# Reduced renal excretion of zalcitabine via the inhibition of organic anion transporters

Organic anion transporters의 억제에 의한
zalcitabine의 신배설 감소

2006년 2월 일

조 선 대 학 교 대 학 원

약 학 과

오 요 한

# Reduced renal excretion of zalcitabine via the inhibition of organic anion transporters

지도교수 한 효 경

이 논문을 약학 석사학위신청 논문으로 제출함.

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약 학 과

오 요 한

# 오요한의 석사학위논문을 인준함

위원장 조선대학교 교 수 최 준 식 

위원 조선대학교 교 수 최 후 균 

위원 조선대학교 교 수 한 효 경 

2005년 11월 일

## 조선대학교 대학원

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## Organic anion transpoters의 억제에 의한 zalcitabine의 신 배설 감소

오 요 한

지 도 교 수 : 한 효 경

조선대학교 대학원 약학과

Zalcitabine과 비스테로이드성 소염제와의 약물 동력학적 관계를 알아보기 위하여 이 연구를 수행하였다. Zalcitabine의 세포 내 흡수에 있어서 ketoprofen과 naproxen의 영향을 알아보기 위하여 hOAT1(사람의 유기 음이온 수송체1)이 과다 발현된 세포(CHO/hOAT1 cells)를 사용하였다. 또한 ketoprofen이나 naproxen (25mg/kg)으로 전처리 하거나 전처리 없이 zalcitabine (20mg/kg)을 쥐에 정맥 투여 함으로써 zalcitabine의 약물 동력학적 변화를 측정하였다. 그 결과 CHO/hOAT1 cells에 있어서 OAT1의 활성이 증가함으로써 zalcitabine의 세포 내 흡수가 현저하게 증가하였다. 반면 OAT1의 inhibitor로 전처리 하였을 경우 zalcitabine의 세포 내 흡수가 현저히 감소하였다. 쥐에 있어서 zalcitabine을 단독으로 정맥 투여한 경우와 ketoprofen이나 naproxen으로 30분 전에 전처리 한 후 zalcitabine을 정맥 투여한 경우를 비교해 볼 때 zalcitabine의 약물 동력학적 변화가 뚜렷이 나타났다. ketoprofen이나 naproxen으로 전처리 한 경우 zalcitabine의 신 클리어런스가 3

배에서 4배까지 감소하였다. 그 결과 ketoprofen과 naproxen으로 전처리 한 쥐에 있어서 zalcitabine의 systemic exposure가 zalcitabine을 단독 투여한 그룹보다 현저히 높아졌다. Zalcitabine의 반감기 또한 ketoprofen이나 naproxen으로 전처리 했을 때 4배에서 5배까지 길어졌다. 이러한 결과는 ketoprofen과 naproxen과 같은 비스테로이드성 소염제가 zalcitabine의 organic anion transpoters에 매개된 세뇨관 분비를 억제함으로써 zalcitabine의 약물 동력학의 변화에 영향을 끼쳤다는 것을 나타낸다. 그러므로, 잠재적인 약물 상호 작용의 임상적인 중요성을 고려할 때, 이러한 약물들을 병용 투여할 경우 주의 깊은 관찰이 필요하다.

# Abstract

## Reduced renal excretion of zalcitabine via the inhibition of organic anion transporters

Yo-Han Oh
Advisor : Prof. Hyo-Kyung Han
Department of Pharmacy
Graduate School of Chosun University

The present study aims to investigate the pharmacokinetic interaction between zalcitabine and nonsteroidal anti-inflammatory drugs (NSAIDs).

The effect of ketoprofen and naproxen on the cellular uptake of zalcitabine was examined using the transfected cell lines overexpressing the human organic anion transporter1 (CHO/hOAT1 cells). Pharmacokinetic parameters of zalcitabine were also determined following a single intravenous administration of zalcitabine (20 mg/kg) to rats in the presence and absence of ketoprofen or naproxen (25 mg/kg).

