Urinary levels of chloroquine in relation to dietary protein

S.A. Adelusi

Department of Pharmacology and Therapeutics, University of Ibadan, Ibadan (Nigeria), 16 November 1981

Summary. When rats suffering from protein-calorie malnutrition (kwashiorkor) were given chloroquine, urinary levels of unchanged drug increased while chloroquine metabolites decreased. This apparent inhibition of chloroquine metabolism was eliminated by refeeding the animals.

Protein-energy malnutrition (kwashiorkor) is rampant in many developing countries as a result of poverty and ignorance[1], large family size[2] and civil war[3]. Most of these countries (particularly those in the tropics) have been decleared to be endemic zones for malaria, and chloroquine is indiscriminately administered to patients without regard to their nutritional status.

The lack of quantitative data on urinary levels of chloroquine and its metabolites in this diseased state, and the influence of nutrition on the drug's metabolism has necessitated this study, using the rat as a model.

Materials and method. White rats of the Wistar strain, aged between 3 and 4 weeks at the beginning of the experiment were used for this study. Chloroquine sulphate was obtained from May & Baker Dagenham, U.K. and the quantities administered are given in terms of the base. The rats were divided into 3 groups A, B and C, each group containing 6 male rats. Group A was fed on a commercially-produced diet, group B was fed on a gari-based diet with the same percentage of protein (21%) as the commercially-produced diet and group C on a gari-based diet with 2% protein. The purpose of feeding group A with the commercially-produced diet was to compare the growth of this group with group B which served as the control rats in this study.

At the end of 24 days when the typical symptoms of kwashiorkor (a protein-energy malnutrition syndrome) had been established in group C, the rats in each group were housed individually in metabolic cages where they were allowed free access to food and water and allowed to become habituated for 2 days. Urine was collected from each rat in groups B and C for 24 h after which the rats were given chloroquine at a dose of 10 mg/kg b.wt. through i.p. administration. Urine was also collected for 24 h after chloroquine administration and 24 h after 7 days of chloroquine administration.

6 male rats were also treatedfor kwashiorkor but allowed to recover from the malnourished state by refeeding them for 50 days with the diet which contains 21% protein. They were housed in cages as before; collection of urine and treatment with chloroquine was as described above.

Chloroquine and its polar metabolites were determined by a modification of the fluorimetric methods described by Rubin et al.[4], and Adelusi and Salako[5]. 1 ml of the urine sample was diluted with 1 ml of 0.1 M HCl and this mixture was made alkaline with 0.5 ml strong ammonia (s.g. 0.91). Chloroquine and its metabolites were extracted twice with 10 ml portions of diethyl ether (analar grade). The ether extracts were bulked together and washed with 5 ml of borate buffer pH 9.5.

Chloroquine assay. 15 ml of the ether layer was shaken with 5 ml of phosphate borate buffer pH 7.85 for 5 min to remove the metabolites. 10 ml of the organic layer was shaken with 5 ml of 0.1 M HCl for 5 min. The acid extract was mixed with 0.2 M NaOH in 50% ethanol (v/v) and the fluorescence of the solution (pH 9.9) was read on a Perkin-Elmer spectrofluorimeter using excitation and emission wavelength of 331 and 386 nm respectively.

Chloroquine metabolites assay. The phosphate borate buffer pH 7.85 extract was mixed with 0.2 M NaOH in 50% ethanol (v/v) and the fluorescence of the final solution (pH 9.9) was read as above.

A calibration curve was determined with standard solutions of chloroquine. The chloroquine metabolites were not individually characterized but were determined in terms of unchanged chloroquine base. The dilution factors were taken into consideration in the final calculation. The amount of chloroquine and the metabolites found in the urine were expressed in μg per 24 h. Urine collected for 24 h before chloroquine administration was used as blank during determination.

Results and discussion. After 24 days, the rats in group C, that is, the rats fed on the gari based diet with 2% protein, presented the clinical picture of protein-energy malnutrition. Those in groups A and B were fed on the commercially produced diet or the gari based diet with 21% protein gained weight steadily.

