An official Publication of the Indian Pharmacological Society
Indian Journal of Pharmacology
Home
Current issue
Instructions
Submit article

Indian J Pharmacol. 2013 Jul-Aug; 45(4): 325–329. PMCID: PMC3757597
doi: 10.4103/0253-7613.114994

Development of an *in vitro* cell culture model to study milk to plasma ratios of therapeutic drugs

Maithili A. Athavale, Anurupa Maitra, Shahnaz Patel, Vijay R. Bhate,[1] and Villi S. Toddywalla

National Institute for Research in Reproductive Health (ICMR), Mumbai, India
[1]Analytical Solutions, Navi Mumbai, India
Correspondence to: Dr. Anurupa Maitra, E-mail: anurupamaitra@yahoo.co.in

Received June 5, 2012; Revised March 22, 2013; Accepted April 23, 2013.

Abstract

Objective:

To create an *in vitro* cell culture model to predict the M/P (concentration of drug in milk/concentration in maternal plasma) ratios of therapeutic drugs viz. rifampicin, theophylline, paracetamol, and aspirin.

Materials and Methods:

An *in vitro* cell culture model using CIT3 cells (mouse mammary epithelial cells) was created by culturing the cells on transwells. The cells formed an integral monolayer, allowing only transcellular transport as it happens *in vivo*. Functionality of the cells was confirmed through scanning electron microscopy. Time wise transfer of the study drugs from plasma to milk was studied and compared with actual (*in vivo*) M/P ratios obtained at reported t_{max} for the respective drugs.

Results:

The developed model mimicked two important intrinsic factors of mammary epithelial cells viz. secretory and tight-junction properties and also the passive route of drug transport. The *in vitro* M/P ratios at reported t_{max} were 0.23, 0.61, 0.87, and 0.03 respectively, for rifampicin, theophylline, paracetamol, and salicylic acid as compared to 0.29, 0.65, 0.65, and 0.22, respectively, *in vitro*.

Conclusion:

Our preliminary effort to develop an *in vitro* physiological model showed promising results. Transfer rate of the drugs using the developed model compared well with the transfer potential seen *in vivo* except for salicylic acid, which was transferred in far lower concentration *in vitro*. The model has a potential to be developed as a non-invasive alternative to the *in vitro* technique for determining the transfer of therapeutic drugs into breast milk.

KEY WORDS: Cell culture, milk-plasma ratio, CIT3 cells, *in vitro*, M/P ratios, reversed phase-High performance liquid chromatography

Introduction

It is well established that almost all drugs taken by nursing mothers pass into breast milk in a “pharmacologically active” form,[1,2] thereby, exposing the nursling to these pharmacologically active drugs. This exposure could be potentially harmful as it occurs during the nursling's rapid growth and development phase, when its drug metabolizing and excretory systems are still immature. One of the ways to minimize infant's exposure to these maternal drugs would be to know the quantity of each drug

transferred to milk.

The potential of a drug to be transferred to milk is expressed as its M/P ratio i.e., the concentration of a drug in breast milk/its concentration in maternal plasma. Conducting *in vivo* studies with all the commonly used therapeutic drugs is no doubt extremely difficult as it involves a number of sequential plasma and milk samples from sick nursing mothers on these drugs. To circumvent the difficulties involved in conducting large scale *in vivo* studies to obtain M/P ratios of drugs, many pharmacologists have proposed different mathematical equations and *in vitro* models to predict these ratios.

Mathematical equations[3,4,5,6,7] and an *in vitro* model[8] are available in the literature to predict the M/P ratios of drugs. Most of these proposed equations/models are cumbersome to use because of the difficulties involved in obtaining certain variables to be substituted in the equation. Even after the cumbersome exercise, the M/P ratios obtained do not always compare well with actual *in-vivo* ratios.[9,10,11]

The transfer of a drug from maternal plasma to breast-milk *in vivo* depends not only upon the physicochemical characteristics of drug as has been considered in the earlier equations/models but also on a number of physiological factors. In 2003 Gerk *et al.*,[12] used CIT3 cells (mouse mammary epithelial cells) to create an *in vitro* M/P model using snapwells to study the transport of drugs. In this paper, we have presented a variation of this model. The paper describes the development and validation of a novel *in vitro* model taking into consideration some of the physiological factors.

