

CHAPTER-V

DISCUSSION

With the rapid growth of knowledge during the last few decades in the field of pharmacology and pharmacotherapeutic sciences, diseases such as leprosy, malaria, amoebiasis and tuberculosis have nearly been eradicated in western countries. This significant achievement is yet to be obtained in a developing country like India. Although, modern drugs have contributed towards improved health standards, because of other closely linked detrimental factors, tropical diseases and the problems associated with them still continue to pose an increasing threat to the welfare of mankind. Dosage of drugs based on western population are inappropriate for use in tropical countries because of differences in genetic profile, body weight, nutritional status and environmental factors. Of all the factors which may influence drug response, nutrition is probably the most important (Krishnaswamy, 1978). Undernutrition and pharmacokinetics of a drug form two sides of a pharmacological coin. There is now increasing awareness of the interplay between nutrition and drug metabolism. However, most of the studies in this area are still confined to experimental situations and there are very limited data on human population. There is some information regarding the role of drugs in modifying nutritional status.

However, there is little information how nutritional status of an individual modifies drug kinetics which in turn has several therapeutic implications. The interplay between undernutrition and a disease like tuberculosis is of considerable importance, one leading to the other through a vicious cycle, wherein the exact relationship is not precisely established. Various drug regimens are available for treatment of tuberculosis. However, such regimen suffer from a serious handicap, in that the nutritional status of the patient is not taken into account in suitably modifying the drug regimen in the treatment of tuberculous patients. Such regimens are established in the well developed western countries where neither tuberculosis nor undernutrition is a problem of significance.

Rifampicin is very important constituent of most, if not all, regimens for tuberculosis. Since it is comparatively a new drug, adequate information including the influence of undernutrition on its pharmacokinetic behaviour, is not available. Various pharmacokinetic parameters of a drug like absorption, gastrointestinal metabolism, distribution, plasma protein binding, uptake and localization within tissues, biotransformation and excretion are affected by undernutrition ultimately modifying therapeutic efficacy and/or toxicity of the drug (Krishnaswamy, 1978).

Scarce reports are available on the influence of undernutrition on the pharmacokinetic behaviour of a drug. Studies on chemotherapeutic agents like chloroquine (Wharton and McChesney, 1970), chloramphenicol (Mehta et al., 1975), tetracycline (Shastri and Krishnaswamy, 1976), streptomycin (Prasad and Krishnaswamy, 1977) and sulfadiazine (Shastri and Krishnaswamy, 1978) have provided interesting information. Significantly decreased half-life and higher elimination rate are observed with tetracycline; however, pharmacokinetic parameters of streptomycin are not significantly altered.

Adequate data are not available on the pharmacokinetic behaviour of rifampicin in undernourished patients. Therefore, it would be gainful to know whether undernutrition affects the pharmacokinetic parameters of rifampicin, a drug which is administered for prolonged periods to a large number of undernourished patients. Further, rifampicin has been previously shown to affect the metabolism and efficacy of various important drugs such as anti-coagulants (Boekhout-Mussert et al., 1974), methadone (Kreek et al., 1976) and steroids (Burley, 1977); mainly due to induction of hepatic microsomal enzymes. This study was designed with one possibility that the information generated may help in determining the appropriate dosage

regimen in order to achieve better therapeutic success with minimal toxicity.

The original design of this study was to include undernourished patients of pulmonary tuberculosis, normally nourished patients of tuberculosis, undernourished non-tuberculous volunteers and normal healthy volunteers. However, for want of adequate number of subjects/patients, only undernourished patients of pulmonary tuberculosis and volunteers were studied.

Most of the patients coming to the Shri Sayaji General Hospital and Smt. Padmavati Sanatorium, Baroda had nutritional score below 0.180.

The available patients were divided into two subgroups based on their nutritional status score. Despite the significant difference in body weights, there was no significant difference in either total protein or serum albumin concentrations. It was also seen that with decreasing nutritional score, there was increase in T_{max} and decrease in C_{max} (Table-31), indicating definite relationship between the nutritional status and these parameters. Subjects having nutritional score above 0.140 showed preponderance for T_{max} of two hours, whereas subjects having nutritional score 0.140 or below demonstrated higher T_{max} . This

reinforced the justification of arbitrary allocation of the available patients into two subgroups (A) with nutritional score below 0.140 (i.e. comparatively more undernourished) and (B) with nutritional score between 0.140 to 0.180 (i.e. comparatively less undernourished group).

