).

3.3. Pharmacokinetic study

3.3.1. In vivo experiments

Male Sprague-Dawley rats weighing 250 ± 20 g were fasted for 12 h prior to the experiments but had free access to water. Tweleve rats were divided into two groups. Each rat, anesthetized in an ether-saturated chamber, was secured on a surgical board in the supine position with a thread. A polyethylene tube was inserted into the right femoral artery of the rat, all of the incision was covered with wet cotton and the cannula was flushed with 0.2 ml of heparinized normal saline (80 U/ml) to prevent blood clotting. Both clotrimazole alone and inclusion compound (0.36 g/kg equivalent to clotrimazole 80 mg/kg) were suspended in 1% povidone solution, and orally administered to rats in each group, respectively. Then, 0.25 ml of blood was collected from the right femoral artery at predetermined time and centrifuged at 3000 × g for 20 min using a centrifuge 5415C (Eppendorf, USA) (Chang et al. 2002; Ficarra et al. 2000).

3.3.2. Blood sample analysis

Plasma (100 μl) was mixed with 10 μl of ethanol solution containing clotrimazole/ibuprofen (100 μg/ml), as internal standard, 50 μl of 85% phosphoric acid, and extracted with 500 μl of dichloromethane. After vor-

texing vigorously for 1 min, it was then centrifuged at $3000 \times g$ for 2 min to separate the organic phase. After evaporation of the organic phase in a centrifugal vacuum concentrator, the residue was reconstituted with 50 µl of the mobile phase. Then, a 20 µl aliquot was analyzed by HPLC (Jasco UV-975, Japan) equipped with an Inertsil ODS-2 C_{18} column (GL science, 0.5 µm, 15 cm × 0.46 cm i.d.) and UV detector (Model L-7450). The mobile phase consisted of a mixture of methanol and 25 mM dibasic potassium phosphate buffer pH (6.3) (70 : 30, v/v) adjusted pH to 4.8 with 1M phosphoric acid. The mobile phase was filtered through a 0.45-µm filter (Millipore, Bedford, MA) and ultrasonically de-aerated prior to use. The eluent was monitored at 230 nm with a flow rate of 1.2 ml/min (Beckers et al. 1991).

3.3.3. Pharmacokinetic data analysis

The non-compartmental pharmacokinetic parameters including area under the drug concentration-time curve (AUC) were calculated using the RSTRIP II program (Salt Lake City, UT, USA). The maximal plasma concentration of drug (C_{max}) and time to reach maximum plasma concentration (T_{max}) were also obtained from plasma data. The data from different formulations were compared for statistical significance by the Student t-test. All results were expressed as mean ± standard deviation (S.D.).

3.4. Hepatotoxicity study

3.4.1. In vivo experiments

Male ICR mice weighing 20–22 g were used for each group in the experiment. Mice were divided into two groups (n = 6), fasted for 24 h prior to the experiments but had free access to water. Clotrimazole powder and inclusion compound (0.36 g/kg equivalent to clotrimazole 80 mg/kg) were suspended in 1% povidone solution, and orally administered to mice in each group. 24 h after drug administration, GOT/GPT (glutamic oxaloacetic transaminase/glutamic pyruvic transaminase) levels were determined by the Reitman-Frankel method (1957). The mouse serum (50 µl) was added to a 250 µl of mixture containing L-aspartic acid and α-ketoglutaric acid for GOT determination or to a 250 µl of mixture containing DL-alanine and α-ketogltaric acid for GPT determination. After the mixture was incubated at 37 °C for 1 h, 250 µl of 2,4-dinitrophenylhydrazine was added. After the mixture was incubated at 25 °C for 20 min, 2.5 ml of 0.4 N NaOH was added. After 10 min, the change of absorbance was measured at 505 nm with an UV-VIS spectrophotometer (Shimadzu, UV-1601, Japan).

3.4.2. Histologic analysis

The liver was isolated, fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS), embedded in paraffin using an embedding center and cut into slices. The paraffin-embedded tissue sections (4 µm) were stained with hematoxylin-eosin (Miyazaki et al. 1998) and observed under a light microscope (Leitz; Laborlux 12 Pols, Germany).

3.4.3. Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM) and A two-tailed Student's t-test was used to examine the significance of the data. The data is presented as a mean ± SEM. A P value < 0.05 was considered significant.

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References

Becket G, Schep LJ, Tan MY (1999) Improvement of the *in vitro* dissolution of praziquantel by complexation with α-, β-, and γ-cyclodextrins. Int J Pharm 179: 65–71.