The cellular uptake of zalcitabine significantly increased by the enhancement of OAT1 activity in CHO/hOAT1 cells, while it was significantly reduced in the presence of OAT1 inhibitors. Compared to the control (given zalcitabine alone), the pretreatment of ketoprofen or naproxen 30 min prior to an intravenous administration (20 mg/kg) of zalcitabine significantly altered the pharmacokinetic profiles of zalcitabine in rats. Renal clearance of zalcitabine was reduced by approximately

three to four folds in the presence of ketoprofen or naproxen. Consequently, the systemic exposure (AUC) of zalcitabine in the rats pretreated with ketoprofen or naproxen was significantly ($p<0.05$) higher than that from the control group given zalcitabine alone. Terminal plasma half-life of zalcitabine was also prolonged by four to five folds in the presence of ketoprofen or naproxen. Those results suggest that NSAIDs such as ketoprofen and naproxen are effective to alter the pharmacokinetics of zalcitabine by inhibiting the organic anion transporter-mediated tubular secretion of zalcitabine.

Therefore, the concomitant use of NSAIDs with zalcitabine should require close monitoring for clinical consequence of potential drug interaction.

# 1. Introduction

Zalcitabine (2',3'-Dideoxycytidine or ddC), a pyrimidine nucleoside, is highly active against human immunodeficiency virus (HIV) and also hepatitis B virus (HBV) (1-3). It has been approved for the treatment of acquired immunodeficiency syndrome (AIDS) and related diseases either alone or in the combination with zidovudine. Zalcitabine is rapidly and extensively absorbed after oral administration, and the primary route of elimination is renal excretion of unchanged drugs with 60-70 % of an oral dose recovered in urine within 24 hours (4-5). Renal clearance of zalcitabine exceeds glomerular filtration rate, implying that the drug undergoes the active tubular secretion in the kidney (4-5).

Zalcitabine was reported to interact with organic anion transporters (6, 7) and the co-administration of probenecid, a competitive inhibitor of organic anion transporters, has been shown to alter the pharmacokinetics of zalcitabine in HIV-positive patients (8). So far, at least, three members of the OAT family have been detected in the rat (rOAT1-3), and five isoforms (hOAT1-5) in humans (9-11). Among those isoforms, OAT1 appears to interact with a broad spectrum of important therapeutics including nonsteroidal anti-inflammatory drugs (NSAIDs), β-lactam antibiotics, diuretics, hippurates and nucleoside antiviral drugs (6, 7, 12-15). Given that considerable number of drugs and toxins can interact with OAT1, potential drug interaction via OAT1-mediated renal excretion may require the close monitoring in the combination therapies of zalcitabine, particularly with commonly prescribed or over-the-counter

drugs for its implication on efficacy and toxicity. Furthermore, considering the short plasma half-life of zalcitabine (1-3hr), the inhibition of renal excretion of zalcitabine by the concomitant use of OAT1 inhibitors may prolong the systemic exposure of zalcitabine, resulting in less frequent dosing. Therefore, the present study aims to evaluate the pharmacokinetic interaction between zalcitabine and NSAIDs (OAT1 inhibitors) in the transfected cells overexpressing human organic anion transporter 1 (CHO/hOAT1) as well as in rats. Since rat OAT1 and human OAT1 share a high sequence homology (91 %) (15), rat was selected as an animal model for the pharmacokinetic studies of zalcitabine.

# 2. Materials and Methods

**2-1 Materials :** Zalcitabine, naproxen, ketoprofen, p-aminohippurate (PAH), 5-Bromo-2'-deoxyuridine (BDU), 6-carboxyfluorescein and BCA protein assay kit were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were reagent grade and all solvents were HPLC grade. CHO cells were purchased from ATCC (Rockville, MD, USA).

**2-2 Cells:** Chinese hamster ovary cells stably expressing hOAT1 (CHO/hOAT1 cells) have been generated and characterized as described by Ho et al. (16). CHO/hOAT1 cells were grown in F-12 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 200 μg/ml G-418. CHO-K1 cells were cultured in the same media without G418.