To create an *in vitro* model to predict M/P ratios, we used CIT3 cells that could mimic two intrinsic factors of mammary epithelial cells i.e. secretion of milk and the tight junction property. The model was further validated by comparing M/P ratios of our study drugs obtained through model with those obtained from our *in vivo* studies obtained at t_{max} of each drug. Four of the commonly administered study drugs were selected namely, rifampicin, theophylline, paracetamol, and aspirin. Selection was based on their different therapeutic requirements, pharmacological properties, and physicochemical characteristics.

Materials and Methods

In vivo Study

The study was approved by the Ethics Committees of the National Institute for Research in Reproductive Health (Erstwhile Institute for Research in Reproduction). Informed consent was obtained in local language from all the participants who volunteered for the study.

Study Drugs

Rifampicin, Theophylline, Paracetamol, and Aspirin.

Study Subjects

All subjects were 20–30 year old nursing women, weighing between 40 kg and 50 kg. Their infants were between 1 month and 5 months of age and all were exclusively breastfed. The subjects were divided into four groups as per the study drug and 15 subjects were studied for each drug.

Group I

Consisted of mothers suffering from tuberculosis. They were on a daily therapy with rifampicin 450 mg + isoniazid 300 mg (Lupin Ltd.) prescribed for a 9 months period. Five of them initiated their therapy during pregnancy and the rest 10 initiated post partum.

Group II

Consisted of mothers who were chronic asthmatics and hence took a daily single dose of theophylline 100 mg + Salbutamol 2 mg (Airomol tab, Zydus Ltd.) as per their physician's advice.

Group III

Consisted of healthy mothers not on any medication. They volunteered to take a single dose of

paracetamol 500 mg (Glaxo Smithkline Ltd.) solely for the study.

Group IV

Also consisted of healthy mothers who did not require any medication. They too, like the mothers in Group III, volunteered to take a single dose of Aspirin (acetyl salicylic acid, Bayer Ltd.) 600 mg (2 tablets of 300 mg) solely for the study.

The subjects in Groups III and IV agreed to take paracetamol/aspirin, since being over the counter drugs, they often ingested these drugs for minor illnesses.

Sampling Schedule

Prior to ingestion of the drug, baseline blood and milk samples were collected from the subjects in Groups I and II, as they were on a daily regimen of rifampicin or theophylline. For the study, a uniform pattern for maternal blood and milk sample collection was followed, for all the subjects, in the four study groups.

It was impressed upon the mothers to breastfeed their infants well, just before taking the study drug, as no feeds could be given till their blood and breast milk sample collection was completed. The time interval between maternal drug ingestion and sample collection was fixed as per the reported t_{max} of the study drugs in plasma.

Maternal blood and foremilk samples were collected two and a half hrs after rifampicin,[13] 2 hrs after theophylline,[14] 1 hr after paracetamol and one and a half hrs after aspirin ingestion by the mothers.[15] They were then instructed to breastfeed their infants. After the infants were fully fed from both the breasts, another milk sample i.e. the hind milk sample was collected. Equal quantities of the fore and hind milk samples were mixed together and the samples immediately taken for analysis. As rifampicin is photo-sensitive, samples containing this drug were immediately shielded from light.

Development of *In vitro* Cell Culture Model

CIT3 cell culture

CIT3 cells (PN 9, a gift from Prof. Margaret Neville, Denver, U.S.A.) were split by trypsinization. The detached cells were then gently removed and neutralized with CIT3 growth medium. The growth medium comprised of Dulbecco's modified Eagle's medium (DMEM) with Ham's 12 (50:50), supplemented with 2% heat inactivated fetal bovine serum (FBS), 5 ng/ml epidermal growth factor (EGF), 10 μg/ml insulin, 100 U/ml penicillin, and 100 μg/ml streptomycin.