The initial few tests showed that the amounts of chloroquine found in the urine of the rats fed on the commercially produced diets were not significantly different from those of the rats fed on the gari-based diet with 21% protein and therefore, results for urinary levels of chloroquine and its metabolites presented in the table are for the rats fed on the gari based-diet with a different amount of protein.

In the table, the amount of chloroquine (μg/24 h) excreted by the rats fed on the gari-based diet with 21% protein (i.e. the normal rats) was significantly lower than that of the rats fed on the gari-based diet with 2% protein (i.e. the kwashiorkor rats) $p < 0.05$. However, the amount of chloroquine excreted by the refed rats showed no significant difference from the values obtained for the normal rats ($p > 0.05$) but were significantly different from the values obtained for the kwashiorkor rats ($p < 0.05$). The amount of the metabolites obtained from the malnourished rats was lower than those for the normal rats ($p < 0.01$) and the refed rats ($p < 0.05$). There is no significant difference

Chloroquine and its metabolites in the urine of normal, kwashiorkor and refed rats

N	Total amount of chloroquine excreted in 24 h (μg/24 h)		Total amount of chloroquine metabolites excreted in 24 h (μg/24 h)	
	24 h after chloroquine administration	24 h after 7 days of chloroquine administration	24 h after chloroquine administration	24 h after 7 days of chloroquine administration
Normal rat	205.61 ± 26.00	1.04 ± 0.13	99.80 ± 8.50	0.6 ± 0.07
Kwashiorkor rat	315.83 ± 32.90	3.17 ± 0.55	58.31 ± 6.44	0.50 ± 0.06
Refed rat	211.02 ± 27.12	1.17 ± 0.27	87.31 ± 10.22	0.72 ± 0.08

Each value is the mean of 6 determinations ± SEM.

between the levels of the metabolites obtained for the normal and the refed rats ($p > 0.05$). These differences were observed 24 h after chloroquine administration and 24 h after 7 days of chloroquine administration. The values in the table are considerably lower after 7 days of chloroquine administration than after 1 day; this is probably due to the fact that the amount of chloroquine and its metabolites excreted in urine decreases with time.

The fact that more chloroquine and less metabolites were excreted by the kwashiorkor rats as compared to the normal and the refed rats showed that chloroquine is probably slowly metabolised during protein-energy malnutrition. It is well known that the rate of metabolism of drugs and foreign compounds in mammals may be altered by changes in the quality and quantity of dietary protein[6-8]. It has been shown that chloroquine is metabolized in the rat via a dealkylation process such that 70% of the excreted quinolines was unchanged chloroquine and the rest consisted of the desethyl and bisdesethyl chloroquine as metabolites[9,10]. The reason for the difference observed in the urinary levels of chloroquine and its metabolites in both the normal and the kwashiorkor rats might be due to the effect of the disease. In the kwashiorkor rats, the effect of the disease might reduce the concentration or the activity of the drug-metabolizing enzymes; thus, when the rats were refed to normal from the malnourished state, the metabolism of chloroquine became normal. This finding is in agreement with a previous study by a group of workers[11] who studied th in vitro and in vivo effects of dietary protein intake on drug metabolism. They found very low drug metabolising activity in rats fed with a protein free diet and progressively higher activity when the protein content of the diet was raised to 50%.

The result from this study therefore shows that the metabolism of chloroquine was impaired in protein-energy malnourished rats, and refeeding corrected this abnormality. However, the measurement of chloroquine and its metabolites in urine might not be a direct evidence for altered metabolism of chloroquine since other changes such as altered binding to serum protein[12], or changes in excretory functions could play some part.

