นิพนธ์ต้นฉบับ

# A study of the bioavailability of four brands of propranolol tablet in normal Thai volunteers.*

Chandhanee Itthipanichpong**
Krisana Kraisintu*** Pirojana Sirivong**
Duangchit Panomvana Na Ayudhya**** Sirimas Nanthasomboon**

**Itthipanichpong C, Kraisintu K, Sirivong P, Panomvana Na Ayudhya D, Nanthasomboon S. A study of the bioavailability of four brands of propranolol tablet in normal Thai volunteers. Chula Med J 1993 Mar; 37(3): 189-196**

*This study was performed in order to evaluate both in vivo and in vitro bioavailability of four brands of propranolol tablet marketed in Thailand. One of the four was the original brand and the others were locally manufactured brands.*

*The in vitro study demonstrated that the disintegration time, dissolution rate, propranolol content, the content uniformity and weight variation of all four products employed in this study met USP XXII specifications.*

*The bioavailability study was carried out in 10 normal volunteers (five males and five females, ranging in age from 20 to 45 years), using a randomized, double blind and cross-over study design. After fasting overnight, each subject was administered two 40 mg tablets of propranolol orally, for a total dose of 80 mg each. Plasma propranolol was determined by specific high-performance liquid chromatography analysis. No significant difference was observed in the extent and rate of propranolol absorption. Therefore, it could be concluded that all four propranolol tablets were bioequivalent.*

*The mean peak plasma propranolol concentration ($C_{max}$) and the time required to reach the peak ($T_{max}$) obtained from this study were 87.79 ± 8.32 ng/ml and 1.99 ± 0.12 hrs, respectively. The area under tha plasma concentration-time curve from 0 to 24 hrs ($AUC_0^{24}$) was 498.50 ± 50.39 ng.ml$^{-1}$ hr. The elimination half-life of propranolol in the Thai volunteers was 2.78 ± 0.16 hrs. It was found that the absorption rate constant (Ka) and the elimination rate constant ($K_{el}$) were 1.05 ± 0.03 hr$^{-1}$ and 0.28 ± 0.01 hr$^{-1}$*

**Key words :** *Bioavailability study, Propranolol.*

Reprint request : Itthipanichpong C, Department of Phrmacology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.
Received for publication. January 25, 1993.

*This work was supported by the Government Pharmaceutical Organization.
**Department of Pharmacology, Faculty of Medicine, Chulalongkorn University.
***Department of Research and Development, The Government Pharmaceutical Organization.
****Department of Pharmacy, Faculty of Pharmaceutical Science, Chulalongkorn University.

**จันทนี อิทธิพานิชพงศ์, กฤษณา ไกรสินธุ์, ไพโรจน์ ศิริวงษ์, ดวงจิต พนมวัน ณ อยุธยา, ศิริมาศ นันทสมบูรณ์. การศึกษาการเอื้อประโยชน์ในร่างกายของยาเม็ดโปรปราโนลอล 4 ตำรับ ในอาสาสมัครไทยปกติ. จุฬาลงกรณ์เวชสาร 2536 มีนาคม; 37(3) : 189-196**

*งานวิจัยนี้มีวัตถุประสงค์ เพื่อศึกษาการเอื้อประโยชน์ในร่างกายของยาเม็ด propranolol สี่ตำรับ โดยมีผลิตภัณฑ์ต้นแบบหนึ่งตำรับและผลิตภัณฑ์ภายในประเทศสามตำรับ โดยการประเมินคุณภาพทั้งในหลอดทดลองและในร่างกาย พบว่า ผลิตภัณฑ์ทั้งสี่ตำรับมีคุณสมบัติภายนอกร่างกาย ได้แก่ การแตกตัว การละลาย ปริมาณตัวยาสำคัญ ความสม่ำเสมอของตัวยาสำคัญ ความแตกต่างของน้ำหนักยา แต่ละเม็ดตรงตามมาตรฐานที่กำหนดในเภสัชตำรับ (USP XXII)*