Seeding CIT3 cells on transwell

The cell suspension after trypsinization was seeded onto transwells (Corning Costar, membrane diameter 24 mm, growth surface area 4.7 cm^2. pore size 0.4 μm). To maintain hydrostatic pressure equilibrium, of the two chambers of the transwell, 1.5 ml of media was added in the filter insert and 2.5 ml in the well. The plates were then incubated at 37°C, 5% CO_2 for 24 hrs. After 24 hrs, the medium was aspirated and discarded and replaced with fresh CIT3 growth medium. The cells were kept in growth medium for 7 days with a media change every alternate day. After 7 days, when the cells became confluent, CIT3 growth medium was changed to “secretion media” (CIT3 growth medium was modified by removal of EGF and addition of 3μg/ml each of ovine prolactin and hydrocortisone from Sigma). The cells were kept in this media for another 7–10 days to become functional (media change was given every alternate day).

To test whether the cells were actually functional, we scanned the cells under the Scanning Electron Microscope. One of the prerequisites for conducting drug transport experiments is to have cells that are functional and form an integral monolayer. An integral monolayer differs from a confluent monolayer in that the former should have no tear or intracellular space at all like a sheath or a membrane. This membrane allows only transcellular transport with negligible paracellular drug transport as it occurs *in vivo*.

Thus, an integral monolayer mimics the mammary alveoli's distinguishing feature of forming tight-

junctions during established lactation. The distinguishing feature of integral monolayer is that the membrane shows a very high resistance when checked with a special instrument called “Epithelial Volt-ohm meter”. For our experiments, only experiments showing a transepithelial resistance greater than 800 ohms/cm^2 were used.

To obtain comparable M/P ratios, we were interested in developing a system that was as close to *in vivo* system as possible. The CIT3 cells used in our model could mimic the mammary alveoli in both function (secretory) and behavior (tight-junction property). We decided to further substitute the secretion media in the upper chamber of the transwell with control milk (breast milk of healthy mother,1.5 ml) and one in the lower with control plasma (2.5 ml) spiked with the study drug to develop comparable *in vivo* system. This was considered as “zero hour” reading. The quantity of drug used to spike the control plasma depended upon the mean concentration of drug in plasma at the t_{max} after maternal ingestion of that drug (i.e t_{max} value obtained by *in vivo* studies in nursing mothers).

At zero hour, 1.0 ml of plasma and milk samples were collected in duplicates, the cluster plates each containing six transwells was then placed on a rocker platform (Cole Parmer) at a speed of 20 r.p.m in a CO_2 incubator (5% CO_2 and 37°C). Thereafter, plasma and milk samples were collected at periodic time intervals. Unlike the *in vivo* study wherein only a single plasma and milk sample was collected at the known t_{max} of drug in plasma, we decided to collect samples before, at and after t_{max}. To assess the integrity of the monolayer the transepithelial resistance was rechecked by carefully removing the milk and plasma samples and replacing them with media. A transepithelial resistance was observed at the end of the experiment for assurance of the integrity of membrane. The collected samples were then analyzed by Reverse Phase High Performance Liquid chromatography (RP-HPLC) method developed for each drug. After estimating the drug's concentration in plasma and milk their respective M/P ratios were calculated and tabulated in a “time-wise” manner.

Analysis of Study Drugs

Sample Extraction and Analysis

For extraction of the study drugs, 1 ml of plasma and milk sample was used. The extracting solvent for plasma was 1 ml of acetonitrile (for rifampicin), 20% perchloric acid (for theophylline and paracetamol), and 6% trichloroacetic acid (for aspirin), respectively. The extracting solvent of the study drug for milk was similar to that of plasma except for aspirin where a mixture of 1 ml of hydrochloric acid and 6 ml of 2:1 cyclohexane:dichloromethane was used. Internal standards used were Amlodipine besylate (25 μg/ml for plasma, 5 μg/ml for milk), Etofyline (10 μg/ml for plasma,7 μg/ml for milk), Theophylline (4 μg/ml for both plasma and milk), and salicylic acid (40 μg/ml for plasma and 10 μg/ml for milk) for rifampicin, theophylline, paracetamol, and aspirin, respectively. The co-drug isoniazid and salbutamol ingested along with the “study” drug rifampicin and theophylline, respectively, did not interfere with the estimation as these do not get extracted in the solvents used. Plasma and milk extracts were then filtered through a 0.2 μ millipore filter and were injected into the HPLC system.