1 Bassir, O., in: Handbook of practical biochemistry, 2nd edn, p.25. Ibadan University Press, Ibadan 1963.
2 Morley, D., Bicknell, J., and Woodland, M., Trans. R. Soc. trop. Med. Hyg. *62* (1968) 164.
3 Wood-Walker, R.B., Lancet *1* (1970) 299.
4 Rubin, M., Zvaifler, N., Bernstein, H., and Mansour, A., in: Proc. 2nd int. Congr. of Pharmac., p.467. Pergamon Press, Oxford 1965.
5 Adelusi, S.A., and Salako, L.A., J. Pharm. Pharmac. *32* (1980) 711.
6 Boyd, E.M., Clin. Toxic. *2* (1969) 423.
7 Smith, J.W., Hill, C.H., and Hamilton, P.B., Poultry Sci. *50* (1971) 768.
8 Sick, D.B., and Carlton, W.W., Am. J. vet. Res. *33* (1972) 107.
9 McChesney, E.W., Conway, W.D., Banks, Jr, W.F., Rogers, J.E., and Shekosky, J.M., J. Pharm. exp. Ther. *151* (1966) 482.
10 Fletcher, K.A., Baty, J.D., Price Evans, D.A., and Gilles, H.M., Trans. R. Soc. Trop. Med. Hyg. *69* (1975) 6.
11 Kato, R., Oshima, T., and Tomizawa, S., Jap. J. Pharmac. *18* (1968) 356.
12 Buchanan, N., and Van der Walt, L.A., Am. J. trop. Med. Hyg. *26* (1977) 1025.

Temporal storage of kynurenine and 3-OH-kynurenine in the fat body of metamorphosing *Ephestia kühniella*

K. Cölln and Eva Hedemann

Zoologisches Institut der Universität zu Köln, Lehrstuhl für experimentelle Morphologie, Weyertal 119, D-5000 Köln 41 (Federal Republic of Germany), 17 March 1982

Summary. During the pharate pupal stage a massive accumulation of kynurenine and 3-OH-kynurenine is observed in the fat body of *Ephestia kühniella*. By injection it can be demonstrated that this organ is capable of sequestering at least 3-OH-kynurenine, the dominating tryptophan metabolite in *Ephestia*. It is suggested that the fat body reduces a possibly harmful excess of tryptophan metabolites at the beginning of metamorphosis. These sequestered metabolites provide a precursor depot for ommochrome synthesis in later development.

During metamorphosis, holometabolous insects can be regarded as closed systems. Chemical precursors required for the development of adult structures can be derived only from histolysis products or from reserves accumulated during the larval feeding period. Kynurenine and 3-OH-kynurenine are intermediates in the tryptophan → ommochrome pathway[1]. In the meal moth *Ephestia kühniella* Z. they are mainly utilized during the second half of the pupal stage for the synthesis of screening pigment inside the compound eyes[2]. But they are already present in relatively high concentrations at the beginning of the pharate pupal stage[3]. Kynurenine and 3-OH-kynurenine, however, are very reactive molecules; 3-OH-kynurenine, especially, causes abnormal development at higher concentrations[1,4,5]. Therefore the internal level of both tryptophan metabolites should be regulated. The aim of this study was to obtain some information about regulatory mechanisms by investigating the developmental patterns and tissue distribution of kynurenine and 3-OH-kynurenine.

Material and methods. Experiments were performed with last instar larvae of *Ephestia kühniella* Z. Feeding larvae 7 days after molt and pharate pupal stages A2 and A5 were analyzed. Timing and sexing have been described elsewhere[6]. Characteristics of the strains used (wild type and mutant *a*) and rearing conditions have been summarized by Caspari and Gottlieb[7]. Injections, ligations, hemolymph sampling, determination of hemolymph volume and the preparation of visceral fat bodies already have been described in detail[6]. Tryptophan, kynurenine and 3-OH-kynurenine were quantified by means of 2-dimensional TLC on cellulose[8,9]. Protein was determined after extraction of tryptophan metabolites with biuret reagent[10], with BSA serving as a standard. Statistical calculations were done using the tests of Mann and Whitney or Kruskal and Wallis as referred to by Campbell[11].

Results and discussion. During developmental phase analyzed here, no apparent sex-specific differences exist with respect to kynurenine and 3-OH-kynurenine. Other investi-