*ผลการศึกษาภายในร่างกาย เมื่อให้อาสาสมัคร 10 คน เป็นเพศชาย 5 คน และเพศหญิง 5 คน อายุระหว่าง 20-45 ปี รับประทานยาเม็ด propranolol ขนาด 40 มิลลิกรัม จำนวนสองเม็ด แล้วเจาะเลือดเพื่อหาระดับของยา propranolol ในพลาสมา โดยใช้ High performance liquid chromatography พบว่ายาเม็ด propranolol ทั้งสี่ตำรับ มีการเอื้อประโยชน์ในร่างกายเท่าเทียมกัน เพราะยาทั้งสี่ตำรับมีอัตราเร็ว และปริมาณยาที่ถูกดูดซึมเข้าสู่ร่างกายไม่แตกต่างกันอย่างมีสัยสำคัญทางสถิติ ($p>0.05$)*

*สำหรับค่าเฉลี่ยของพารามิเตอร์ทางเภสัชจลนศาสตร์ในอาสาสมัครทั้ง 10 คน พบว่าระดับยาสูงสุดในพลาสมา ($C_{max}$) คือ $87.79 \pm 8.32$ นาโนกรัมต่อมิลลิลิตร ส่วนเวลาที่ระดับยาในเลือดสูงสุด ($T_{max}$) คือ $1.99 \pm 0.12$ ชั่วโมง ค่ากึ่งชีพ ($T_{1/2}$) คือ $2.78 \pm 0.16$ ชั่วโมง พื้นที่ภายใต้เส้นกราฟระหว่างความเข้มข้นยา propranolol ในพลาสมา กับเวลา ($AUC_0^{24}$) เท่ากับ $498.50 \pm 50.39$ นาโนกรัม.มล$^{-1}$.ชั่วโมง และค่าคงที่อัตราเร็วการดูดซึมยาคือ $1.05 \pm 0.03$ ชั่วโมง$^{-1}$ ส่วนค่าคงที่อัตราเร็วการกำจัดยาคือ ($K_{el}$) เท่ากับ $0.28 \pm 0.01$ ชั่วโมง$^{-1}$*

Propranolol, a beta-adrenergic blocking drug, is wildly used throughout the world as antihypertensive, antianginal and antiarrhythmic drugs. A large number of propranolol products are available in the market where the price competition between the original product and the locally manufactured ones is keen. Physicians have questioned the therapeutic equivalency of the differences brands, which led us to study the bio-availability of each product. Moreover, great inter-individual variability in the disposition of propranolol has been observed and differences in propranolol plasma levels between patients receiving identical oral doses have been reported.[1-3] Through study of the pharmacokinetic process, the individual and inter-individual variability in absorption, distribution and excretion of the drug can be defined.[4]

This study was performed to evaluate the bio-availability of four commercial brands of propranolol tablet marketed in Thailand and also the pharmacokinetic profile of propranolol in normal Thai volunteers.

## Materials and Methods

### Materials

Four brands of propranolol hydrochloride tablet marketed in Thailand were designated as drugs A,B,C,D. One of them was the original product; the others locally manufactured products.

DL-propranolol hydrochloride, labetalol and 1-heptane-sulfonic acid were purchased from Sigma Co U.S.A. Diethyl ether and methanol were obtained from May and Baker (UK) and sodium metabisulfite was purchased from Merck Co. (Germany). All of the reagents used in this study were HPLC grade except diethyl ether and sodium metabisulfite.

### Study subjects

Ten healthy Thai volunteers (five males and five females) gave written consent to participate in this study, which was approved by the Ethical Board of the Faculty Committee. None of the subjects had any abnormal finding on routine laboratory testing, history taking, physical examination and electrocardiography. All subjects were non-smokers and had not taken any medication or alcoholic drink for at least three days prior to the study. The mean age of the subjects was $28.96 \pm 5.56$ years (range 20 to 45). Their mean weight was $55.95 \pm 8.56$ kg (range 40-75 kg).