The mobile phase comprised of 66 mM potassium dihydrogen phosphate:acetonitrile:methanol (40:2:58 v/v) adjusted to pH 6.0 with dilute orthophosphoric acid for rifampicin, 50 mM potassium dihydrogen phosphate:acetonitrile:methanol (90:3:7 v/v) with pH adjusted to 4.7 with orthophosphoric acid for theophylline, 50 mM potassium dihydrogen phosphate:acetonitrile:methanol (95:2:5 v/v) with pH adjusted to 6 with triethylamine for paracetamol and 35% methanol, pH 2.5, adjusted with orthophosphoric acid for aspirin, respectively. The flow rate was adjusted to 1 ml/min and the λ_{max} was 340 nm for rifampicin, 272 nm for theophylline, paracetamol, and aspirin, respectively.

The processed samples were then analyzed by the Liquid RP-HPLC method. Analysis was done on DIONEX-500 HPLC system. The column used was Hypercil reverse phase $BD_S - C_{18}$ column (5 μ × 15 cm × 4.6 mm) linked to a pre-column frit.

Results

Assay Characteristics

The retention times of the test drugs as well as their internal standards were 5 and 7 mins for rifampicin

and amlodipine, 8 and 12 mins for theophylline and etofyline, 9 and 18 mins, for paracetamol and theophylline, and 14.5 and 12 mins for salicylic acid and para-nitro benzoic acid RP-HPLC methods used for analyzing the drugs showed good linearity (R^2 = 0.999), accuracy (recoveries greater than 95%), and good sensitivity (5 ng/ml). Baseline levels of the subjects in Groups I and II did not show any detectable amount of drug confirming no residual contamination at the time of blood sampling.

Table 1 shows the transfer of the study drugs from maternal plasma to breast milk. It shows the mean plasma and milk concentrations of the study drugs along with their M/P ratios. It is clear from the table that the transfer of each drug to milk varies. i.e, theophylline and paracetamol are transferred in far larger quantities as compared to rifampicin and salicylic acid.

Table 2 indicates the transfer of study drugs from plasma to milk across the CIT3 monolayer at different time intervals. The underlined M/P values indicate the time intervals when the plasma and milk samples were collected for our *in vivo* study. The transfer of the four test drugs from circulation to breast milk, as indicated by M/P ratios obtained *in vitro* showed a trend similar to that seen *in vivo* at t_{max} of each drug.

Figure 1 shows the scanning electron micrograph of the CIT3 cells cultured *in vitro*. The cells proliferated in the growth media and showed secretory deposits when cultured in secretory media.

Drugs are passed into milk as per their physicochemical characteristics, mammary epithelial cell factors, and different routes of drug transport etc. Aim of our study was to make a *in vitro* model that would closely simulate the *in vivo* situation. The samples were collected at different time points around the t_{max} of the study drug. This enabled us in understanding the rate of transfer of each drug. To validate our model, the M/P ratio obtained at the reported t_{max} of the study drug [Table 2] was compared with the respective *in vivo* M/P ratios actually obtained in our study [Table 1].

Discussion

It is well accepted that the risk posed by “maternal drugs” to the nursling can be minimized by exposing the nursling to the least quantity of maternal drugs. In this regard, realizing the importance of *in vivo* M/P ratios for the commonly prescribed drugs to breast feeding mothers, and associated difficulties in obtaining them, many pharmacologists have proposed different mathematical equations and *in vitro* models to predict them. However, most of these mathematical equations and *in vitro* models failed to predict *in vivo* M/P ratios.

