Unidirectional Inversion of Ibuprofen in Caco-2 Cells: Developing a Suitable Model for Presystemic Chiral Inversion Study

Haiping HAO, a Guangji WANG, a,b Jianguo SUN, a Zuoqi DING, a Xiaolan Wu, a and Michael ROBERTS b

a Key Lab of drug Metabolism & Pharmacokinetics, China Pharmaceutical University; 1 Shennong Road, Nanjing 210038, China: and b Princess Alexandra Hospital, Therapeutics Research Unit, University of Queensland; 4102 Ipswich Road, Woolloongabba, Qld., Australia. Received September 28, 2004; accepted January 14, 2005

Intestinal chiral inversion of ibuprofen is still lacking direct evidence. In a preliminary experiment, ibuprofen was found to undergo inversion in Caco-2 cells. This investigation was thus conducted to determine the characteristics and influence of some biochemical factors on the chiral inversion of ibuprofen in Caco-2 cells. The effects of substrate concentration (2.5—40 µg/ml), cell density (0.5—2×10^6 cells/ well), content of serum (0—20%), coexistence of S ibuprofen (corresponding doses), sodium azide (10 mM), exogenous Coenzyme A (CoA) (0.1—0.4 mM), and palmitic acid (5—25 µM) on inversion were examined. A stereoselective HPLC method based on the Chromasil-CHI-TBB column was developed for quantitative analysis of the drug in cell culture medium. The inversion ratio (F) and elimination rate constant were calculated as the indexes of inversion extent. Inversion of ibuprofen in Caco-2 cells was found to be both dose and cell density dependent, indicating saturable characteristics. Addition of serum significantly inhibited the inversion, to an extent of 2.7 fold decrease at 20% content. Pre-existence of S enantiomer exerted a significant inhibitory effect (p<0.01 for all tests). Sodium azide decreased the inversion ratio from 0.43 to 0.32 (p<0.01). Exogenous CoA and palmitic acid significantly promoted the inversion at all tested doses (p<0.01 for all tests). This research provided strong evidence to the capacity and capability of intestinal chiral inversion. Although long incubation times up to 120 h were required, Caco-2 cells should be a suitable model for chiral inversion research of 2-APAs considering the human-resourced and well-defined characteristics from the present study.

Key words ibuprofen; presystemic inversion; Caco-2 cell; Coenzyme A (CoA); palmitic acid

Ibuprofen is one of the well known non-steroid anti-inflammation drugs (NSAIDs) and it is clinically the first choice among its own class, 2-arylpropionic acids, due to its low incidence of unwanted effects. It contains a chiral center adjacent to the carboxyl group and thus existed as (−) R and (+) S enantiomers. Ibuprofen was originally marketed as racemate and later in 1994 dexibuprofen was brought into the market in Austria and Switzerland based on the findings that the pharmacological activity exclusively resided in the (+) S enantiomer. Despite this, racemate is still extensively used worldwide and there is no indication of replacing the racemate with the single S enantiomer.

One important characteristics of ibuprofen pharmacokinetics is that the R enantiomer undergoes metabolic chiral inversion to its antipode but not vice versa. Since its first observation in 1967 by Adams, the pathway and involved biochemical mechanisms of ibuprofen inversion had been fundamentally elucidated in the past forty years. But reports concerning the site of inversion in the current literature still seemed to be contradictory. Mehvar and Jamali claimed that this unidirectional inversion occurred predominantly and presystemically in the gut based from a pharmacokinetics model study. Later observations that formulations with prolonged absorption exhibited greater extent of inversion supported the presystemic inversion of ibuprofen. To the contrary, no significant differences of the area under curve values (AUC) for S-ibuprofen was observed between oral and intravenous administration of racemic ibuprofen to humans, which precluded the existence of presystemic inversion. Similar research conducted in dogs led to the same conclusion. Furthermore, no evidence of intestinal inversion of ibuprofen was observed in an in situ isolated perfused rat intestine/liver system. To date, only one report gave direct evidence to the presystimic inversion of ibuprofen in humans. When (−) R ibuprofen was incubated with ileum or colon segments excised from the cancer patients, substantial inversion of up to 30% was observed within 200 min. Thus, the site of inversion remained controversial. Although (−) R ibuprofen was demonstrated to be inverted in all species tested, the inversion extent and site may vary from species to species, thus the results obtained from other species can not be extrapolated to humans. Unfortunately, human intestinal segments could only be obtained from patients who required intestinal removal, which limited the extension of research and might lead to high inter-assay variability. Therefore, development of a human-resourced model for research of the inversion of ibuprofen and other 2-arylpropionic acid drugs is of special importance.