## Methods

### In vitro study

The following tests were carried out in order to determine whether or not all the propranolol hydrochloride tablets used in this study met the requirement' of the United States Pharmacopeia (XXII)[5]:

1. weight variation
2. disintegration
3. dissolution
4. propranolol content of each tablet
5. content uniformity

## Invivo study

### Bioavailability study

After an overnight fast, blood samples (8 ml each) were drawn from the subject for control purposes (time 0). They then were administered 80 mg of propranolol hydrochloride ($2 \times 40$ mg tablets) each with a glass of water (250 ml). Blood samples were taken at 1,1.5,2,2.5,3,4,6,8 and 24 hours after dosing. The subjects were allowed to have breakfast two hours after drug administration. Blood samples were collected in heparinized tubes and centrifuged at 2,500 rpm for five minutes. Then the plasma was seperated and frozen at $-20°C$ until the time of sample analysis. The study was a double-blind, randomized and cross-over design.

Plasma propranolol determination was done by modified HPLC method described by Drummer OH.[6] To the aliquot portion of the plasma sample (900 $\mu$l) was added 100 $\mu$l of internal standard labetalol (400 $\mu$g/ml) and 100 $\mu$l of 20% sodium metabisulfite. The solution was then alkalinized by adding 1 ml of 1 M sodium carbonate and extracted by diethyl ether (7 ml). The organic phase was separated by centrifugation and evaporated to dryness in a nitrogen atmosphere. The residue was then dissolved in 0.8 ml of the mobile phase and 50 $\mu$l of each sample was injected into the chromatograph.

The high-performance liquid chromatography system consisted of a radial pack cartridge (C18) 8 mm ID plastic column and the radial compression separation system (RCM 100) from Water Associated Pty. Ltd. U.S.A. The absorbance of the eluent was determined using spectrofluorometer (Jasco Model FP210) with the excitation and emission wavelengths at 296 and 340 nm, respectively. The mobile phase was 0.006 M 1 heptane-sulfonic acid sodium salt in 1% glacial acetic acid : methanol 40 : 60. The flow rate was 1.2 ml/min.

The following pharmacokinetic parameters were determind :

$C_{max}$ = peak plasma concentration
$T_{max}$ = time of peak plasma concentration
$T_{1/2}$ = half-life

AUC = area under plasma concentration-time curve
$K_{el}$ = elimination rate constant
$K_a$ = absorption rate constant

Mean and standard deviation were used for the in vitro data presentation. Mean and standard error were for the in vivo.

Comparison of each parameter of the four individual products was done by analysis of variance.

## Results

### In vitro study

The in vitro study demonstrated that the four preparations of propranolol tablet employed in this study (A,B,C,D) fulfilled the requirements of the United States Pharmacopeia XXII. The disintegration time, dissolution rate, propranolol content, the content uniformity and weight variation of each preparation are shown in tables 1 and 2.

**Table 1.** The qualitative and quantitative study in vitro of the four preparations of propranolol tablets marketed in Thailand (mean ± SD).

| Drugs | Weight variation ($\bar{x}$ ± SD, n = 10) (mg) | Disintegration time (min, n = 6) | Dissolution at 30 min (% LA ± SD, n = 10) | Propranolol content (% LA ± SD, n = 3) |
|---|---|---|---|---|
| A | 206.18 ± 2.66 | 6 | 106.26 ± 2.09 | 96.57 ± 1.18 |
| B | 190.90 ± 2.08 | 8 | 100.48 ± 1.74 | 96.35 ± 0.93 |
| C | 195.70 ± 3.63 | 12 | 105.34 ± 3.13 | 100.60 ± 0.95 |
| D | 202.74 ± 5.38 | 20 | 97.80 ± 2.55 | 95.37 ± 0.83 |