The main focus of this study was to check the effect of undernutrition on rifampicin pharmacokinetics, to overcome any bias because of disease state. To rule out the role of any pathological factor in altering pharmacokinetics of rifampicin in undernutrition, experiments with human healthy volunteers were done. Subjects having nutritional score below 0.180 were grouped as undernourished volunteers and those scoring more than 0.180 were grouped as normally nourished volunteers. Although, there was statistically significant difference in nutrition score, total proteins and serum albumin levels were not different.

Undernutrition is a complex phenomenon. Changes occur in almost every organ of the body. The functional status of gastrointestinal tract is markedly altered in undernourished individuals and as a consequence, the rate and extent of drug absorption is likely to be altered. First pass metabolism is also likely to be altered. Excellent review by Viteri and Schneider (1974) and Suskind (1975) discuss how nutritional deficiencies produce alterations in the digestive system.

Absorption of a drug from gastrointestinal tract is an important aspect of drug therapy. Many characteristics like pH in the lumen, gastric emptying time, intestinal transit time, surface area and intestinal villi in the gastrointestinal tract determine drug absorption. All these factors considerably influence the rate and extent of absorption of drugs.

Very early studies indicate that gastric emptying time and intestinal transit time are significantly prolonged. Gastric dilatation and ptosis, gastric mucosal atrophy, hypochlorhydria and a reduction in the gastric bacterial barrier have been reported by Mata et al.(1972).

The role of chronic protein malnutrition in the causation of the malabsorption syndrome has been studied by many scientists, reporting almost similar structural and functional changes. Mayoral et al.(1967) reported changes of small bowel with malabsorption, due to protein malnutrition, in eight patients. These investigators have suggested certain clinical and biochemical differences between protein malnutrition-induced malabsorption versus tropical sprue, which need critical evaluation in India where both conditions are prevalent.

A study by Tandon et al.(1968) showed intestinal mucosal alterations in the form of blunting of villi, fusion at their bases and variable inflammatory cell infiltration of lamina propria. These findings suggest that experimental protein deficiency in adults is associated with and probably leads to alterations, in structure and function, thus setting up a vicious circle of malabsorption and nutritional deficiency. It is well known that epithelial cells lining the intestinal villi have a high rate of turnover and require building material in the form of protein to replace shed cells (Crosby, 1961).

The small intestine in protein calorie malnutrition (PCM) depicts a wide variety of changes. The mucous membrane is thinner with villous atrophy and increased intestinal transit time (Mayoral et al., 1972).

In the present study, the significantly lower serum concentrations of the drug in undernourished group as compared to levels seen in normally nourished group, when drug was given orally alone or along with INH, in man or in rats (acutely or chronically) provide strong support

that between normal and malnourished animals or humans, there are differences in pharmacokinetic parameters.

In our study, the lower levels of rifampicin in under-nourished humans and LP rats may be due to (i) decreased extent and rate of absorption, (ii) increased rate of elimination and/or (iii) both.

In animal studies, using the isolated intestinal loop in vitro, the total amount of rifampicin absorbed over a period of four hours and the amount absorbed at any given time was always significantly higher in NP group than in LP group. Thus, the net amount of rifampicin absorbed and rate at which rifampicin is absorbed from the intestines is very likely to contribute to the low rifampicin levels observed in humans and also the delay in attaining C_{max} as evidenced by a tendency to larger T_{max} values in humans (Fig.10).

As opposed to the studies in humans however, there are no differences obtained in T_{max} values in normal and LP group of rats, although the C_{max} values are significantly different. One possible explanation may be, that in animals, the doses of rifampicin are quite large and likely to minimize any change in T_{max}. Perhaps, more number of observation points around C_{max} may have revealed the exact situation. The comparatively poor absorption of rifampicin

in more undernourished persons is probably a reflection of the structural and functional changes in the absorbing surface of the intestines due to undernutrition.

To rule out the influence of disease per se on the absorption of rifampicin similar studies were carried out in non-tuberculous volunteers with different nutritional status (Table-36 and Table-37).

