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An Investigation into the Effects of Ivermectin and Praziquantel on the Intestinal Transport and Metabolism of Albendazole

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Preliminary investigation into the effects of ivermectin and praziquantel on the intestinal transport and biotransformation of albendazole was carried out on the rat using the *in-vitro* gut-sac method. Drug detection was carried out by high performance liquid chromatography. Ivermectin caused a 3-5-fold ($P<0.01$) increase in serosal concentrations of albendazole over the control at both the 60 and 120-minute study while the two higher concentrations of ivermectin resulted in a 3-fold ($P<0.0001$) increase in albendazole biotransformation campared to the control at all time points. Praziquantel had no effect on albendazole or albendazole sulphoxide.

As both albendazole and ivermectin are substrates of cytochrome 3A4 (CYP3A4) and P-glycoprotein transporter protein, the data is suggestive of a strong interaction between albendazole and ivermectin at P-glycoprotein level that results in an increased absorption albendazole as well as the transformation of albendazole sulfoxide.

Key Words: Ivermectin, Praziquantel, intestinal transport, Albendazole metabolism, interactions.

INTRODUCTION

The assortment of anthelmintic drugs is currently limited in number and diversity. A number of drug combinations are being tried out with favourable outcome. In onchocercrasis, co-administration of albendazole and ivermectin has been shown to produce a more pronounced effect than the use of ivermectin alone [1] while combination of praziquantel and albendazole gave better results than the use of either drug alone in the treatment of hydatid disease [2,3] and neuro cysticerosis [4].

Both albendazole and ivermectin are substrates of CYP 3A4 isoenzyme [5,6] and the efflux transporter P-glycoprotein (P-gp) [7,9]. Albendazole is also metabolised by flavin monoxygenases (FMO) [5]. CYP 3A4 and P-gp are both present in the gastrointestinal tract (GIT). Consequently albendazole undergoes intestinal sulfoxidation and P-gp secrection [10,11]. Its metabolite, albendazole sulfoxide possesses antihelmintic activity, is the active compound systematically and is further degraded in the liver to an inactive sulfone [12]. Praziquantel is extensively metabolized probably by CYP 2B1 and CYP 3A isoenzymes [13]. Praziquantel has been noticed to increase systemic availability of albendazole when the two are administered together [14,15].

Considering these shared factors there is a high probability of an interaction between the drugs that may contribute to the enhanced pharmacological outcome observed. This study carried out an initial investigation into the effects of ivermectin and praziquantel on the disposition of albendazole at the GIT.

Material and Methods

All chemicals used were HPLC grade. Albendazole and albendazole sulfoxide were obtained from SmithKline Beecham (UK), proguanil from Zeneca (Macclesfield, UK),

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ivermectin from MERCK (UK) and praziquantel from Sigma (Germany). methanol, orthophosphoric acid, triehylamine (TEA), acetonitrile, dicloromethane, and phosphate buffered saline tablets were obtained from Sigma Chemicals (Germany). Nitrogen gas was from BOC gases (Surrey, UK). Wistar rats with an average weight of 300 g were obtained from Charles River.

A 10 cm length everted gut sacs sections were prepared from rat intestines by the Wilson and Wiseman method [16]. 1 ml of Krebs solution was introduced into the sac that was then immersed into 10 ml of the drug buffer solution being tested. It was aerated, then incubated in an oscillating water bath at 37^0C for the test duration. At the end of the incubation period the volume of the sac (seronal) contents and the flask (mucosal) contents were noted and quantities of albendazole and albendazole sulfoxide in each determined.

EXTRACTION PROCEDURE

The extraction procedure described was then reconstituted with the mobile phase and run on HPLC. The mobile phase consisted of 73% by volume water containing 0.5% TEA, and 27% acetonitrile at pH~2.8 run in a pre-packed Hypersil BDS C18 column (120 mm x 4.6 mm i.d.) of 5 μ particle size at a wavelength of 254 nm and flow rate of 1 ml/min. Retention times in minutes were as follows; albendazole (11.5), proguanil (9) and albendazole sulfoxide (2.55).

Data Analysis

Microsoft Excel was employed to plot the calibration curves and analyze the raw data. Linear regression was used to determine the slope and correlation coefficient. Acceptable calibration curves had linear correction values (r^2) greater than 0.970. Statistical analysis was performed by means of ANOVA followed by bonferroni modified t-test (ARCUS PRO-II software).

RESULTS

Due to the poor solubility of albendazole and presence of the sulfoxide (≅ 4% w/w) as an impurity in albendazole powder, the starting concentrations of both albendazole and albendazole sulfoxide were not uniform as expected.

All ivermectin drug concentrations produced an increase in amounts of serosal albendazole compared to control (Figure 1).