**2-3 Uptake studies**: Cells were seeded in 6-well culture plates at a density of $10^5$ cells/$cm^2$. At 4 days post-seeding, the cells were washed twice with pH 7.0 uptake buffer containing 1 mM $CaCl_2$, 1mM $MgCl_2$, 150 mM NaCl, 3 mM KCl, 1 mM $NaH_2PO_4$, 5 mM D-glucose, and 5 mM HEPES. Zalcitabine solution (0.5 mM) in the presence or absence of inhibitors was added to each well and incubated for 15 min at 25 ℃. At the end of incubation, drug solution was removed and the cells were washed three times with ice- cold PBS. After cell lysis by adding 1 mL of Milli-Q water, cells were harvested and sonicated for 1-2 min.

Trichloroacetic acid (3-5%) was added to the cell lysate, vortexed rigorously, and centrifuged for 5 min at 3000 rpm. After filtration of the supernatant through a membrane filter (0.45 μm), samples were analyzed by HPLC. The protein amount of each sample was determined with BCA protein assay kit following manufacturer's instruction (Sigma Chemical Co., St. Louis, MO, USA).

**2-4 Animal Studies :** All animal studies were performed in accordance with the experimental protocols approved by the animal care committee of Chosun University.

Male Sprague-Dawley rats weighing 280-300 g were obtained from Samtako Bio Co., Ltd (Osan, Korea). At the experiment, rats were divided into three groups, comprising 5 rats per each group. Group 1 – 3 were given intravenously 20 mg/kg of zalcitabine with either (i) naproxen sodium (20 mg/kg), (ii) ketoprofen (20 mg/kg) 30 min prior to the administration of zalcitabine, or (iii) no concomitant treatment (control). Blood samples were collected from the right femoral artery at 0, 0.083, 0.16, 0.33, 0.5, 1, 2, 4, 8, 12 and 24 hr following a zalcitabine administration. Urine was also collected for 24 hrs from the same group of rats. Blood samples were centrifuged at 3000 rpm for 10 min to obtain the plasma for the HPLC assay. Urine samples were centrifuged for 10 min at 3000 rpm and then passed through a membrane filter (0.45 μm). All samples were stored at - 70 ℃ until analyzed.

**2-5 HPLC Assay:** The plasma and urine concentrations of

zalcitabine were determined by a HPLC assay described as follows. Briefly, the internal standard (20 μg/mL of BDU) was added to plasma samples and then samples were deproteinized by adding acetonitrile. After centrifugation of the samples for 10 min at 3000 rpm, the supernatant was evaporated to dryness under Nitrogen stream. The residue was reconstituted with 100 μL of mobile phase and then a 50 μL of aliquots was injected directly into the HPLC system. The filtered urine samples (50 μL) were injected into HPLC system after appropriate dilution. The chromatographic system was consisted of a pump (LC-10AD), an automatic injector (SIL-10A) and a UV detector (SPD-10A) (Shimadzu Scientific Instruments, Japan) set at 254 nm. An octadecylsilane column (Gemini C18, 4.6 × 250 mm, 5μm; Phenomenex, Torrance, CA, USA) was eluted with a mobile phase consisting of 10% methanol in phosphate buffer, pH 6.8 at a flow rate of 1.0 mL/min. The calibration curve from the standard samples was linear over the concentration range of 0.1 μg/mL to 20 μg/mL. The limit of detection was 0.1 μg/mL.

**2-6 Data Analysis**

**Pharmacokinetic Analysis**: Non-compartmental pharmacokinetic analysis was performed using Kinetica-4.3 (InnaPhase Corp., Philadelphia, PA, USA). The area under the plasma concentration-time curve (AUC) was calculated using the linear trapezoidal method. The terminal elimination rate constant ($\lambda z$) was estimated from the slope of the terminal phase of the log plasma concentration-time points fitted by the method of least-squares, and then the terminal elimination half-life ($T_{1/2}$) was

calculated as 0.693/ $\lambda z$. Total clearance (CL) was estimated by dividing Dose by AUC and the renal clearance ($CL_R$) was determined as $CL_R$ = Ae/AUC, where Ae (amount of unchanged drug eliminated in urine) and AUC are measured over the same time interval.

**Statistical analysis**: All the means are presented with their standard deviation. The pharmacokinetic parameters were compared with a one-way ANOVA, followed by a posteriori testing with the use of the Dunnett correction. A $P$ value $< 0.05$ was considered statistically significant.