A lower peak serum concentration obtained in undernourished volunteers is observed, whereas, there is not much difference in T_{max} . However, as the degree of undernutrition became worse, the T_{max} gradually increased, indicating a poor rate of absorption.

Since, all volunteers were given rifampicin powder in exactly weighed amounts to be swallowed directly, the question of differences in formulation does not arise in this study. Any difference in disintegration time is bypassed and the same powder of rifampicin was given from one batch of rifampicin only.

Any changes on the peak concentration and the T_{max} are therefore entirely due to the process of absorption. These results are also substantiated by the in vitro absorption studies done on isolated loop of intestine in rats (Table-18, Fig.2).

Another possibility for the decreased rifampicin values, is an increase rate of elimination. An increased rate of elimination may be due to increased rate of metabolism and/or increased amount of renal and faecal elimination.

In our study, as far as the acute studies are concerned, the question of increased rate of metabolism does not arise. In fact, the undernourished animals have lesser cytochrome P-450 than normal and are therefore unlikely to metabolize rifampicin faster. Hence lower peak rifampicin values due to increased rifampicin metabolism in undernourished animals is not a possibility.

Another factor of lower peak values, could be due to increased rate of renal and faecal elimination during the first 3 hours post-rifampicin administration. A study of the urine excretion data in animals reveals that at least during the first 12 hours there is significant change in rifampicin excretion between NP and LP group of rats when noted as percentage of dose administered. Thus, it appears that two factors could be responsible for low peak rifampicin concentrations in man and animals in a state of under-nutrition. These could be due to the decreased rate of absorption from the intestine and the time to attain peak concentrations. Another factor could be due to increased rate of urinary elimination as evidenced by higher percentage of dose of rifampicin eliminated during first 12 hours.

Rat has been used as an experimental model for studying rifampicin kinetics as serum concentration of this antibiotic in man closely resembles that in rat more than in other animals like dog and mouse (Furesz, 1970). It is easier to bring up the colony of rats and it is possible to induce protein-undernutrition in rats by feeding a low protein diet in control group, for a few weeks only in post-weaning period (Rajlakshmi et al., 1974).

When experimentally, the undernutrition was induced, the rats showed significantly lower body weight compared to the control group. However, this growth retardation was not accompanied by reduction in weight of various tissues in undernourished rats except liver which weighed significantly less than in the control group (Table-17). Six weeks long administration of INH and rifampicin to rats undernourished for 8 weeks in the post-weaning period did not alter body or tissue weights (Table-16 and Table-17).

In patients of pulmonary tuberculosis, usually one of the signs is loss of body weight. Chemotherapy produces improvement including weight gain which appears to be due to improvement in the disease and not because of the direct drug effect. The same fact emerges in the present study on rats, since when they were subjected to undernutrition and chronic treatment with rifampicin, twice weekly and INH daily for 6 weeks, the weight gain was not modified.

The amount absorbed is not the only factor which is modified by undernutrition, others like its distribution, plasma protein binding, uptake and localization within tissues, biotransformation, excretion both biliary and renal and drug interactions may also be modified by undernutrition leading to altered response to the drug.

A significant observation made in this study is that the serum half-life of rifampicin is considerably lower in undernourished group. In patients of pulmonary tuberculosis, following a single dose of rifampicin, the aV_d was significantly higher in group B i.e. less undernourished than in group A i.e. comparatively more undernourished while elimination rate constant was significantly higher in group A than in group B. Along with reduced serum concentration, the half-life was also lower in group A than in group B (Table-32b).

Besides alteration in the absorption rate and its extent (as described above), another important factor which appears responsible for reduced half-life of rifampicin is clearance of the drug from the body.

The rate of absorption and elimination rate constant are two critical factors in determining plasma concentration of any drug. Since in present study, the dose interval and dose per kg body weight were same, any variation in rate of absorption and rate of elimination or both would have contributed to reduced serum concentrations, changes in aV_d and reduced half-life.

One of the important parameter which can lead to change in clearance is the rate of excretion of the drug through kidney.

The 24 hour urinary excretion of rifampicin (mg/day) was 154.80 ± 3.3 in group A patients and 121.5 ± 3.8 in group B patients.