% LA = % of labelled amount
Labelled amount = the amount of drug labelled in each tablet

**Table 2.** The content uniformity study of the four preparations of propranolol tablets.(n = 10).

| | A % LA | B % LA | C % LA | D % LA |
|---|---|---|---|---|
| 1 | 96.20 | 102.80 | 106.44 | 99.26 |
| 2 | 96.20 | 103.63 | 104.07 | 102.96 |
| 3 | 96.08 | 106.11 | 106.38 | 99.38 |
| 4 | 92.54 | 106.70 | 102.79 | 97.38 |
| 5 | 97.38 | 100.09 | 107.59 | 99.38 |
| 6 | 95.49 | 101.03 | 103.95 | 102.92 |
| 7 | 100.92 | 105.76 | 106.02 | 99.03 |
| 8 | 98.91 | 101.62 | 113.63 | 101.27 |
| 9 | 94.19 | 101.39 | 103.28 | 104.46 |
| 10 | 96.20 | 99.85 | 105.41 | 99.03 |
| $\bar{X}$ | 96.42 | 102.90 | 105.96 | 100.28 |
| SD | ± 2.32 | ± 2.54 | ± 3.11 | ± 2.08 |
| % CV | 2.41 | 2.47 | 2.94 | 2.07 |

% CV = % coefficient of variation.

## In vivo study

### The bioavailability study of four propranolol preparations

Figure 1 shows the chromatogram of propranolol and labetalol (the internal standard). The rentention time for labetalol and propranolol were 4.33 and 6.76 minute respectively. The limit of detection was 10 ng/ml. The precision was high as the mean coefficient of variation was less than 7% and the percentage of recovery of propranolol in the plasma was between 92 and 105%.

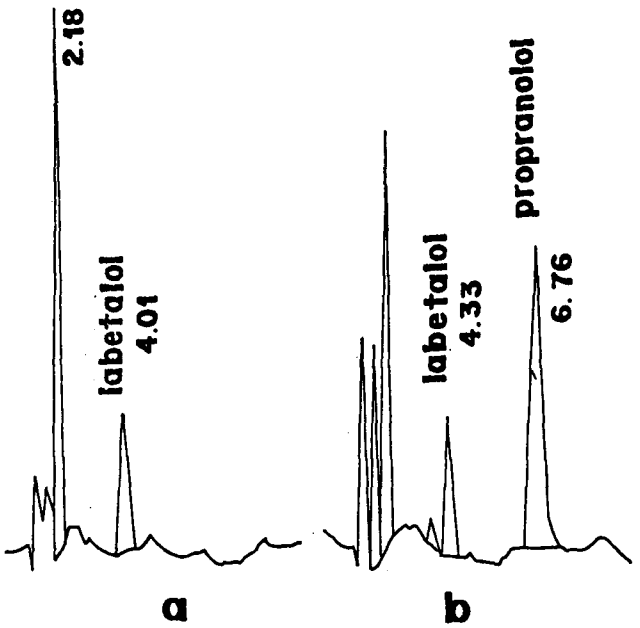


**Figure 1.** The chromatogram of propranolol and the internal standard (labetalol)
a. plasma blank with internal standard
b. propranolol and labetalol in plasma

Figure 2 shows that the mean plasma propranalol concentration and the time profile after oral administration of four propranolol preparations gave quite similar patterns (table 3). The pharmacokinetic parameters, $C_{max}$, $T_{max}$, $AUC_0^{24}$, $K_a$, $K_{el}$ and $T_{1/2}$ are demonstrated in table 4.

The mean peak propranolol concentration ($C_{max}$) of the four preparations of the drug was between 82.34 and 96.96 ng/ml. The mean peak time concentration was between 1.90 and 2.05 hours. By using the trapezoidal rule, it was found that the area under the plasma concentration-time curve from 0 to 24 hours ($AUC_0^{24}$) was between 429.83 and 557.42 $ng.ml^{-1}$ hr. The elimination half-life ($T_{1/2}$) was between 2.27 and 3.14 hrs.

