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ZIDOVUDINE-CYCLODEXTRIN INCLUSION COMPLEX AND ITS PERMEABILITY ACROSS RAT STOMACH AND INTESTINAL COMPARTMENTS

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Abstract

The permeability of zidovudine in zidovudine-cyclodextrin inclusion complex across stomach and intestinal compartments of rat was investigated spectrophotometrically. The absorption maximum (λ_{max}) for zidovudine in HCl buffer (pH 1.2) and in phosphate buffer (pH 7.4) were 267 and 268 nm respectively. The inclusion complex was formed by freeze-drying method and the stoichiometric ratio was determined to be 1:1. Infra-red spectrophotometry was used to confirm the formation of the inclusion complex. Buffered solutions of the inclusion complex were introduced into the stomach (HCl buffer, pH 1.2) and intestine (phosphate buffer, pH 7.4) immersed in appropriate buffer solution. In the stomach, after 60 min, the transport of zidovudine increased by between 50 and 55 % above that of zidovudine alone; while in the intestine the increase was between 10 and 15 %. In both tissues the steady state condition for zidovudine alone occurred after 4 h while that for the inclusion complex occurred after 3 h. These figures suggest that B - cyclodextrin facilitated the transport of zidovudine across these tissues and so we intrapolate that B-cyclodextrin can improve the bioavailability of zidovudine

Key words: *Zidovudine; inclusion complex; cyclodextrin; bioavailability*

INTRODUCTION

Zidovudine or Azidothymidine (AZT) was originally developed for cancer treatment but was later adapted for antiretroviral therapy. Mechanistically, AZT is a member of the nucleoside reverse transcriptase inhibitor (NRTI). They are converted into their triphosphates by a system of kinase enzymes in the body before they are incorporated into their viral RNA. In HIV/ AIDS patients, the activity of this enzyme is reduced by as much as 10 % (Nortemans *et al.,* 1998). The monotherapy of zidovudine in HIV/AIDS patients proved to be a complete failure because of viral resistance, hence the resort to combination therapy. In combination therapy drugs are selected in such a fashion that their individual mechanisms of action are not at variance with each other. The unfortunate phenomenon of resistance was intensively investigated over a period of 10 years (Tambussi *et al.,* 1998) and it was discovered that viral resistance was extremely rampant. Currently, combination therapy has become the norm rather than the exception where a combination of between 2 and 5 drugs are administered. As would be expected, the larger the cocktail, the more rapidly the viral load was reduced (Weverling *et al.,* 1998).

Antiretroviral drugs (ARVs) currently used for the management of HIV/AIDS amongst adults in particular are administered in fixed doses as against concentration-controlled

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doses. It has been reported that fixed doses result in great variability in pharmacologic response (Fletcher *et al.,* 1998) as compared to concentration-controlled doses. This heterogeneity in response has been attributed to cellular and systemic differences in concentration which by themselves may arise from kinase enzyme activity (Fletcher, *et al.,* 1998). Concentration-controlled doses suffer the disadvantage of higher regimens but they are without any difference in safety and tolerance. This suggests that concentration-controlled regimens are superior to fixed doses.

Cyclodextrins generally are cyclic oligossacharides arranged in such a way that there is a cavity in the middle carrying lipophilic groups and the hydroxyl groups on the exterior. When complexes are formed, no bonds are established and none is broken and the complex is easily dissociated. Indeed, in solution, equilibrium exists between the drug and the complex. The inclusion may be complete or partial thus giving rise to different stiochiometric ratios. The formation of inclusion complex between beta-cyclodextrin and drug molecules has extensively been reported in the literature (Uekama, 1981; Kralova *et al.*,1995; Ito *et al.,* 1995, Ammar *et al.,* 1996; Ghorab *et al.,* 2004; Koester *et al.,* 2004). Cyclodextrins have the advantage of improving the chemical stability of drugs (Jadgwign, 1998), improving solubility in aqueous media (Uekama *et al.,* 1985; Ammar *et al.,* 1996) and bioavailablity (Ghorab *et al.,* 2004). Even liquid drugs can be transformed into crystalline molecules which can be formulated into tablets (Atwood *et al.,* 1984).

The authors have not cited any reference in the literature on inclusion complexes of zidovudine with cyclodextrins and possible effects on bioavailablity. The observation that pharmacologic variability is a function of systemic concentration and the fact that cyclodextrins have been shown to improve bioavailability of several other drugs suggest that beta-cyclodextrin inclusion complex can in some way diminish variability.

The design of this investigation was to establish whether an inclusion complex of zidovudine and beta-cyclodextrin can increase its transport across the gut compartments. Since the transport of drugs from site of application to the systemic circulation is the major determinant of biolavailablity, this study will help to extrapolate into the in vivo transport of zedovudine in human systems. In fact the influence of cyclodextrin-zidovudine inclusion complex on bioavailability and pharmacokinetic profiles in HIV/AIDS patients will be the logical extension of the present study.