In rats, after single dose administration, the urinary excretion values expressed as percentage of dose administered were 9.2 ± 0.4 and 7.7 ± 0.4 per 24 hours in undernourished and in normally nourished group respectively. After chronic treatment with rifampicin, the values were 7.3 ± 0.2 and $6.3 \pm 0.2\%$ per 24 hours in undernourished and normally nourished group respectively.

Ideally, the excretion of rifampicin not only from the urine but that of unabsorbed rifampicin in gastrointestinal tract and unabsorbed rifampicin due to biliary excretion could have helped to determine the exact amount of rifampicin absorbed in normal and undernourished volunteers. Since rifampicin itself is degraded partly in faeces (Acocella, 1978), this study would have been difficult and hence only rifampicin excretion in urine was undertaken for the study.

The mean AUC of undernourished subjects was significantly lower than that of healthy subjects (Table-38). The mean serum rifampicin concentration at 2 hours in healthy subjects was 16.1 ± 1.2 mg/ml serum while in undernourished group, it was 11.2 ± 1.1 mg/ml serum (Table-39b). These results indicate that absorption could have been impaired in undernutrition. On the other hand, observations on apparent renal clearances indicate that in the undernourished the drug is extracted/metabolised at a higher rate than in the healthy subjects. Excretion of desacetyl rifampicin as percentage of total rifampicin excretion is higher in undernourished group. The difference is greater after chronic rifampicin treatment (Table-28). A comparatively faster excretion of the drug could have contributed to both smaller area under the curve and lowered serum peak levels as mentioned previously. Lowered serum peak levels could also be due to the decreased rate and extent of absorption. (However, limitations of the design of the experiments do not allow for a clearcut comment.)

Thus, it becomes clear that the urinary excretion of rifampicin is higher in the undernourished group, which is also supported by the animal experiment results (Table-26).

Higher urinary excretion, lower serum concentration along with rapid clearance of rifampicin in more undernourished than in less undernourished subjects could be because of lower plasma protein binding along with decreased rate of absorption or less complete absorption. Drug protein binding interactions frequently determine the rate at which drugs are absorbed from the gastrointestinal tract, transported to tissues and eliminated from the body. Drug protein interactions are usually reversible with the free drug in plasma being in equilibrium with that in tissue fluids. Protein energy malnutrition alters plasma and tissue proteins quantitatively and also probably qualitatively (Krishnaswamy, 1978).

The enhanced clearance of rifampicin in undernourished human subjects and rats could be a consequence of decreased plasma protein binding. In acute studies in rats, a possible uptake by various tissue systems resulting into decreased free level of drug in serum may also be contributory. Altered characteristics of hepatic microsomal enzymes in undernourished state can further complicate the pharmacokinetics. Available literature presents limited work done in this regard on human subjects. For obvious reasons, it is not feasible to study the tissue levels and hepatic microsomal enzyme contents in human subjects. To overcome this limitation, simultaneous

animal studies on albino rats were carried out (i) to check the tissue distribution of rifampicin along with the study on serum and urine concentrations of rifampicin, (ii) to explore hepatic microsomal enzyme system and (iii) to observe in vitro pattern of absorption.

After a single dose administration, tissue concentrations of rifampicin were high only in liver and kidney of undernourished group of rats. These are two main organs for hepatobiliary and urinary excretion of rifampicin which is almost equally excreted in bile and urine, the recovery in the two fluids being of the same order of magnitude (Acocella, 1978). This might account for the possibility of increased excretion of rifampicin in urine and possibly increased metabolism and biliary excretion from liver. The latter could have been studied based on faecal excretion but due to limitation of collecting uncontaminated faeces, it could not be done.

In acute experiments, peak rifampicin concentrations in lung in undernourished group were lower than in control group and tended to be slightly higher at 10 hour interval only in undernourished group. This may be expected since lung is considered to be in the central compartment. In other organs differences were either insignificant or highly variable. Thus, from the results of the study on urinary excretion and possibly lower protein binding, it appears that more free

rifampicin was cleared in undernourished group possibly due to high levels of rifampicin in liver and kidney.