The absorption rate constant (Ka) and the elimination rate constant ($K_{el}$) obtained from the computerized CSTRIP program were between 0.98 and 1.10 $hr^{-1}$, 0.23 and $0.32^{-1}$, respectively. There was no significant difference between the four preparations in all the pharmacokinetic parameters studied.

**Table 3.** The mean plasma propranolol concentration and time profile after 80 mg oral dose of the four brands propranolol tablet. (mean ± SE) in 10 subjects.

| | mean plasma propranolol concentration | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Time (hr) | | | | | | | | | |
| Drugs | 0 | 1 | 1.5 | 2 | 2.5 | 3 | 4 | 6 | 8 | 24 |
| A | 0 | 51.49 ± 12.61 | 64.06 ± 10.12 | 67.26 ± 11.79 | 71.10 ± 19.61 | 65.79 ± 15.23 | 51.77 ± 11.13 | 30.45 ± 5.94 | 20.59 ± 4.06 | 0.73 ± 0.61 |
| B | 0 | 60.86 ± 15.04 | 68.86 ± 15.61 | 74.24 ± 13.22 | 67.98 ± 11.51 | 59.26 ± 10.58 | 48.95 ± 9.29 | 30.31 ± 5.59 | 17.24 ± 3.92 | 1.36 ± 0.98 |
| C | 0 | 44.07 ± 11.12 | 56.62 ± 13.34 | 70.41 ± 12.19 | 70.79 ± 13.46 | 55.21 ± 11.38 | 45.23 ± 10.80 | 26.99 ± 5.85 | 13.15 ± 3.36 | 2.05 ± 1.27 |
| D | 0 | 67.60 ± 19.24 | 72.27 ± 18.98 | 81.38 ± 16.48 | 77.95 ± 16.09 | 66.04 ± 12.89 | 55.26 ± 11.87 | 32.26 ± 6.63 | 19.38 ± 4.02 | 2.45 ± 1.57 |

**Table 4.** The pharmacokinetic profile from 10 subjects following the oral administration of 80 mg propranolol. (mean ± SE).

| Drugs | $C_{max}$ (mg/ml) | $T_{max}$ (min) | $AUC_0^{24}$ ($ng.ml^{-1}.hr$) | Ka ($hr^{-1}$) | $K_{el}$ ($hr^{-1}$) | $t_{1/2}$ (hr) |
|---|---|---|---|---|---|---|
| A | 85.95 ± 15.82 | 2.05 ± 0.17 | 495.71 ± 94.22 | 1.06 ± 0.06 | 0.23 ± 0.02 | 3.14 ± 0.20 |
| B | 85.96 ± 19.09 | 2.00 ± 0.25 | 510.10 ± 105.10 | 1.03 ± 0.05 | 0.27 ± 0.03 | 2.83 ± 0.38 |
| C | 82.34 ± 12.67 | 1.90 ± 0.22 | 429.83 ± 96.30 | 0.98 ± 0.08 | 0.32 ± 0.02 | 2.27 ± 0.21 |
| D | 96.90 ± 17.14 | 2.00 ± 0.22 | 557.42 ± 117.85 | 1.10 ± 0.05 | 0.28 ± 0.04 | 2.87 ± 0.40 |
| **$\bar{X}$ ± SE** | **87.79 ± 8.32** | **1.99 ± 0.12** | **498.50 ± 50.39** | **1.05 ± 0.03** | **0.28 ± 0.01** | **2.78 ± 0.16** |