# 3. Results

**3-1 Cellular uptake Studies**: PAH and 6-carboxyfluorescein exhibited the significant inhibition effect on the cellular uptake of zalcitabine in the concentration dependent manner as shown in Fig. 1. The uptake of zalcitabine by CHO/hOAT1 cells was also significantly ($p<0.05$) reduced in the presence of naproxen or ketoprofen. Furthermore, the cellular accumulation of zalcitabine was three fold higher in CHO/hOAT1 cells than the uptake in the untransfected CHO-K1 cells, implying that the cellular uptake of zalcitabine was related to the enhancement of the organic anion transport activity in CHO/hOAT1 cells (Fig. 2).

**3-2 Pharmacokinetic Studies in rats**: The mean plasma concentration-time profiles of zalcitabine in the presence and absence of ketoprofen or naproxen were evaluated in rats and illustrated in Fig. 3. The mean pharmacokinetic parameters of zalcitabine were also summarized in Table I. Renal clearance of zalcitabine accounted for approximately 70 % of the total clearance in all cases, as consistent with previous reports (4, 5). As summarized in Table I, the pretreatment of naproxen 30 min prior to zalcitabine administration significantly ($p<0.05$) altered the pharmacokinetics of zalcitabine in rats, compared to the control given zalcitabine alone. Renal clearance and total clearance of zalcitabine decreased by about three to four folds in the presence of naproxen or ketoprofen. Consequently, the systemic exposure (AUC) of

zalcitabine was significantly ($P<0.05$) higher than the control given zalcitabine alone (Fig. 3). The terminal plasma half-life ($T_{1/2}$) of zalcitabine increased by four to five folds in the presence of naproxen or ketoprofen. In the control group, urinary excretion of zalcitabine was rapid, where approximately 84 % of the dose was excreted into urine within the first 8hrs. Following the co-administration of naproxen, the urinary excretion of zalcitabine was 58, 25 and 6 % of the administered dose in the 8 hr-, 12 hr- and 24 hr-urine samples, respectively (Fig. 3). In the presence of ketoprofen, the urinary excretion of zalcitabine was similar to that from the co-administration of naproxen.

Collectively, pretreatment of naproxen or ketoprofen prior to zalcitabine administration significantly altered the renal elimination and pharmacokinetic profiles of zalcitabine.

# 4. Discussion

In addition to a change in drug metabolism, particularly via cytochrome P450-mediated metabolism, there are increasing evidences suggesting that clinically important drug interactions can be caused by the modulation of drug transporters. For example, the oral exposure of talinolol decreased under the pretreatment of rifampicin, a P-glycoprotein inducer, while the concomitant administration of P-gp inhibitors such as verapamil increased the oral exposure of talinolol (17, 18). For cationic drugs, the decrease in renal excretion of dofetilide and procainamide by the co-administration of cimetidine can be explained by the inhibition of organic cation transporter-mediated active secretion at the basolateral membrane of renal proximal tubules (19, 20). While the majority of drug interactions has the potential to cause adverse effects, some interactions mediated by organic anion transporters have a positive impact such as combination therapy of cidofovir with probenecid that significantly reduces the nephrotoxicity of cidofovir (21). Therefore, transport proteins can play an important role in many clinical drug interactions, either negatively or positively.

The organic anion transporter family has been implicated in the distribution of zalcitabine to the CNS and the proximal tubular cells in the kidney [7-8]. However, previous studies were mainly relied on the inhibition studies to demonstrate the interaction of zalcitabine with organic anion transporters and the cellular uptake characteristics of zalcitabine need to be clarified in more details. Hence, the present study examined

the transport of zalcitabine across the cellular membrane by using transfected cell lines overexpressing human organic anion transporter 1 (CHO/hOAT1). As illustrated in Fig. 1 and 2, the cellular uptake of zalcitabine significantly increased by the enhancement of OAT1 activity in CHO/hOAT1 cells, while it was significantly reduced in the presence of OAT1 inhibitors such as ketoprofen, naproxen, PAH and 6-carboxyfluorescein. These results indicated that zalcitabine should be translocated by OAT1 across the cellular membrane. Based on these results, the potential drug interaction of zalcitabine via the OAT1-mediated transport pathway was evaluated in rats by using nonsteroidal anti-inflammatory drugs such as ketoprofen and naproxen as the potent inhibitors against OAT1.