Caco-2 cells, a cell line derived from human colon carcinoma, were extensively applied and proved a suitable model in the research of absorption characteristics and mechanisms of drugs. When Caco-2 cells reach confluence, the cells differentiate morphologically into polarized, columnar cells with organized microvillus membranes and also acquire many biochemical characteristics of enterocytes. Most importantly, long chain acyl-CoA synthetase (EC 6. 2. 1. 3), which was previously demonstrated to be stereoselective and the rate controlling enzyme in the ibuprofen inversion catalyzing ibuprofen-CoA formation was reported to be active on catalyzing fatty acid metabolism in Caco-2 cells.

Taking all information into account, a preliminary experiment had been conducted to test whether ibuprofen could undergo chiral inversion in Caco-2 cells. As a result, substantial inversion of (−) R ibuprofen (a 20% inversion was observed within 24 h coincubation) was observed when incubated in Caco-2 cells. The present investigation was then designed to:

* To whom correspondence should be addressed. e-mail: hhp770505@yahoo.com.cn © 2005 Pharmaceutical Society of Japan
1) present direct evidence to the intestinal inversion of ibuprofen; 2) develop Caco-2 cells into a model system in presystemic inversion research of ibuprofen and other 2-arylpropionic acid drugs by optimizing culture conditions; and 3) determine whether such an inversion occurred in Caco-2 cells through the same biochemical processes as that in the hepatocyte by cross-comparison with previous reports.

MATERIALS AND METHODS

Chemicals and Reagents 
(\(-\)R (ee%\(>98\%)), \((+)S\) ibuprofen (ee%\(>98\%)), and \(S(+)\) naproxen (ee%\(>98\%)) were purchased from BIOMOL Research Laboratories Inc. Coenzyme A disodium salt (CoA) and palmitic acid were obtained from Sigma Chemical Co. Dulbecco’s modified Eagle’s medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco. All other reagents and solvents were of analytical and HPLC grade.

Cell Culture and Various Treatments Coincubation

The human colon carcinoma cell line was obtained from ATCC (Rockville, MD, U.S.A.). Cells between passages 60—80 were used in all experiments. Cells were grown in dishes in DMEM supplemented with 10% FBS and 1% essential amino acids, and when 80% confluence reached, subcultured into 24, 12, or 6 well plates for experimental use. Four days after confluence was reached, the culture medium was removed and washed twice with Hank’s buffer solution (ph 7.4) before addition of fresh DMEM solution containing different treatments. After that, 3, 2 and 1.5 ml of culture medium contained various treatments was added into each well of the 6, 12, and 24 well culture plates, respectively.

Stock solution of \((-\)R, \((+)S\) and racemic ibuprofen was prepared in 100% ethanol at concentration of 8 mg/ml. Palmitic acid at concentration of 10 mM was prepared in 100% ethanol with slight heating while CoA disodium salt and sodium azide was directly dissolved in DMEM to yield the concentration of 12.3 mM and 1 mM, respectively.

The effect of serum content on inversion was investigated at FBS concentrations of 0, 2.5, 5, 10, and 20% with 10 \(\mu\)g/ml of racemic ibuprofen coincubation. Based on this research, all other experiments were conducted without addition of FBS into the culture medium. To determine whether the inversion of ibuprofen was saturated, inversion ratio of series concentrations of \((-\)R ibuprofen at 2.5, 5, 10, and 40 \(\mu\)g/ml were observed. Furthermore, influence of different cell density set at 0.5, 1, and 2\(\times\)10^6 cells per well were evaluated at \((-\)R ibuprofen concentration of 10 \(\mu\)g/ml. Racemic ibuprofen at concentrations of 5, 10, 20, 40, and 80 \(\mu\)g/ml were also administered to the culture system for determining the effect of the preexistent \(S\)-enantiomer on inversion. Sodium azide at a final concentration of 10 mM was coincubated with ibuprofen (10 \(\mu\)g/ml) to determine the role of ATP during the inversion process. Influence of CoA and palmitic acid on the inversion was also observed at final concentrations of 0.1, 0.2, and 0.4 mM for CoA, and 5, 10, 25 \(\mu\)M for palmitic acid coincubating with 10 \(\mu\)g/ml of \(R\)-ibuprofen, respectively. All experiments were conducted in triplicate. 100 \(\mu\)l of drug-containing medium was removed at 24, 48, 72, 96 and 120 h and stored at \(-20^\circ\)C before stereoselective HPLC analysis.