When chronic treatment with rifampicin was given twice a week for 6 weeks, the tissue levels remained significantly higher in NP group at 5, 10 and 20 hours compared to that found after a single dose administration (acute treatment). From the results obtained in LP group of rats, it appears that the tissues of undernourished rats extract lesser rifampicin. This should have been reflected as higher rifampicin concentration in blood, however, due to faster elimination through kidney, rifampicin concentrations in undernourished group were lower.

In the chronic rifampicin study, only kidney level appeared to be higher in undernourished group at all the time points studied except during absorption phase, indicating that more free rifampicin was available for excretion. Levels in liver were not significantly different. On the other hand, in this tissue, differences as noted in acute studies tended to be minimal. No differences were observed in levels of rifampicin between normal and undernourished groups, in lung, heart and skeletal muscle.

Liver, lung and heart rifampicin levels were generally higher in chronic study than in acute study. In chronic study, there was also increased AUC, indicating some degree of tissue saturation. In the case of skeletal

muscle not much differences were present. In spleen, in control group levels were generally lower and in undernourished group, the levels were comparatively higher, following chronic treatment. It is difficult to give any explanation.

The urinary excretion of rifampicin in rat (expressed as percentage of dose administration) was higher in undernourished group following both acute or chronic treatment. However, at all the time points checked for urinary excretion, lower values were seen when expressed as ug/ml/kg (Table-27). It is possible that after chronic treatment, there is higher tissue capture of rifampicin in all tissues including kidney and liver. It is also possible that this decrease in urinary excretion of rifampicin (as percentage of total dose) after chronic treatment is the final result of less complete absorption. This may be because of saturation of the absorption processes, as noted in serum levels also (Table-19). Serum rifampicin levels in undernourished group were lower at all the time points which may be the result of lower plasma protein binding. It would thus appear that lower serum levels in chronic therapy in LP group could be due to (a) saturation of absorption process, (b) lowering of plasma protein binding (c) increased levels in tissues and (d) increased rate of excretion.

The faster rate of clearance of the drug from the body may be related to its relatively poorer protein binding observed in the undernourished subjects. Values for plasma protein binding observed in undernourished patients in in vivo experiments were lower than those reported by Bowman and Reinberger (1974). However, an in vitro technique for estimation of protein binding with high concentrations of the antibiotic showed comparatively higher binding. Such high concentrations are not attained during therapy and values reported here, may, therefore represent more closely the physiological state.

Albumin is the main binding protein in plasma and hypoalbuminaemia is a characteristic finding in malnutrition. The rate of albumin synthesis is directly related to the level of protein intake and amino acid supply (Waterlow, 1975). An extensive study by Buchnan (1977) has indicated that protein binding in vitro of a number of drugs such as salicylate, warfarin, digoxin, thiopentone, is altered in undernourished children.

In vitro plasma protein binding of rifampicin at the 24 hours of incubation period was 23% to albumin and 76.95% to α_2 protein fraction. In vivo plasma protein binding of rifampicin to albumin and α_2 protein fractions at 4 hours were 20% and 79.93% respectively (Table-40).

Limited knowledge exists regarding the relationship of concentration of free drug to total drug as a function of exact drug concentration, total protein concentration and albumin concentration. In general undernutrition alters the plasma protein profile. The decreased plasma protein binding of some drugs such as streptomycin, cloxacillin, digoxin, thiopentone, phenylbutazone, sulfadiazine and sulfisoxazole is presumably due to lower serum albumin levels (Buchanan, 1977).

Several investigators (Boman and Reinberger, 1974; Chonjnowsky and Gralewicz, 1976) found that 30-41% of the protein bound rifampicin is associated with the serum albumin fraction. Buchanan (1977) has reported no differences in total rifampicin protein binding in the sera of albumin-depleted or kwashiorkor (2-2.4 g albumin/dl serum) and normal (4.1 g albumin/dl serum) subjects, suggesting that the gamma-globulins rather than albumin may be the major serum binding site for rifampicin.

In more undernourished patients, higher urinary excretion and lower plasma protein binding observed support the contention that in undernourished subjects, rapid clearance of rifampicin is responsible for decreased half-life and lower serum concentration.