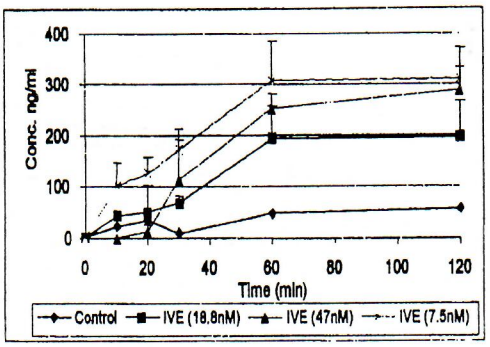

Figure 1: Effect of different concentration of ivermectin on the concentration of serosal albendazole. Ivermectin (IVE) (n=3-5). Figures in brackets indicate the concentration of the added drug. The means are ±1 s.d

All ivermectin concentrations caused a decrease in mucosal albendazole that was not significantly different from the control (Figure 2).

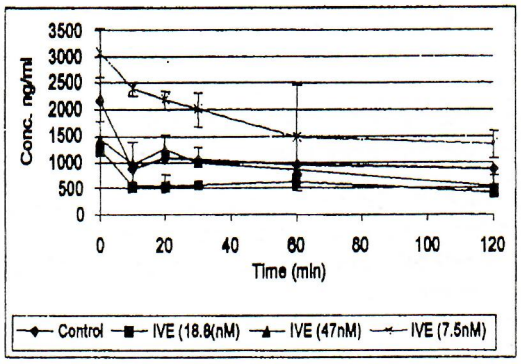

Figure 2: Effect of the different concentration of ivermectin on the concentration of mucosal albendazole as a function of time. Ivermectin (n=3-5) Figures in brackets indicate the concentration of the added drug. The means are ±1 s.d

Sulfoxidation occurred linearly in the control experiment as similarly observed by Villaverde and others [10]. In contrast to the control, the two higher concentrations of ivermectin caused an unexpected increase in both serosal and mucosal albendazole sulfoxide determined at each time point as indicated in Figures 3 and 4.

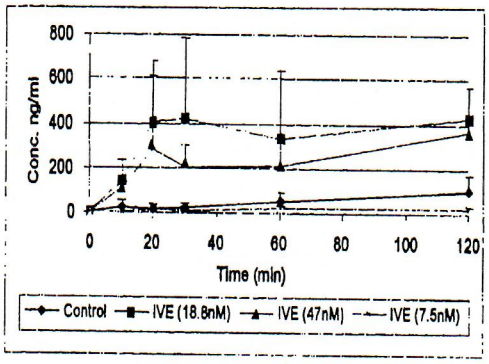

Figure 3: Effect of the different concentration of ivermectin on the concentration of mucosal albendazole sulfoxide as a function of time. Ivermectin (n=3-5)

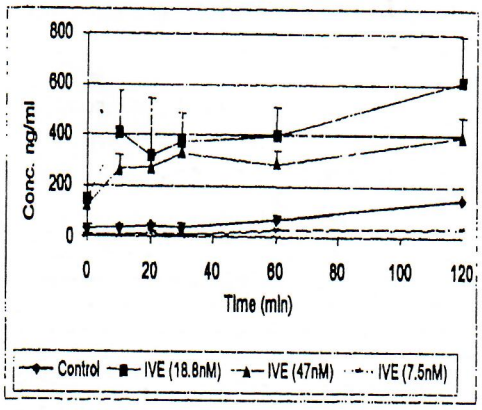

Figure 4: Effect of the different concentration of ivermectin on the concentration of mucosal albendazole sulfoxide as a function of time. Ivermectin (n=3-5)

Newly formed albendazole sulfoxide made up between 0.5-12% (control), 23-46% (ivermectin 18.8 μM) ($p<0.0001$) and 10-27% (ivermectin 47 μM) ($p<0.0001$ of the total albendazole sulfoxide detected at the end of the set time. The increase was immediate and sustained. The lower concentration of ivermectin did not produce any significant difference in total albendazole sulfoxide detected compared to the control.
Addition of praziquantel did not produce any significant change relative to the control in all the experiments.

Average dose-mass balance recovery figures ranged from 40% to 81%. The amount unaccounted for is presumed to be bound to the intestinal tissue (this was not analyzed) and may be a factor to consider when interpreting these data as it is not known whether much of what is bound is the parent albendazole or its metabolite.

DISCUSSION

Regions of the GIT lining differ in the absorptive surface area (greatest at the jejunum and ileum), permeability properties, presence and distribution of transporter proteins and biotransforming enzymes [18,19]. These variations contribute to the differences observed in absorption of drugs from diverse regions of the GIT [20,21]. In particular, P-gp present on the luminal surface and acting as a secretory transporter of CYP 3A4 involved in metabolism of drugs, are abundant in epithelial cells of the GIT [22]. These two act as absorption barriers and determinants of oral bioavailability of a number of drugs.