MATERIALS AND METHODS

Materials

Beta-cyclodextrin (Sigma Chem. Co.; St Louis Missouri, USA), Retinovir^{R} (Welcome, England). The following solvents were products of BDH, England: Hydrochloric acid, sodium hydroxide pellets, phosphoric acid, chloroform, absolute ethanol. Absolute ethanol and chloroform were re-distilled before use. Spectrophotometer (Jennway, Model 6405, England). IR spectrophotometer (Perkin Elmer, USA).

Methods

Isolation of drug from the formulation: Ten capsuls of retinovir^{R} (Zidovudine) each containing 100 mg of the drug base were emptied into 250 ml beaker and 100 ml chloroform added. The mixture was stirred vigorously for 20 min. with a magnetic stirrer and allowed to stand for another 10 min. The chloroform layer was filtered and the residue further extracted with more chloroform (2 x 50 ml). The pooled solution was evaporated at 50 ^{o}C. The recovered crystals were repeatedly recrystallized with hot water and dried in the oven for 72 hr at 50^{o}C. The melting point was determined and compared with the literature value (Merck Index, 1996). The purity of the crystals was further confirmed by thin layer

chromatography.

Optimization of the spectrophotometric procedure

The analytical technique was optimized by the method previously described (Onah, 2005). Essentially, the procedure involves determining the wavelength at absorption maximum for zidovudine (λ_{max}), determining the limit of detection and quantitation; measuring absorbance at different concentrations with different plots and regression equations. The mathematical equations that satisfied the various conditions were developed.

Calibration curve and its validation

Stock solution of zidovudine containing 0.01 mg/ml was prepared in both hydrochloric acid (pH 1.2) and phosphate buffer (pH 7.4). Different concentrations (arithmetic progression) between 0.01 mg/ml and 0.10 mg/ml were prepared by dilution method and their absorbances determined. Five replicate determinations were made for each concentration from which standard deviations were calculated and regression plots generated. The plots show that Beer-Lambert law was obeyed. Further variations in concentrations were analyzed and errors in slope and intercept determined. Repeating the entire procedure and spiking with known concentrations and converting absorbances to other corresponding concentrations using the calibrated curve validated the plots.

Preparation of zidovudine- beta-cyclodextrin inclusion complex

A mixture of zidovudine and beta-cyclodextrin in equimolar ratio (1:1) was dissolved in 50 % aqueous ethanol (Odzemir and Ordu (1998). The mixture was stirred vigorously with a magnetic stirrer for 90 minutes; the ethanol portion was evaporated under reduced pressure while the aqueous portion was freeze-dried. To confirm that the inclusion complex was formed, beta-Cyclodextrin and the inclusion complex were separately subjected to FT-IR spectrophometry.

Determination of stoichiometry of complexation

The stoichiometric ratio between beta-cyclodextrin and zidovudine was carried out by phase-solubility studies as reported by Higuchi and Connors (1965). Graded concentrations of beta-cyclodextrin (1.0×10^{-3} M to 5.0×10^{-3} M) were prepared and placed in screw-cap test-tubes at pH 7.0 and excess zidovudine (50 mg was added to each tube. The test tubes were placed in a thermostated water bath (28°C) and rocked continuously for 2 hr for equilibrium to be attained. The samples were allowed to stay at room temperature for 72 hr before the solutions were filtered through 0.85 mm millipore membrane. A portion of the solution (0.1 ml) from each tube was transferred to a volumetric flask and made-up to 10 ml mark. Spectrophotometric method was used to analyze each tube for the concentration of zidovudine.

Determination of permeability of zidovudine and its inclusion complex

The animals used for this experiment were kindly provided from Animal House of the Department of Pharmacology, University of Jos, Jos, Nigeria. The animals weighed between 250 g and 300 g and were killed by a sharp blow to the head. The stomach and the intestine were cleaned out with saline water and used immediately. The pyloric end of the stomach was tied up neatly while 3.0 ml of the stock solution, pH 1.2 (1 mg/ml or 3.7411×10^{-3} M) was introduced from the upper outlet. The arrangement was examined to ensure that there were no leakages at all. The stomach and its content was submerged in 50 ml of the HCl buffer (pH 1.2) contained in a conical flask. At zero time, 1.0 ml of the buffer was withdrawn from the flask into a test tube and replaced immediately with fresh buffer. The withdrawal continued at regular intervals for 600 min. The

solutions withdrawn were made up to 10 ml mark and absorbance read at the absorption maximum of zidovudine. The above procedure was repeated for the inclusion complex. The entire experiment was again repeated using the small intestine but with phosphate buffer (pH 7.4). Each experiment was replicated three times and the standard error of the mean calculated.