Considering that naproxen and ketoprofen are eliminated more slowly than zalcitabine in rats (23, 24), the inhibition effect of NSAIDs on the renal secretion of zalcitabine was examined following a single intravenous administration of naproxen or ketoprofen 30 min prior to zalcitabine administration. Since plasma concentrations of zalcitabine decline very rapidly in the rat, larger doses must be administered to adequately characterize the disposition of zalcitabine as reported similarily in the previous pharmacokinetic studies (4, 25). Thus, in the present study, the dose of zalcitabine administered to rats was relatively large in comparison to those given to patients in clinical trials. No obvious toxicity was noted at this dose during the studies.

As illustrated in Fig. 3 and 4, concurrent use of naproxen or ketoprofen significantly altered the disposition of zalcitabine in rats. The

increase in zalcitabine's AUC with co-administration of naproxen or ketoprofen could be entirely accounted for by the reduction in renal clearance ($CL_R$) of zalcitabine. Considering that (i) zalcitabine undergoes the active tubular secretion in the kidney and (ii) both zalcitabine and NSAIDs could interact with organic anion transporters in the renal tubular cells, the reduction in $CL_R$ of zalcitabine in the presence of naproxen or ketoprofen might be resulted, at least in part, from the inhibition of organic anion transporters by naproxen or ketoprofen. The terminal plasma half-life ($T_{1/2}$) of zalcitabine increased about four to five folds in the rats pretreated with naproxen or ketoprofen, resulting in the delayed urinary excretion of zalcitabine.

Taken all together, the systemic exposure of zalcitabine increased with the slower renal excretion of zalcitabine when the drug was given with OAT1 inhibitors such as naproxen or ketoprofen. Although potential adverse effects, this interaction may provide a therapeutic benefit whereby it prolongs the duration of action of zalcitabine with longer plasma half-life, resulting in less frequent dosing of zalcitabine, and also lowers the dose administered. Therefore, clinical significance of this finding need to be further evaluated at the therapeutic dose levels in the clinical studies

# 5. Conclusion

Zalcitabine appeared to be translocated by OAT1 across the cellular membrane. Pretreatment of naproxen or ketoprofen, OAT1 inhibitors, prior to zalcitabine administration significantly altered the renal elimination and pharmacokinetic profiles of zalcitabine. Therefore, concomitant use of NSAIDS with zalcitabine should require close monitoring for potential drug interaction.

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# Ⅶ. References


1. M.A. Waqar, M.J. Evans, K.F. Manly, R.G. Hughes, and J.A. Huberman. Effects of 2',3'-dideoxynucleosides on mammalian cells and viruses. *J Cell Phys.* **121**: 402-408 (1984).
2. J.E. Dahlberg, H. Mitsuya, S.B. Blam, S. Broder, and S.A. Aaronson. Broad spectrum antiretroviral activity of 2',3'-dideoxynucleosides. *Proc Natl Acad Sci. USA.* **84**: 2469-2473 (1987).
3. K. Ueda, T. Tsurimoto, T. Nagahata, O. Chisaka, and K. Matsubara. An in vitro system for screening anti-hepatitis B virus drugs. *Virology* **169**: 213-216 (1989).
4. R.W. Klecker, J.M. Collins, R.C. Yarchoan, R. Thomas, N. McAtee, S. Broder, and C.E. Myers. Pharmacokinetics of 2',3'-dideoxycytidine in patients with AIDS and related disorders. *J Clin Pharmacol.* **28**: 837-842 (1988).
5. L.E. Gustavson, E.K. Fukuda, F.A. Rubio, and A.W. Dunton. A pilot study of the bioavailability and pharmacokinetics of 2',3'-dideoxycytidine in patients with AIDS or AIDS-related complex. *J Acquir Immun Defic Synd.* **3**: 28-31 (1990).
6. S. Wada, M. Tsuda, T. Sekine, S.H. Cha, M. Kimura, Y. Kanai, and H. Endou. Rat multispecific organic anion transporter 1 (rOAT1) transports zidovudine, acyclovir, and other antiviral nucleoside analogs. *J Pharmacol Exp Ther.* **294**: 844-849 (2000).
7. J.E. Gibbs, S.A. Thomas. The distribution of the anti-HIV drug, 2',3'-dideoxycytidine (ddC), across the blood-brain and blood-cerebrospinal

fluid barriers and the influence of organic anion transport inhibitors. *J Neurochem.* **80**: 392-404 (2002).