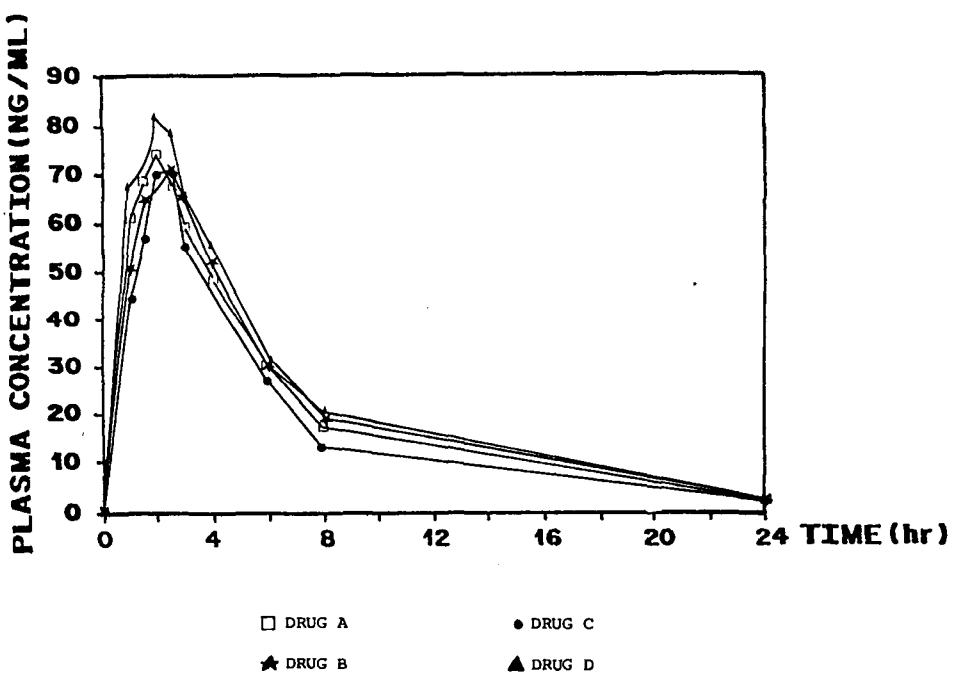


**Figure 2.** The mean plasma concentration-time curve from 10 subjects after the oral administration of 80 mg propranolol. (Drug A, B, C, D)

## Discussion

The four preparations of propranolol tablets met the specification of the United States Pharmacopeia XXII including the disintegration time which was less than 30 minutes, the dissolution rate in 30 minute was moe than 75% of the labelled amount, the content of propranolol was not less than 90% and not more than 110% in each tablet. The content uniformity was in the range 90-110% labelled amount and the weight variation was less than 7.5%. It indicated that the four products of 40 mg propranolol tablets were equivalent in both qualitative and quantitative study in vitro.

The bioavailability study in vivo was considered from the extent and rate of drug absorption into the body. Thus the pharmacokinetic parameters concerned were $T_{max}$, $C_{max}$, AUC and Ka.

The maximum plasma drug concentration ($C_{max}$) represented the highest plasma concentration acheived after drug administration and it also reflected both the rate and extent of drug absorption. The result shown in table 4 indicated that drug A, B, C and D displayed the same rate and extent of propranolol absorption since there was no significant difference in $C_{max}$ between the four products. The other parameters, $T_{max}$ which reflected the rate of propranolol absorption into the body and $AUC_0^{24}$ which determined the extent of the drug absorption also showed no significant difference between each preparations.

On the assumption that the pharmacokinetics of propranolol was one-compartment model, the elimination rate constant ($K_{el}$), and the absorption rate constant ($K_a$); then from this study no statistical difference was observed between drugs A, B, C, D. Therefore it could be concluded that the four preparations of propranolol 40 mg tablets were bioequivalent since the extent and rate of drug absorption of each product were equivalent.