Analytical Method

One hundred microliters of drug containing medium was pretreated according to the previous report^9 with slight modifications. Briefly, 10 \(\mu\)l of the naproxen solution was added into the medium as an internal standard to yield the concentration of 10 \(\mu\)g/ml, after which 40 \(\mu\)l of 1 M HCL was added followed by vortexing for 30 s. n-Hexane-isopropanol (95 : 5, v/v/v : 1 ml) was then added into the tube followed by vortexing for 2 min. 800 \(\mu\)l of the upper organic layer was transferred into a new tube and evaporated to dryness under vacuum pump. The residue was reconstituted in 100 \(\mu\)l mobile phase.

All of the HPLC analysis was conducted in a LC-2010 liquid chromatograph system (SHIMADZU). The chiral separation was obtained on a Chromsil-100-5CHI-TBB column (250\(\times\)4.6 mm i.d., 5 \(\mu\)M ) protected with a guard column (50\(\times\)4.6 mm). The mobile phase consisted of \(n\)-hexane, methyl \(t\)-butyl ether, isopropanol (74 : 25 : 1, v/v/v) containing 0.1% galactic acid, at a flow-rate of 1ml/min at ambient temperature. The UV wavelength was set at 220 nm and 20 \(\mu\)l of each sample was injected. Baseline separations of both enantiomers and internal standards were retrieved within 10 min. Calibration curves for both enantiomers were established daily over the range of 0.3 to 80 \(\mu\)g/ml. All of the correlation coefficients were greater than 0.995, which indicated a good linearity over this range. The coefficient of variation for replicated analysis was less than 8% at each concentration of the calibration curve.

Data Analysis

The area under the concentration curves was calculated according to the linear trapezoidal rule. The elimination rate constants \((k_{\omega})\) of \((-\)R enantiomer were calculated from the first-order elimination equation. Assuming that \((-\)R ibuprofen undergoes no other metabolism except for inversion, the elimination constant can be approximately regarded as the inversion rate constants \((k_{inv})\). The fraction inverted \((F_i)\) when \((-\)R ibuprofen administered was calculated based on the principles that assuming no other metabolism occurred in Caco-2 cells (\(S\)-ibuprofen undergo negligible metabolism in Caco-2 cells evidenced from the fact that no significant decrease of concentration was observed during the 120 h coincubation), the inversion ratio can be calculated from the equation as follows:

\[
F_i = \frac{AUC_S}{AUC_R + AUC_S}
\]

(1)

where \(AUC_S\) is the AUC of the \(S\)-enantiomer while \(AUC_R\) is the AUC of the \(R\)-enantiomer following incubation with \(R\)-enantiomer. For racemic ibuprofen investigation, the inversion ratio was calculated based on the principles discussed by Friham^10 fitted to the \(in vitro\) model as follows:

\[
F_i = \frac{AUC_S - AUCT/2}{AUCT/2}
\]

(2)

where \(AUC_S\) and \(AUCT/2\) indicated the AUC of the \(S\)-enantiomer and half of AUC sum of both enantiomers following incubation with racemic ibuprofen.

Statistical Analysis

All statistical analysis was performed using SPSS software (SPSS 10.0 for windows). All data were expressed as means\(\pm\)S.D. Statistical significance was set at 0.05 or 0.01. A Student’s \(t\)-test (independent, two-tailed) was applied to the comparison between two groups, while one-way ANOVA followed by LSD test was introduced into multi-group analysis.
RESULTS

Effect of Serum Content on the Inversion of Racemic Ibuprofen in Caco-2 Cells In a preliminary experiment, (−)-R ibuprofen was observed to be inverted in Caco-2 cells cultured in normal culture conditions (DMEM supplemented with 10% FBS) albeit to a relatively low extent. The possible influence of the content of FBS on the inversion was subsequently evaluated in order to optimize the culture condition. The results were depicted in Fig. 1. A significant decrease of the inversion ratio was observed even with addition of 2.5% FBS (p<0.01). The inversion ratio was lowered to a greater extent with the increase of FBS content, and a 2.7 fold inhibition was demonstrated with addition of 20% FBS. Based on this result, all other experiments conducted in this paper were carried out in a serum-free culture system.