8. J.W. Massarella, L.A. Nazareno, S. Passe, and B. Min. The effect of probenecid on the pharmacokinetics of zalcitabine in HIV-positive patients. *Pharm Res.* **13**: 449-452 (1996).
9. J.E. Race, S.M. Grassl, W.J. Williams, and E.J. Holtzman. Molecular cloning and characterization of two novel human renal organic anion transporters (hOAT1 and hOAT3). *Biochem Biophys Res Comm.* **255**: 508-514 (1990).
10. T. Sekine, S.H. Cha, and H. Endou. The multispecific organic anion transporter (OAT) family. *Pfugers Arch-Eur J Physiol.* **440**: 337-350 (2000).
11. W. Sun, W.R. Rong, P.D. van Poelje, M.D. Erion. Isolation of a family of organic anion transporters from human liver and kidney. *Biochem Biophys Res Comm.* **283**: 417-422 (2001).
12. N. Apiwattanakul, T. Sekine, A. Chairoungdua, Y. Kanai, N. Nakajima, S. Sophasan, and H. Endou. Transport properties of nonsteroidal anti-inflammatory drugs by organic anion transporter 1 expressed in Xenopus laevis oocytes. *Mol Pharmacol.* **55**: 847-854 (1999).
13. S. Jariyawat, T. Sekine, M. Takeda, N. Apiwattanakul, Y. Kanai, S. Sophasan, and H. Endou. The interaction and transport of β-lactam antibiotics with the cloned rat renal organic anion transporter 1. *J Pharmacol Exp Ther.* **290**: 672-677 (1999).
14. Y. Uwai, H. Saito, Y. Hashimoto, and K.I. Inui. Interaction and transport of thiazide diuretics, loop diuretics, and acetazolamide via rat

renal organic anion transporter rOAT1. *J Pharmacol Exp Ther.* **295**: 261-265 (2000).

15. B.C. Burckhardt, and G. Burckhardt. Transport of organic anions across the basolateral membrane of proximal tubule cells. *Rev Physiol Biochem Pharmacol.* **146**: 95-158 (2003).
16. E.S. Ho, D.C. Lin, D.B. Mendel, and T. Cihlar. Cytotoxicity of antiviral nucleotides adefovir and cidofovir is induced by the expression of human renal organic anion transporter 1. *J Am Soc Nephrol.* **11**:383-393 (2000).
17. K. Westphal, A. Weinbrenner, M. Zschiesche, G. Franke, M. Knoke, R. Oertel, P. Fritz, O. von Richter, R. Warzok, T. Hachenberg, H.M. Kaumann, D. Schrenk, B. Terhaag, H.K. Kroemer, and W. Siegmund. Induction of P-glycoprotein by rifampin increases intestinal secretion of talinolol in human beings: a new type of drug/drug interaction. *Clin Pharmacol Ther.* **68**: 345-355 (2000).
18. T. Gramatte, and R. Oertel. Intestinal secretion of intravenous talinolol is inhibited by luminal R-verapamil. *Clin Pharmacol Ther.* **66**: 239-245 (1999).
19. S. Abel, D.J. Nichols, C.J. Brearley, and M.D. Eve. Effect of cimetidine and ranitidine on pharmacokinetics and pharmacodynamics of a single dose of dofetilide. *Br J Clin Pharmacol.* **49**: 64-71 (2000).
20. C.D. Christian, C.G. Meredith, and K.V. Speeg. Cimetidine inhibits renal procainamide clearance. *Clin Pharmcol Ther.* **36**: 221-227 (1984).
21. K.C. Cundy. Clinical pharmacokinetics of the antiviral nucleotide analogues cidofovir and adefovir. *Clin Pharmacokinet.* **36**: 127-143

(1999).