As a substrate of CYP enzymes and P-gp, albendazole has an extremely poor oral bioavailability. In man it is metabolized to albendazole sulfoxide in the liver by FMO and CYP enzymes in a 30:70 % ratio respectively [5]. In rats both systems seem to contribute similarly to albendazole sulfoxidation in the intestine [11].

In a study using an isolated rat intestine, Lawrenz and others [23] observed the absorption of albendazole sulfoxide but not of albendazole, which was detected only sporadically and at very low concentrations. In this study all ivermectin solutions caused an increase in serosal albendazole compared to the control ($p<0.01$). Therefore ivermectin must be having an inhibiting effect on a factor

or factors that normally restricts the passage of albendazole across the intestinal mucosa. The two higher concentration of ivermectin caused a surprising and significant increase in the total albendazole sulfoxide formed at each time point compared to the control. Since ivermectin is also a substrate of CYP3A4, one would expect competition with albendazole for the enzyme that should result in decreased formation of albendazole sulfoxide. It appears that, (i) some other process contributing to the presence of the albendazole sulfoxide was greatly increased and (ii) effects resulting from co-sharing CYP3A4 were insignificant. Probably ivermectin is not metabolized to a great extent at this level. Another contributory factor could be the presence and participation of FMO enzymes [11] that may well compensate any reduction in CYP activity. This can be confirmed by determining the enantiomeric ratio of the resultant albendazole sulfoxide since FMO is enantio-selective and produces the (+) enantiomer [24]. Indeed inhibition of CYP3A4 by erythromycin or prolonged administration of albendazole did not significantly affect intestinal sulfoxidation [10,11].

In normal circumstances albendazole is transported into enterocytes and some of the ensuing metabolite transported out at both the basolateral and apical membranes raising the serosal and mucosal albendazole sulfoxide concentrations, respectively. Transport into and out of cells of albendazole and the albendazole sulfoxide is partly by passive diffusion [26] and will therefore be dictated by the concentration gradient. As a substrate of P-gp [7], albendazole is normally extruded from enterocytes back into the lumen. This effect reduces the amount immediately available for conversion to albendazole sulfoxide or further transportation to the liver and hence diminishes the amount available systemically on the short term. On a long-term basis it means prolonged uptake into enterocytes with a possibly more complete conversion to albendazole sulfoxide and less absorption of the parent drug into the portal system i.e. P-gp maximizes drug exposure to intestinal enzymes by allowing only small quantities to be present at any one particular time but over a prolonged period, thus decreasing the importance of enzyme quantity [27].

Presence of ivermectin, which has a greater affinity for P-gp than albendazole [8], inhibits P-gp secretion of albendazole into the lumen and consequently permitting presence of increased amounts of albendazole within enterocytes over a time period. This results in a rapid rise in levels of the metabolite and the passage of more parent drug into serosal fluid. This mechanism would account for the increase in total albendazole sulfoxide and serosal albendazole seen in presence of equimolar and higher concentrations of ivermectin.

Secretion of drugs by P-gp is a saturatable and energy dependent process [28]. Enzymes are also saturatable systems. In this study the higher concentration of ivermectin produced significantly less (p-0.0001) albendazole sulfoxide than the equimolar concentration suggesting some concentration dependent effects. Given that concentration of albendazole sulfoxide was still increasing at 120 minutes, it is unlikely that the enzyme system was exhausted by the end of the two-hour study. It is probable that ivermectin at this high concentration was providing appreciable competition for CYP enzymes thus reducing conversion of albendazole.

There was no obvious difference between the mucosal and serosal concentrations of albendazole sulfoxide. Findings by Redondo and colleagues [11] indicate similar proportions of albendazole sulfoxide in the mesenteric and intestinal lumen of the rat. This agrees with the passive diffusion mode of transport suggested above. That ivermectin had an effect on the P-gp is further confirmed by the drastic reduction towards unity in the concentration gradient of albendazole.

Praziquantel had no significant effect on the intestinal disposition of albendazole though it is documented to increase serum and cyst

fluid levels of albendazole when the two are co-administered.

CONCLUSION

The effects of P-gp and metabolizing enzymes on the pharmacokinetics of albendazole are complex. P-gp by secreting albendazole back into the lumen reduces its rate of biotransformation and absorption into hepatic portal vein but permits it to act on intestinal worms and facilitates prolonged exposure to intestinal enzymes [29]. Ivermectin acts to increase the rate of albendazole sulfoxide formation as well as boost the amount of albendazole and albendazole sulfoxide reaching the portal vein. This may, the effects of the liver on albendazole disposition not withstanding, translate into higher concentrations in circulation that probably contribute to the enhanced effect seen when albendazole is co-administered with ivermectin in filariasis.