The transfer of drugs from maternal plasma to breast-milk *in vivo* is a physiological process, involving many intrinsic and extrinsic factors, namely maternal factors, mammary epithelial cell factors, the physicochemical characteristics of drugs, and different routes of drug transport like passive, facilitated, and active.

Our aim was to develop a model which could simulate the physiological situation. The method developed successfully mimicked two of the intrinsic factors of the mammary epithelial cells viz. secretory property of mammary cells and tight junction property as well as the passive route of drug transport.

To validate this model, *in vivo* M/P ratios of the study drugs were needed, for which sensitive analytical methods were required for estimating the drug concentrations in plasma and milk. Though a number of analytical methods such as Bioassays, Enzyme linked immunosorbant assay, Radioimmunoassay, Gas liquid chromatography, etc. are available for drug analysis, each has its own limitations. We opted for HPLC not only due to its specificity and sensitivity but also because virtually all types of drugs can be analyzed by this method. The time interval between maternal drug ingestion and sample collection was set as per the reported t_{max} of the study drug in plasma, which is indicative of its highest concentration in plasma.

Tuberculosis is primarily a respiratory infection, and is rampant in India. Rifampicin is an amphoteric lipid soluble first-choice anti-tuberculosis drug and is also prescribed to nursing mothers. Asthma is another commonly encountered respiratory disorder for which theophylline, an amphoteric water-soluble bronchodilator, is commonly prescribed even to nursing mothers. Thus, rifampicin and theophylline are chronically taken drugs by afflicted mothers. Paracetamol and aspirin are among the

most commonly prescribed and self-administered acidic water soluble analgesic and antipyretic drugs, also ingested during breast feeding period.

As can be seen from Table 1, the mean M/P ratio for rifampicin is 0.23, indicating a very low transfer of rifampicin to milk. Further, the drug is also prescribed prophylactically (10 mg/kg body weight) to the nurslings of mothers on rifampicin therapy. As the quantity of the drug transferred to milk is very low, these mothers can safely breast-feed their infants.[16] The average M/P ratio at t_{max} for theophylline obtained both *in vivo* and *in vitro* was 0.6 indicating that it is transferred to milk in larger quantities. As generally accepted, due to the toxicity posed by theophylline, it is not to be prescribed even to asthmatic infants, making it essential to minimize the nursling's exposure to it through breast milk. It is generally recommended that these mothers should breast-feed their infants before ingesting the drug and expel out the drug from the milk at t_{max} (2 hrs).

With regard to aspirin and paracetamol, our *in vivo* studies indicated that paracetamol passes into milk in greater quantities compared to aspirin. The M/P ratios 0.87 and 0.03, respectively obtained are in conjunction with that reported in literature.[17,18] However, in spite of its low transfer to milk, salicylic acid is not recommended to nursing mothers as it could have potential adverse effects on the platelet function of the infant.[17] In case of paracetamol, the drug is transferred to milk in larger quantities and it undergoes phase-I biotransformation by N-oxidation and phase-II biotransformation by coupling with glucuronic acid. The infant is able to excrete only a part of paracetamol metabolite by coupling with sulfuric acid.[19] Thus, the possibility of paracetamol accumulating in young infant seems very real, especially, if ingested over a long time. It is, therefore, recommended that the mothers on this drug should expel out the milk at $t_{max.}$ (1 hr) before nursing the infant.

The *in vitro* sampling time as can be seen in Table 2 was decided according to the reported t_{max} of study drug. Collection of samples at different time points enabled us in understanding the rate of transfer of each drug. The M/P ratio of lipid soluble drugs rifampicin and theophylline at t_{max} (2.5 hrs and 2 hrs, respectively) were 0.29 and 0.65. For the remaining two water soluble drugs salicylic acid and paracetamol, the M/P ratios at t_{max} were 0.22 and 0.65, respectively. The trend of transfer of these drugs reflected through their M/P ratios showed similarity through the *in vitro* model for all the study drugs except Salicylic acid. In case of salicylic acid, its transfer to breast milk is in far lower concentration *in vivo*, which may be attributed to a high plasma protein binding (85%) of the drug.[20] However, though the plasma was used in *in vitro* model, it did not show such a low transfer as observed *in vivo*; this could be because apart from plasma binding proteins some intrinsic factors could be playing a role in the *in vivo* transfer which needs to be understood. At present, the discrepancy of *in vivo* and *in vitro* M/P ratios of aspirin is beyond our scope of understanding.