22. A.S. Mulato, E.S. Ho, and T. Cihlar T. Nonsteroidal anti-inflammatory drugs efficiently reduce the transport and cytotoxicity of adefovir mediated by the human renal organic anion transporter 1. *J Pharmacol Exp Ther.* **295**: 10-15 (2000).
23. J.H. Satterwhite, F.D. Boudinot. Age- and Dose-Dependent Naproxen Disposition in Fischer 344 rats. *J Gerontol.* **46**: 222-227 (1991).
24. J.H. Satterwhite, F.D. Boudinot. Pharmacokinetics of Ketoprofen in Rats: Effect of Age and Dose. *Biopharm Drug Dispo.* **13**: 197-212 (1992).
25. S.S. Ibrahim, and F.D. Boudinot. Pharmacokinetics of 2',3'-dideoxycytidine in rats: Application to interspecies scale-up. *J Pharm Pharmacol.* **41**: 829-834 (1989).

**Table** Ⅰ. Mean pharmacokinetic parameters of zalcitabine following an intravenous administration (20 mg/kg) of zalcitabine to rats in the presence and absence of ketoprofen or naproxen (Mean ± SD, n = 5)

| Parameters | Zalcitabine alone | Zalcitabine + Naproxen | Zalcitabine + Ketoprofen |
|---|---|---|---|
| $T_{1/2}$ (hr) | 1.9±1.3 | 10±1.8* | 8.4±3.0* |
| CL (L/hr/kg) | 0.67±0.11 | 0.20±0.09* | 0.25±0.06* |
| $CL_R$ (L/hr/kg) | 0.47±0.09 | 0.12±0.07* | 0.15±0.09* |
| Vdss (L/kg) | 1.0±0.3 | 1.3±0.8 | 1.5±0.4 |
| AUC (μg*hr/mL) | 46.6±21.9 | 191±50.9* | 140±22.5* |
| Ae (% dose) | 88±10 | 89±6.2 | 91±8.4 |

*: $p < 0.05$, significant difference compared to the control (given zalcitabine alone)

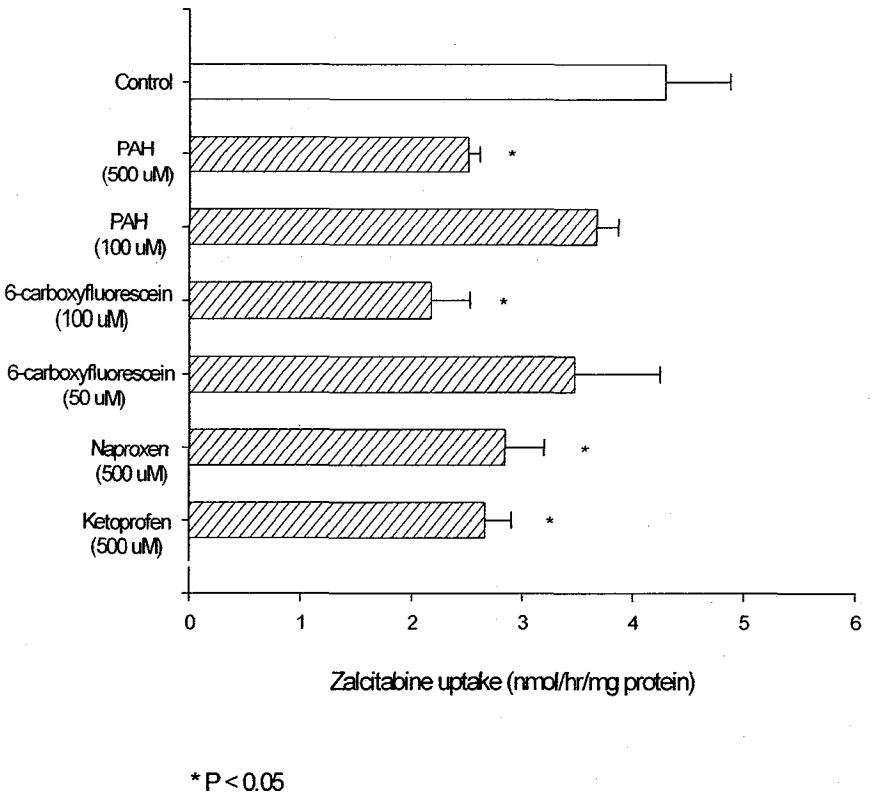


Fig. 1: Inhibition on the cellular uptake of zalcitabine in CHO/hOAT1 cells (Mean ± SD, n = 6)