Further investigations need to be carried out exploiting the availability of cell cultures e.g. Caco-2 cells, with varying expressions of P-gp or CYP enzymes, to confirm the role of these factors in albendazole disposition.

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REFERENCES

[1] K. Awadzi, E.T. Addy, N.O. Opoku, A. Plenge-Boing and D.W. Buttner. Trop. Med. Parasitol. 46 (1995) 213-220.

[2] F. Cobo, C. Yarnoz, B. Sesma, P. Fraile, M. Aizorbe, R. Trujillo, A. Diaz-de-Liano and M.A. Ciga. Trop Med. and Int. Health, 3 (1998) 462-466.

[3] A.E. Mohamed, M.I. Yasawy and M.A. Al Karawi. Hepatogastroenterolgy, 45 (1998) 1690-1694.

[4] H. Jung, M. Sanchez, A. Gonzalez-Astiazaran, J.M. Martinez, R. Suastegui and D.F. Gonzalez-Esquivel. Am. J. Ther. 4 (1997) 23-26.

[5] H.C. Rawden, G.O. Kokwaro, S.A. Ward and G. Edwards. Br. J. Clin. Pharmacol. 49 (2000) 313-322.

[6] Z. Zeng, N.W. Andrew, B.H. Arison, D. Luffer-Atlas and R.W. Wang, Xenobiotica. 28 (1998) 313-321.

[7] B. Nare, Z. Liu, R.K. Prichard and E. Georges. Biochem, Pharmacol. 48 (1994) 2215-2222.

[8] J.F. Pouliot, F. L'Heureux, F. Liu, R.K. Prichard and E.Georges. Biochem Pharmacol. 53 (1997) 17-25.

[9] G. Fricker, H. Gutmann, A. Droulle, J. Drewe and D.S. Miller. Pharm. Res. 16 (1999) 1570-1575.

[10] C. Villaverde, A.I. Alvarez, P. Redondo, J. Voces, J.L. Del Estal And J.G. Prieto. Xenobiotica. 25 (1995) 433-441.

[11] P.A. Redondo, A.I. Alvarez, J.L. Garcia, O.M. Larrode, G. Merino and J.G. Preito. Drug Metab. Dispos. 27(1999) 736-740.

[12] D.W. Gottschall, V.J. Theodorides and R. Wang. Parasit. Today. 6 (1990) 115-124.

[13] C.M. Masimirembwa and J.A. Hasler. Biochem. Pharmacolo. 48 (1994) 1779-1783.

[14] J. Sotelo and H. Jung. Clin. Pharmacokinet. 34 (1998) 503-515.

[15] M. Homeida, W. Leahy, S. Copeland, M.M. Ali and D.W. Harron, Ann. Trop. Med. and Parasitol. 88 (1994) 551-559.

[16] T.H. Wilson and G. Wiseman. J. Physiol. 123 (1954) 116-125.

[17] P.E. Hoaksey, K. Awadzi, S.A. Ward P.A. Coventy, M.L. Orme and G. Edwards. J. of Chromatogr. Biomed. Appl. 566 (1991) 244-249.

[18] Q.Y. Zhang, D. Dunbar, A. Ostrowska, S. Zeiloft, J. Yang, L.S. Kaminsky. Drug Metab. Dispos. 27 (1999) 804-9.

[19] R. Hakkak, M.J. Ronis and T.M. Badger. Gastroeterology. 104 (1993) 1611-1618.

[20] C.F. George. Pharmacokinet. 6 (1981) 259-274.

[21] A.L. Ungell, S. Nylander, S. Bergstrand, A. Sjoberg and H. Lennernas. J. Pharma. Sci. 87 (1997) 360-366.

[22] L.Z. Benet, T. Izumi, Y. Zhang, J.A. Silverman and V. J. Wacher. J. Controlled Release. 62 (1999) 25-31.

[23] A. Lawrenz, S. Eglit and R. Kroker, DTW 99 (1992) 416-418.

[24] S. Larsen-Su and D. Williams. Drug Metab. Dispos. 24 (1996) 927-931.

[25] P. Moroni, T. Buronfose, C. Longin-Sauvageon, P, Delatour and E. Benoti. Drug Metab. Dispos. 23 (1995) 160-165.

[26] H. Jung, L. Medina, L. Garcia, I. Fuentes and R. Moreno-Esparza. J. Pharm. Pharmacol. 50 (1998) 43-48.

[27] M.F. Hebert. Adv. Drug Deliv. Rev. 27 (1997) 201-214.

[28] H. Sphahn-Langguth, G. Bakir, A. Radschuweit, A. Okyar, B. Terhang, P. Ader, A. Hanafy and P. Langguth. Int. J. Clin. Pharmacol. Ther. 36 (1998) 16-24.

[29] P.B. Watkins. Adv. Drug. Deliv. Rev. 27 (1997) 161-170.