In conclusion, transfer of the study drugs as reflected by their M/P ratios using the *in vitro* model showed a trend similar to that seen *in vivo*. Using Wilson's mathematical formula the M/P ratios obtained for the same drugs were 0.63,1.55, 1.58, and 0.63 for rifampicin, theophylline, paracetamol, and salicylic acid respectively. Thus the M/P ratios obtained through mathematical model are significantly different ($P < 0.05$) compared to that *in vivo*. The model developed by us is potentially much better than the mathematical model predicting *in vivo* M/P ratios.

However, further work would be required for this cell culture model to be an acceptable *in vitro* tool for easy screening of drugs.

Footnotes

Source of Support: Indian Council of Medical Research

Conflict Interest: None declared

References

1. Berlin CM., Jr Drugs and chemicals: Exposure of the nursing mother. Pediatr Clin North Am. 1989;36:1089–97. [PubMed: 2677937]

2. Toddywalla VS, Patel SB, Betrabet SS, Kulkarni RD, Kombo I, Saxena BN. Can chronic maternal drug therapy alter the nursing infants hepatic drug metabolizing enzyme pattern? J Clin Pharmacol. 1995;35:1025–9. [PubMed: 8568011]

3. Koshimichi H, Ito K, Hisaka A, Honma M, Suzuki H. Analysis and prediction of drug transfer into human milk taking into consideration secretion and reuptake clearances across the mammary epithelia. Drug Metab Dispos. 2011;39:2370–80. [PubMed: 21940904]

4. Wilson JT, Brown RD, Cherek DR, Dailey JW, Hilman B, Jobe PC, et al. Drug excretion in human breast milk: Principles, pharmacokinetics and projected consequences. Clin Pharmacokinet. 1980;5:1–66. [PubMed: 6988135]

5. Fleishakar JC, Desai N, McNamara PJ. Factors affecting the milk-to-plasma drug concentration ratio in lactating women: Physical interactions with protein and fat. J Pharm Sci. 1987;76:189–93. [PubMed: 3585733]

6. Atkinson HC, Begg EJ. Prediction of drug distribution into human milk from physicochemical characteristics. Clin Pharmacokinet. 1990;18:151–67. [PubMed: 2318008]

7. Begg EJ, Atkinson HC, Duffull SB. Prospective evaluation of a model for the prediction of milk: Plasma drug concentrations from physicochemical characteristics. Br J Clin Pharmacol. 1992;33:501–5. [PMCID: PMC1381436] [PubMed: 1524962]

8. Notarianni LJ, Belk D, Aird SA, Bennet PN. An *in-vitro* technique for the rapid determination of drug entry into breast milk. Br J Clin Pharmacol. 1995;40:333–7. [PMCID: PMC1365152] [PubMed: 8554935]

9. Wilson JT, Brown RD, Hinson JL, Dailey JW. Pharmacokinetic pitfalls in the estimation of breast milk/plasma ratio for drugs. Annu Rev Pharmacol Toxicol. 1985;25:667–89. [PubMed: 3890712]

10. Lau RJ, Emery MG, Galinsky RE. Unexpected accumulation of acyclovir in breast milk with estimation of infant exposure. Obstet Gynecol. 1987;69(3 Pt 2):468–71. [PubMed: 3808527]

11. Oo CY, Kuhn RJ, Desai N, McNamara PJ. Active transport of cimetidine into human milk. Clin Pharmacol Ther. 1995;58:548–55. [PubMed: 7586949]

12. Gerk PM, Moscow JA, McNamara PJ. Basolateral active uptake of nitrofurantoin in the CIT3 cell culture model of Lactation. Drug Metab Dispos. 2003;31:691–3. [PubMed: 12756197]

13. Mandell GL, Petri WA., Jr . Antimicrobial agents: Drugs used in chemotherapy of tuberculosis and leprosy. In: Hardman JG, Molinoff PB, Goodman LS, Gilman A, editors. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 9th ed. New York City: McGraw Hill Companies; 1996. pp. 1159–61.