Effect of Substrate Concentration on Inversion To determine whether the inversion of (−)-R ibuprofen was saturated in cultured Caco-2 cells, five series doses from 2.5 to 40 μg/ml of R ibuprofen were coincubated with cells at a density of 2×10⁶ cells per well. The result was shown in Fig. 2. The highest inversion ratio was acquired at the lowest concentration of ibuprofen, indicating that such an inversion was saturated in Caco-2 cells cultured in the present conditions. The decreasing trend was apparent with the increasing substrate concentration, despite no significant difference being observed between ibuprofen concentration of 20 and 40 μg/ml. The inversion ratio was lowered 2.03 fold from 2.5 to 40 μg/ml of substrate concentration. Similar results were obtained from statistical analysis of the elimination rate of (−)-R ibuprofen. However, contradictory results were reached when plotting AUCS or AUCR against the substrate concentration followed by linear regression analysis. Good linearity was obtained for curve of both enantiomers (r²=0.997 and 0.9968 for AUCS and AUCR, respectively). To explain and resolve such a contradictory result, we further calculated the ratio of AUCS:AUCR and plotted it against the substrate concentration; a substantial decrease of the AUCS:AUCR ratio was observed accompanied with increasing substrate concentration which is consistent with the Fᵢ value calculated (Fig. 3).

Effect of Cell Density on Inversion To further optimize the culture condition and testify the saturation characteristics, the influence of cell density on the inversion was evaluated (Fig. 4). A significant and increasing inversion was demonstrated at cell density of 2×10⁶ cells per well as compared to that of 0.5, 1×10⁶ cells per well (p<0.01 for both), though the inversion ratio between 0.5 and 1×10⁶ cells per well was of no significance (p>0.05). This result further supported that the inversion of (−)-R ibuprofen was saturated.

Effect of the Preexistence of S-Enantiomer on Inversion Previous reports concerning the influence of S-enantiomer over the inversion of (−)-R ibuprofen seemed to be contradictory. Therefore, racemic ibuprofen at concentrations of 5, 10, 20, 40 and 80 μg/ml (corresponding to the concentration of R ibuprofen investigated) were coincubated with Caco-2 cells in the same condition as mentioned above to observe the effect of S-enantiomer on the inversion. The results were presented in Fig. 5. Statistical comparisons were conducted between racemic ibuprofen and the R-enantiomer at corresponding doses separately. Significant differences were obtained at all doses investigated (p<0.01, for all doses), indicating that the preexisting S-enantiomer can substantially inhibit the unidirectional inversion of R ibuprofen.

Effects of Sodium Azide, and Exogenous CoA and
Palmitic Acid on Inversion of Ibuprofen  

ATP and CoA were previously proved to play an important role on the formation of ibuprofen-CoA, the prerequisite intermediary on the process of inversion, catalyzed by the LACS.19,20) However, no research was conducted to observe the effect of ATP and CoA on inversion directly up to now. Thus, the role of the inhibitor of energy metabolism, sodium azide (10 mM), and exogenous CoA (0.1, 0.2, 0.4 mM) were evaluated in the present study to provide direct evidence. Sodium azide at a final concentration of 10 mM was found to inhibit the inversion significantly (Fig. 6a, p<0.01). In contrast, addition of exogenous CoA at all doses significantly increased the inversion ratio (Fig. 6b, p<0.01 for all doses), despite not being dose-dependent.

Palmitic acid was the preferred substrate of long chain acyl-CoA synthetase (LACS). It was predicted to competitively inhibit the ibuprofen inversion mediated by the LACS catalysis in the formation of ibuprofen-CoA. In an attempt to ascertain whether the inversion in the cultured Caco-2 cells was catalyzed by the same LACS that is responsible for the palmitic acid metabolism, the effect of palmitic acid at concentrations of 5, 10 and 25 μM over the inversion were investigated. These results were shown in Fig. 6c. Instead of inhibiting the inversion competitively, significant increases of inversion ratio were obtained by addition of palmitic acid at all doses investigated (p<0.01).