Considering of the elimination rate constant ($K_{el}$) of the four preparations in 10 volunteers, it was $0.28 \pm 0.01$ $hr^{-1}$ (0.23-0.32 $hr^{-1}$) and the elimination half life was $2.78 \pm 0.16$ hr (2.27 - 3.14 hr). Since the elimination half life was the time taken for the concentration to halve[7], its value was inversely proportional to the elimination rate constant ($t_{1/2} = 0.693/k_{el}$)[8]. Any drug with a low value of $K_{el}$ will be eliminated more slowly and the elimination half life will be prolonged. The elimination half life of propranolol obtained in this study was found to be a little bit shorter than that reported by Walle et al[9] in Europeans whose elimination half life was between 3 and 4 hours. However the elimination half life of propranolol in Indians reported by Biswas NR et al[10] was $2.41 \pm 0.65$ hr which was quite similar to that of Thai volunteers. This variability resulted from the difference in drug metabolising enzyme which depended on many factors such as genetics, diet, environment.[11,12]

## Conclusion

The result of the biovailability study of four propranolol tablets demonstrated that all drugs were bioequivalent in vivo and all of them met the USP XXI specification for tablets in vitro. The therapeutic effect of the four propranolol tablets should be similar and they may be used instead of each other.

## References

1. Briggs WA, Lowenthal DT, Cirksena WJ, Price WE, Gibson TP, Flamenbaum W. Propranolol in hypertensive dialysis patients : efficacy and compliance. Clin Pharmacol Ther 1975 Nov; 18(5pt1) : 606-12
2. Chidsey CA. Morselli P. Bianchetti G. Morganti A. Leonetti G. Zanchetti A. Studies of the absorption and removal of propranolol in hypertensive patients during therapy. Circulation 1975 Aug 1; 52(2) : 313-8
3. Esler M. Zweifler A. Randall O. DeQuattro V. Pathophysiologic and Pharmacokinetic determinants of the antihypertensive response to propranolol. Clin Pharmacol Ther 1977 Sep; 22(3) : 299-308
4. Grahame-Smith DG. Aronson JK. The four process of drug therapy. In:Grahame-Smith DG. Aronson JK, eds. Oxford Textbook of Clinical Pharmacology and Drug Therapy. London : Oxford University Press, 1984. 4-5
5. Propranolol in the United State Pharmacopeia. XXII. Printed by Mack Printing Company, Easton Pa. 18042, 1990. 1175-81
6. Drummer OH. Mcneil J. Pritchard E. Louis WJ. Combined high performance liquid chromatographic procedure for measuring 4-hydroxypropranolol and propranolol in plasma: Pharmacokinetics measurement following conventional and slow release propranolol administration. J Pharm Sci 1981 Sep; 70(9) : 1030-2
7. Grahame-Smith DG. Aronson JK. The pharma-

cokinetic process : Is the drug getting to its site of action. In : Grahame-Smith DG. Aronson JK, eds. Oxford Textbook of Clinical Pharmacology and Drug Therapy. London : Oxford University Press, 1984. 32

8. Gibaldi M. Introduction to Pharmacokinetics. In : Gilbaldi M. eds. Biopharmaceutics and Clinical Pharmacokinetics. 4th ed. Philadelphia : Lea & Febiger, 1992. 16
9. Wall T, Conradi EC, Walle UK, Fagen TC, Gaffney TE. 4-Hydroxy propranolol and its glucuronide after single and long term doses of propranolol. Clin Pharmacol Ther 1980 Jan; 27(1) : 22-31
10. Biswas NR, Gary SK, Kumar N, Mukhejee S, Sharma PL. Comparative Pharmacokinetic and pharmacodynamic study of four different brands of propranolol in normal volunteers. Int J Clin Pharmacol Ther Toxicol 1989 Oct; 27(10) : 515-9
11. Walle T, Walle UK, Cowart TD, conradi EC, Gaffney TE. Selective induction of propranolol metabolism by smoking : Additional effects on renal clearance of metabolites. J Pharmacol Exper Ther 1987 Jun; 241(3) : 928-33
12. Kalow W. Ethnic differences in drug metabolism. Clin Pharmacokinet 1982 Sep-Oct; 7(5) : 373-400