14. Serafin WE. Drugs used in the treatment of Asthama. In: Hardman JG, Molinoff PB, Goodman LS, Gilman A, editors. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 9th ed. New York City: McGraw Hill Companies; 1996. pp. 659–82.

15. Insel PA. Analgesic-antipyretic and anti-inflammatory agents and drugs. In: Hardman JG, Molinoff PB, Goodman LS, Gilman A, editors. Goodman and Gilman's The Pharmacological Basis of therapeutics". 9th ed. New York City: McGraw Hill Companies; 1996. pp. 625–33.

16. Tran JH, Montakantikul P. The safety of antituberculosis medications during breastfeeding. J Hum Lact. 1998;14:337–40. [PubMed: 10205455]

17. Unsworth J, d'Assis-Fonseca A, Beswick DT, Blake DR. Serum salicylate levels in a breast fed infant. Ann Rheum Dis. 1987;46:638–9. [PMCID: PMC1002216] [PubMed: 3662653]

18. Notarianni LJ, Oldham HG, Bennett PN. Passage of paracetamol into breast milk and its subsequent metabolism by neonate. Br J Clin Pharmacol. 1987;24:63–7. [PMCID: PMC1386281] [PubMed: 3620287]

19. Levy G, Khanna NN, Soda DM, Tsuzuki O, Stem L. Pharmacokinetics of acetaminophen in human neonate: Formation of acetaminophen glucuronide and sulfate in relation to plasma bilirubin concentration and D-glucaric acid excretion. Pediatrics. 1975;55:818–25. [PubMed: 1134883]

20. Lee S, Johnson D, Klein J, Eppler J. Protein binding of acetylsalicylic acid and salicylic acid in porcine and human serum. Vet Hum Toxicol. 1995;37:224–5. [PubMed: 7571349]

Figures and Tables

Table 1

Drug	Mean and Range at t_{max} in Plasma and Milk (n = 15)		
	Plasma µg/ml	Milk µg/ml	M/P ratio
Rifampicin			
Mean	6.88	1.57	0.23
(±S.E.M.)	(1.72)	(0.41)	
Range	2.76–13.1	0.51–3.54	
Theophylline			
Mean	4.45	2.71	0.61
(±S.E.M.)	(1.13)	(0.70)	
Range	1.98–8.24	0.99–4.44	
Paracetamol			
Mean	11.45	10.01	0.87
(±S.E.M.)	(2.81)	(2.58)	
Range	3.59–18.38	4.57–18.01	
Salicylic acid			
Mean	18.05	0.63	0.03
(±S.E.M.)	(4.49)	(0.16)	
Range	7.16–31.10	0.28–1.50	