DISCUSSION

The results obtained from the present study conducted in Caco-2 cells provided strong evidence to the capability of intestinal chiral inversion. Regardless of whether administration was performed using racemic ibuprofen or the R enantiomer, substantial inversion of the R enantiomer to its antipode was demonstrated, whereas no inversion of S to R enantiomer was observed, consistent with the previous researches.19,21) Since the inversion of 2-arylpropionic acids was both species and substrates dependent, the development and establishment of human-resourced researching model is of particular importance. To date, only one report provided direct evidence of intestinal inversion of ibuprofen in humans.91 Only three samples of the intestine were under investigation in that research because of the limitation of human intestinal tissue. Such a report was therefore deemed to be less informative and conclusive. In the present study, we provided relatively complete information in terms of the charac-
The presence of palmitic acid (5—25 mM) was incubated in Caco-2 cells with or without the presence of sodium azide (SA, 0.1—0.4 mM). (c) Effect of palmitic acid on chiral inversion of ibuprofen in Caco-2 cells. (d) Inhibitory effect of ibuprofen (IBU) at various concentrations on the chiral inversion of ibuprofen in Caco-2 cells. (b) Effect of palmitic acid on chiral inversion of ibuprofen. (-)-R ibuprofen (IBU) at 10 µg/ml was incubated in Caco-2 cells in the absence or presence of CoA (0.1—0.4 mM). (c) Effect of palmitic acid on chiral inversion of ibuprofen in Caco-2 cells. (-)-R ibuprofen (IBU) at 10 µg/ml was incubated in Caco-2 cells in the absence or presence of palmitic acid (5—25 µM).

Fig. 6

Data were expressed as means ± S.D. of triplicate samples. ** p<0.01, as compared to the IBU group, determined from the independent Student’s t-test. (a) Inhibitory effect of sodium azide on chiral inversion of ibuprofen. (-)-R ibuprofen (IBU) at 10 µg/ml was incubated in Caco-2 cells with or without the presence of sodium azide (SA, 10 mM).

Characteristics and biochemical influence over the intestinal chiral inversion of ibuprofen by adopting the Caco-2 cell model. In spite of the limitation that long incubation times up to at least 24 h are required to achieve detected inversion which can already be observed within 3 h of coincubation with the freshly prepared segments of intestine, the advantages of the continuous cultured Caco-2 cells were apparent. As discussed above, the resource of human intestine was limited and other species replacement was not suitable since the chiral inversion of 2-APAs was both species and substrate dependent. Caco-2 cells in continuous culture were previously well proved to be morphologically and physiologically similar to the intestine. Thus, developing the Caco-2 cells into a model in researching the chiral inversion of 2-APAs should be a good alternative to the human intestine. Furthermore, application of Caco-2 cells does not demand time-consuming and technically complicated procedures involved in the preparation of fresh intestinal segments. In addition, numerous parallel experiments can be conducted with a homogenous cell population and thus exclude the high inter-subject variance associated with the freshly prepared cells or the whole organ model. Similar research had been conducted by Menzel-Soglowek who developed the human hepatoma cell line (HepG2) into a suitable model of chiral inversion research as a replacement of freshly prepared hepatocells.

Three kinds of enzymes, LACS, epimerase and hydrolase were necessary for the chiral inversion of 2-APAs, and the LACS was considered to be the stereoselective and rate-controlling enzyme. Entailing long incubation times to acquire substantial inversion in the present study and in the research conducted in HEP G2 cell lines suggested that the involved enzymes expressed (or active) in the immortal cell lines were much lower than that of the freshly prepared or in vivo cells. The LACS responsible for the palmitic acid metabolism was identified in Caco-2 cells and its activity was determined to be about 5.5 nmol/min/mg protein (formation of palmityl-CoA) on four days after confluence. If the LACS was actually the rate-controlling enzyme, the data obtained from the present study can possibly be extrapolated quantitatively to the capacity of intestinal chiral inversion in vivo, by comparing the LACS activity detected in Caco-2 cells with that of the freshly prepared intestinal cells.