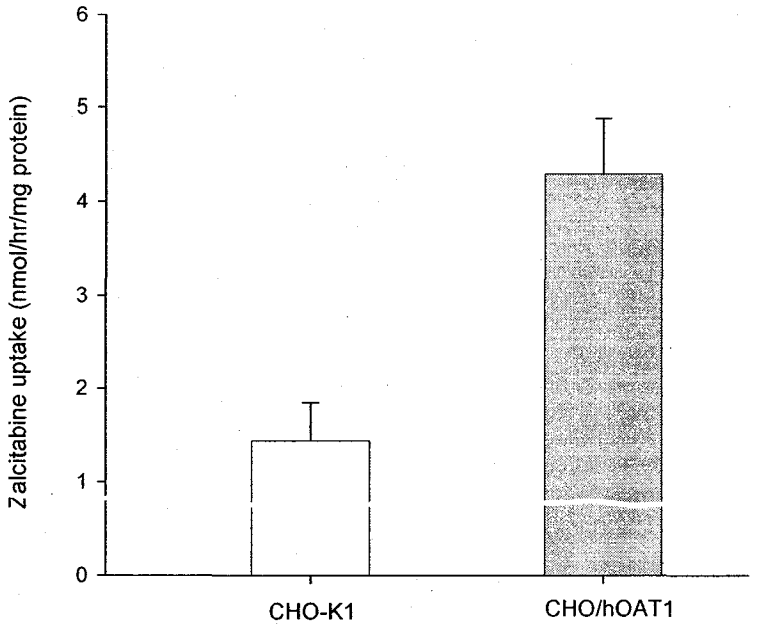


Fig. 2: Cellular accumulation of zalcitabine in CHO/hOAT1 cells and in the untransfected CHO-K1 cells (Mean ± SD, n = 6)

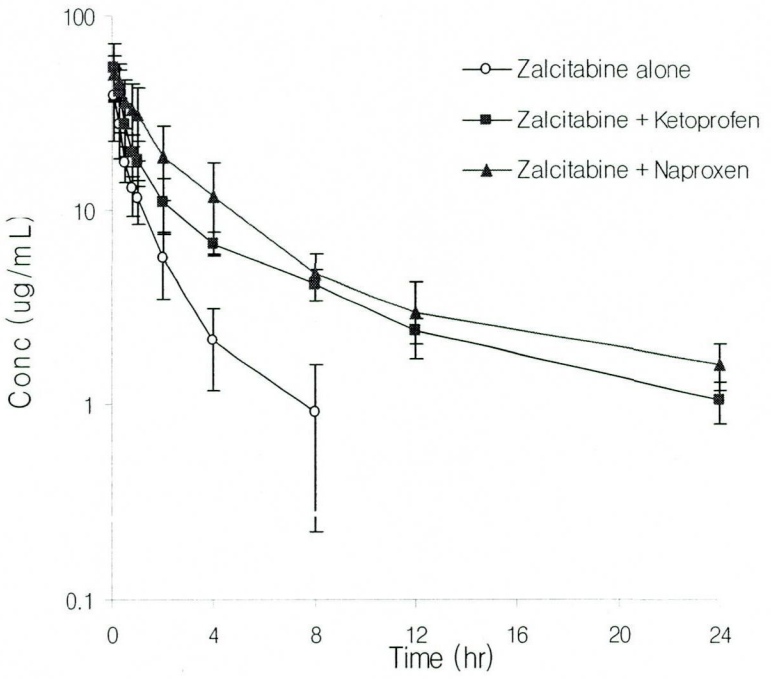


Fig. 3: Mean pharmacokinetic profiles of zalcitabine following an intravenous administration (20 mg/kg) of zalcitabine to rats in the presence and absence of ketoprofen or naproxen (Mean ± SD, n = 5)