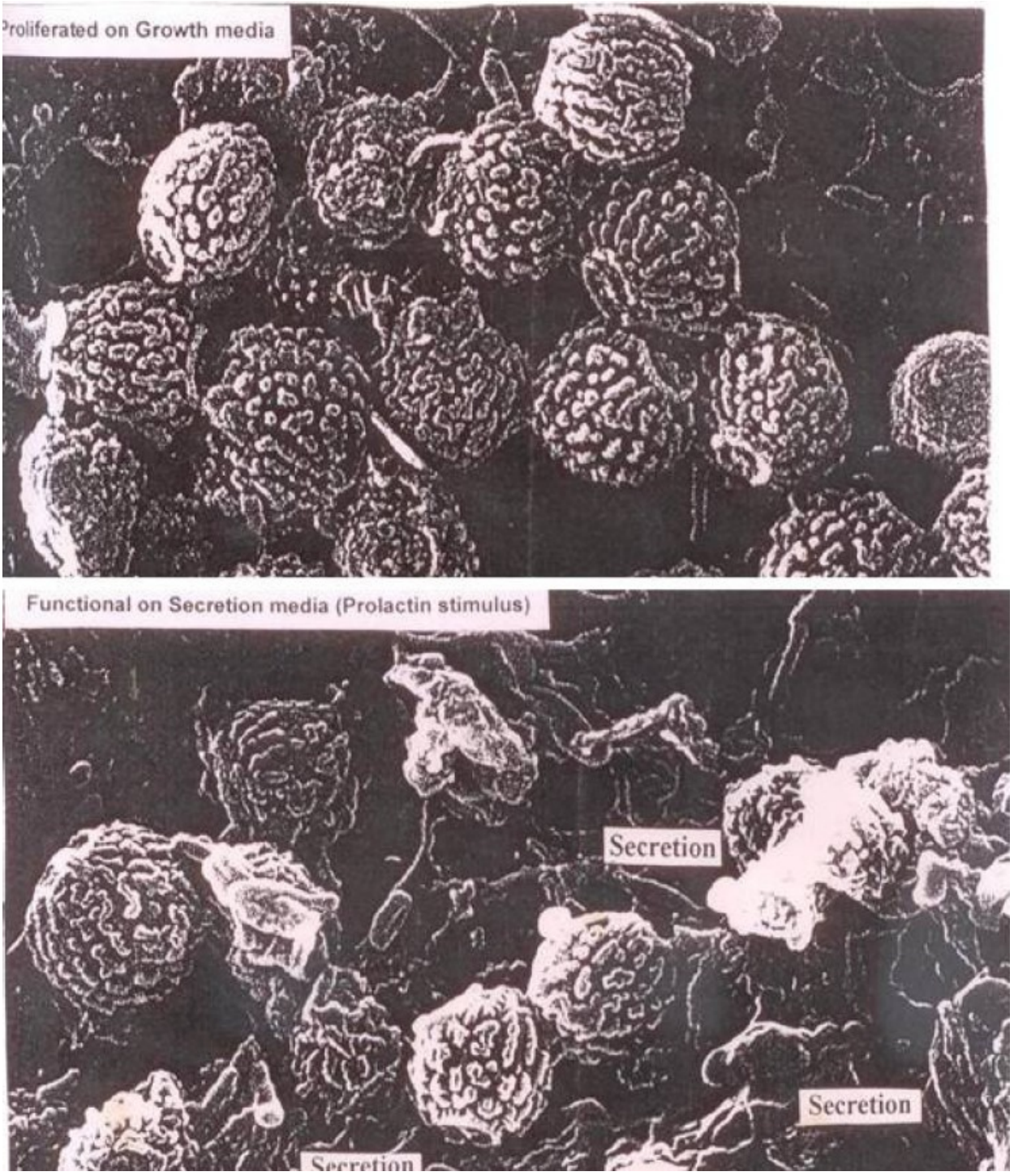
Plasma and milk concentrations of study drugs obtained at t_{max}

Table 2

Drug	Sampling time (hrs) *n* = 6							
	0	0.25	0.5	1	1.5	2	2.5	3
Rifampicin	–	–	–	0.16 (0.004)	–	0.26 (0.008)	0.29 (0.012)	0.32 (0.016)
Theophylline	–	–	–	0.45 (0.004)	–	0.65 (0.012)	–	0.74 (0.008)
Paracetamol	–	–	0.43 (0.028)	0.65 (0.016)	–	0.76 (0.020)	–	0.88 (0.06)
Salicylic acid	–	–	0.15 (0.01)	0.17 (0.008)	0.22 (0.008)	0.25 (0.008)		

M/P ratios (±S.E.M.) obtained at various time points from the *in vitro* model

Figure 1



Scanning Electron micrograph of CIT3 cells when cultured with growth media and Secretory media. Upper panel shows proliferation of the cells in growth media. Secretory deposits are clearly observed when the cells are cultured in CIT3 secretion media seen in lower panel