Bekers O, Uijtendaal EV, Beijnen JH, Bult, A, Underberg WJM (1991) Cyclodextrins in the pharmaceutical field. Drug Dev Ind Pharm 17: 1503–1549.

Chang JY, Oh YK, Kong HS, Kim EJ, Jang DD, Nam KT, Kim CK (2002) Prolonged antifungal effects of clotrimazole-containing mucoadhesive thermosensitive gels on vaginitis. J Control Release 82: 39–50.

Choi HG, Lee BJ, Yong CS, Rhee JD, Han JH, Lee MK, Park KM, Kim CK (2001) Terfenadine-β-cyclodextrin inclusion complex with the anti-histaminic activity enhancement. Drug Dev Ind Pharm 27: 857–862.

Coe KJ, Nelson SD, Ulrich RG, He Y, Dai X, Cheng O, Caguyong M, Roberts CJ, Slatter JG (2006) Profiling the hepatic effects of flutamide in rats: a microarray comparison with classical aryl hydrocarbon receptor ligands and atypical CYP1A inducers. Drug Metab Dispo 34: 1266–1275.

Ficarra R, Ficarra P, Di Bella MR, Raneri D, Tommasini S, Calabrò ML, Villari A, Coppolino S (2000) Study of the inclusion complex of atenolol with β-cyclodextrin. J Pharm Biomed Anal 23: 231–236.

Gandhi RB, Karara AH (1988) Characterization, dissolution and diffusion properties of tolbutamide-β-cyclodextrin complex system. Drug Dev Ind Pharm 14: 657–682.

Jarvinen T, Jarvinen K, Schwarting N, Stella VJ (1995) β-Cyclodextrin derivatives, SBE4-β-CD and HP-β-CD, increase the oral bioavailability of cinnarizine in beagle dogs. J Pharm Sci 84: 295–299.

Kimura E, Bersani-Amado CA, Sudo SU, Santos SRJ, Oga S (1997) Pharmacokinetic profile of piroxicam-β-cyclodextrin, in rat plasma and lymph. General Pharmacol 28: 695–698.

Lee SW, Kim MH, Kim CK (1999) Encapsulation of ethanol by spray drying technique: effects of sodium lauryl sulfate. Int J Pharm 187: 193–198.

Miranda CL, Henderson MC, Buhler DR (1998) Evaluation of chemicals as inhibitors of trout cytochrome P450s. Toxicol Appl Pharmacol 148: 237–244.

Nakai Y, Yamamoto K, Terada K., Akimoto K. (1984) The dispersed states of medicinal molecules in ground mixtures with α- or β-cyclodextrin. Chem Pharm Bull 32: 685–691.

Ning M, Gu Z, Pan H, Yu H, Xiao K (2005) Preparation and in vitro evaluation of liposomal/niosomal delivery systems for antifungal drug clotrimazole. Indian J Exp Biol 43: 150–157.

Pedersen M, Bjerregaard S, Jacobsen J., Sørensen AM (1998) A genuine clotrimazole γ-cyclodextrin inclusion complex-isolation, antimycotic activity, toxicity and an unusual dissolution rate. Int J Pharm 176: 121–131.

Pedersen M (1993) Effect of hydrotropic substances on the complexation of clotrimazole with b-cyclodextrin. Drug Dev Ind Pharm 19: 439–448.

Pedersen M (1997) The bioavailability difference between genuine cyclodextrin inclusion complexes and freeze-dried or ground drug cyclodextrin samples may be due to supersaturation differences. Drug Dev Ind Pharm 23: 331–335.

Stella VJ, Rajewski RA (1997) Cyclodextrins: their future in drug formulation and delivery. Pharm Res 14: 556–567.

Stuchal LD, Kleinow KM, Stegeman JJ, James MO (2006) Demethylation of the pesticide methoxychlor in liver and intestine from untreated, methoxychlor-treated, and 3-methylcholanthrene-treated channel catfish (*Ictalurus punctatus*): evidence for roles of CYP1 and CYP3A family isozymes. Drug Metab Dispos 34: 932–938.

Wojtulewski JA, Gow PJ, Walter J, Grahame R, Gibson T, Panayi GS, Mason J (1980) Anti-inflammatory effect of clotrimazole. Ann Rheum 39: 469–472.

Wong JW, Yuen KH (2001) Improved oral bioavailability of artemisinin through inclusion complexation with β- and γ-cyclodextrin. Int J Pharm 227: 177–185.

Yun MO, Choi HG, Jung JH, Kim CK (1999) Development of thermoreversible insulin liquid suppository with sodium salicylate. Int J Pharm 189: 137–145.