In the present study, 36.5% reduction was observed in the cytochrome P-450 concentration in the undernourished group. As a result of drug treatment (rifampicin + INH), cytochrome P-450 concentration was apparently increased in both low protein and control group but the increase was not significant ($P > 0.05$) (Table-30a). There had been no quantitative change in the cytochrome P-450 enzyme system. However, there appeared to be increased degradation of rifampicin as reflected by increased excretion of the metabolite desacetyl rifampicin (Table-28) suggesting that the enzyme activity was increased. Thus, indirectly rifampicin induced enzyme system induction may be presumed. The inducibility of hepatic microsomal system in the rat by rifampicin has been shown by some (Periou et al., 1983) and not found by others (Barone et al., 1972; Otani, 1975). Induction of microsomal enzymes by rifampicin has been shown by various workers in man (Zilly et al., 1977) in mice (Tredger et al., 1981) and in rabbit (Kergueres et al., 1982). In rats however, rifampicin was reported to be ineffective for inducing cytochrome P-450 (Barone et al., 1972; Otani and Remmer, 1975). Rifampicin in high dosage (40 mg/kg/day) has been shown to induce cytochrome P-450 by Piviou et al. (1983). It has also been reported that INH (10 mg/kg and/or rifampicin (13 mg/kg) oral treatment for 13 days) in rabbits can induce cytochrome P-450 (Kergueres et al., 1982).

Using tritiated rifampicin, it has been shown that rifampicin-quinone, an auto-oxidation product of rifampicin binds to microsomal macromolecules in the rat liver and NADPH cytochrome C-reductase converts this quinone form back to rifampicin and thus prevents a possible cytotoxicity (Bolt and Remmer, 1976).

Thus, the present stage of knowledge regarding the effects of rifampicin in rat liver microsomal system is inconclusive. Therefore, the result (Table-30) on cytochrome P-450 and cytochrome b_5 from rat liver can, at the best be judged as preliminary on the mixed effects of INH and rifampicin warranting a further extension of the work.

Chemotherapy of tuberculosis has significantly changed in the past 10 years with newer chemotherapeutic agents, improved pharmacologic knowledge and expanding therapeutic regimens. The first line antituberculous drugs are isoniazid, rifampicin, ethambutol and streptomycin while second line antituberculous drugs include pyrazinamide, cycloserine, ethionamide, viomycin, capreomycin and kanamycin.

That undernutrition does not affect streptomycin pharmacokinetics and the pharmacokinetic interaction between rifampicin and INH is debatable. INH has been

previously shown to form acetylhydrazine which can bind to macromolecules in liver cells which is followed by necrosis and this incidence is found to ^{be} increased by rifampicin (Mitchell and Jollows, 1975). The metabolism of these two drugs could affect each other and a detailed observation of liver function is required when these two drugs are combined (Zilly et al., 1977). Thus, it was of interest to examine how concurrent administration of other antituberculous drugs like INH, streptomycin, ethambutol and pyrazinamide affects pharmacokinetic profile of rifampicin in undernourished subjects.

Results of this study showed that neither INH nor the INH and streptomycin (or ethambutol or pyrazinamide) simultaneously administered with rifampicin changed the rate of absorption and serum concentration levels of rifampicin significantly.

The urinary excretion of rifampicin was also not modified significantly because of the presence of other antituberculous drugs together with rifampicin. Concurrent administration of other commonly used antituberculous drugs failed to change the rate of absorption and serum concentration or urinary excretion of rifampicin.

On concurrent administration with INH along with rifampicin, in rats, passage towards different tissues was not much altered but the peak serum rifampicin level reached and total amount absorbed were lower than after rifampicin treatment alone though not significantly. The absolute quantity going to tissues remained almost the same leaving the net absolute amount of rifampicin to be excreted by the kidney in the less quantity. This is supported by the observation that the amount excreted by the kidney was lesser. This also may indicate that the dosage of rifampicin in NP and LP groups need not be changed.

The results in the undernourished groups patients or volunteers, suggest that though the total availability is lower due to either impaired drug absorption and/or elevated renal clearance, increased free drug levels (because of comparatively lower plasma protein binding) may compensate for these factors. Therefore, it is possible that therapeutic adequacy may still remain similar and any alteration in rifampicin dosage regimens may not be necessary in the undernourished groups.