Consistent with the inversion which occurred in HEP G2 cell lines, addition of FBS into the culture medium significantly inhibit the chiral inversion of ibuprofen content dependently. This may be explained by the high binding ratio of ibuprofen to serum albumin which may reduce the bioavailability of this compound subject to inversion. Unlike that observed in the HEP G2 cells, the inversion of ibuprofen was found to be both dose and cell density dependent in Caco-2 cells. Such a discrepancy may be attributed to the higher cell density observed in that research (6×10⁶ cells/dish as compared to 2×10⁶ cells/well), and possibly higher enzyme activity in HEP G2 cells. However, it was important to point out that the unsaturated characteristics of inversion concluded in the HepG2 research were simply deduced from the good linearity from plotting the AUC against the dose. In fact, better linearity was obtained from both the AUCS and AUCR plotted against the dose in our study (Fig. 3). In contradiction, the F values were significantly different among different doses. This analytic result indicates that the AUCS is not a good index of inversion. In this investigation, we developed a new and simplified equation to calculate the inversion ratio of ibuprofen in the in vitro model (Eq. 1). Overestimation of the F values was found in the present case by adopting the equation developed by Pang and Kwan and applied in the HEP G2 research as follows:

\[ F_i = \frac{AUCS(R)}{AUCS(S)} \]

where AUCS(R) is the area under the curve of the S-enantiomer following incubation with R-enantiomer while AUCS(S) is that of the S-enantiomer following incubation with S-enantiomer. The limitation of this equation is that two different administrations are required for the calculation, thus incorporating the variance.

The pre-existing S-enantiomer was found to significantly inhibit the inversion which occurred in Caco-2 cells, which was inconsistent with the previous in vivo study. Although
no similar research was found in the current literature, S ibuprofen was previously demonstrated to be a mix-type inhibitor of the LACS,20 which provided a good explanation for the present result. No substantial metabolism (elimination) of S-ibuprofen was detected in Caco-2 cells (data not shown), which meant that high concentration of the S-enantiomer was persistently presented in the culture system following incubation with the racemate. However, it is not the case in vivo due to the rapid metabolism and binding to serum albumin, leading to a much lower inhibitory effect on the inversion than that observed in the present study.

Formation of ibuprofen-CoA, the prerequisite intermediary to initiate the inversion, involved the usage of CoA, ATP, and Mg2+.27 Addition of exogenous CoA into the culture medium significantly increased the inversion ratio, supporting the evidence that the intracellular level of CoA plays a pivotal role on the inversion of 2-APs. Clofibrate acid was previously reported to increase the undirectional chiral inversion of ibuprofen in rat liver preparations or in hepatocytes by a postulated mechanism that clofibric acid could transiently increase the intracellular concentration of CoA making up for the transient decrease caused by R-ibuprofen inversion.21,28 Sodium azide, one of the inhibitors of energy metabolism, was observed to exert a significant inhibition on the inversion for the first time, supporting the concept that ATP was also an important cofactor mediating 2-APs inversion.

Palmitic acid, the preferred substrate of LACS, was predicted to competitively inhibit the ibuprofen inversion assuming that the same enzyme was responsible for palmitic acid metabolism and ibuprofen-CoA formation in the Caco-2 cells. Interestingly, palmitic acid at all doses investigated significantly increased the inversion ratio of ibuprofen, contradictory to all of the previous reports.29—31 This contradictory result should not indicate that the different enzyme was involved, because the LACS activity in Caco-2 cells had been tested.14 and the LACS5 isoform which metabolizes the palmitic acid efficiently was predominantly and strongly expressed in intestinal epithelial cells.32,33 It is useful to emphasize that the much longer time entailed in the present investigation compared to previous research may explain such a reversal result. The relatively low concentration of palmitic acid added (25 μM) can be rapidly consumed within a short time by either incorporating into the cellular triglycerides and phospholipids or entering into β-oxidation in Caco-2 cells.11,34 It was well known that oxidation metabolism of palmitic acid can generate a vast quantity of ATP which may promote the ibuprofen inversion in Caco-2 cells.

In summary, we found that the ibuprofen underwent unidirectional inversion in Caco-2 cells for the first time in this investigation, adding evidence to the capability of intestinal chiral inversion. By optimizing the culture conditions, the Caco-2 cells can be developed into a suitable model for the chiral inversion research of 2-APs. It was found that the chiral inversion of ibuprofen was saturable in Caco-2 cells. Addition of exogenous CoA can significantly increase the inversion ratio while sodium azide can inhibit the chiral inversion to a significant extent.

Acknowledgements This work was partially supported by “863” grant (2003AA2Z347A) from State Ministry of Science and Technology of China.

REFERENCES