RESULT AND DISCUSSION

The crystals of zidovudine isolated from the formulation was reasonably pure judging by the chromatographic (TLC) and melting point of 119 - 121 °C, which compared favorably with the literature value of 120 -122 °C (Merck Index, 1996). The construction of the calibration curve and its validation was critical to the accurate determination of transport of zidovudine across the gut compartments. Therefore, the technique was fully optimized by standard protocols (Fasamade, 1993; Onah, 2005). The absorption maximum (λ_{max}) in HCl buffer (pH 1.2) and phosphate buffer (pH 6.8) were 268 nm and 266 nm respectively (Fig.1). The limit of detection (LOD) and quantitation (LOQ) in both buffers were approximately equal i.e. 15 ng and 18 ng respectively. The mathematical model equations that satisfied linearity of both data from optimization and calibration processes are represented respectively as follows :

$A_{pH\,1.2} = 32.7x\ 0.0502$ (corr. coefficient = 0.998)
$A_{pH\,6.8} = 33.1\ \ 0.0555$ (corr. coefficient =0.999)
Where A represents absorbance in the buffers, while x represents the concentration element in mg/ml.

The relative standard deviation of the intercepts and slopes were 0.09 % and 1.1 % respectively for 5 replicate determinations. Previous experiments have shown this technique to be accurate, reproducible and sensitive.

We are convinced that the inclusion complex was formed as evidenced from FT-IR spectrophotometry (Fig. 2 to Fig. 4). The IR spectrum of zidovudine alone is represented in Fig 2 while that of beta-cyclodextrin is shown in Fig.3. The huge and broad envelope in Fig.3 is due to hydroxyl groups characteristic of beta-cyclodextrin. The spectrum of the inclusion complex (Fig.4) contrasted well with Fig 2 and Fig.3. Other absorption bands at 2953 cm^{-1} (strong) in Fig.2 and 3 remain sharp in Fig.4, while sharp absorptions at 1671 cm^{-1}, 1458 cm^{-1} in fig 2 and 3 are retained in fig.4 but with diminished intensity. Absorption below 1019 cm^{-1} in Fig 3 disappeared in Fig.4. Preliminary investigation showed that solubility of zidovudine complex with beta-cyclodextrin increased between 45 % and 50 %. The permeability of zidovudine and its inclusion complex in the rat stomach and small intestine are shown in Fig. 5 and 6. These figures show that beta-cyclodextrin facilitated the permeability of zidovudine to a greater extent than zidovudine alone. There didn't seem to be any difference between the effects of beta-cyclodextrin on the stomach and intestinal walls.

The optimization of the analytical procedure was critical particularly in spectrophotometry and this has been fully utilized in this investigation. Several techniques such as X-ray crystallography (Ito *et al.,* 1995); differential scanning calorimetry (Ammar *et al.,* 1996), vapour pressure osmometry (Ammar *et al.,* 1995) and IR spectrophotometry (Ozdemir and Ordu, 1998) have been reported and they all compare favorably with spectrophotometric method.

Transport of drugs across stomach and intestinal membranes or walls occur by direct diffusion and not by active or facilitated mechanisms. Cyclodextrins are known to interact with biological membranes making them more fluid (Van Deenen, 1969), thus leading to increased pore size for easy transport of molecules. The improved permeability of zidovudine in both stomach and intestinal walls observed in this study can thus be explained. In the stomach beta-cyclodextrin increased the transport of zidovudine by 42 % within 60 min,

while in the intestine, for the same time, the increase was 23,5 %. These figures suggest that zidovudine would naturally have a slow absorption in the stomach than in the small intestinal. It can also be suggested that in the acid pH of the stomach (pH 1.2), the drug becomes protonated at the azide group converting it into a cation. Cations generally have lower passage across biological membranes. The influence of zidovudine in this connection was to increase the membrane pores, thus enabling the increased transfer of the drug. In the alkaline pH of the intestine, the drug molecules remain essentially neutral and so the drug diffuses relatively readily (Fig.3). Again the increase in permeability may be due to the effect of cyclodextrin. The differences between the increase in transport across the stomach and intestinal walls may be due to physiologic or structural differences.

It is justifiable to conclude from these *in vitro* studies that better permeability would be expected in *in- vivo* models because equilibrium will be in favor of permeability processes since the permeated drugs would be absorbed into the circulation. It could also be suggested that bioavailabilty of zidovudine would be enhanced in *in-vivo* model. The earlier observation that systemic variations in the concentration of anti retroviral (ARV) drugs contribute to differences in pharmacological responses can be minimized if formulations of ARVs are carried out as inclusion complexes of cyclodextrins. The advantage of oral administration of inclusion complexes is that cyclodextrins are not absorbed into the circulation and are not irritating to the gut walls. It has been speculated that cyclodextrins have potential for use as sustained release vehicle.

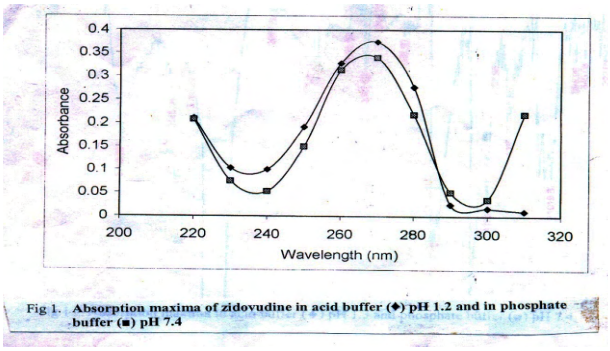

Fig 1. Absorption maxima of zidovudine in acid buffer (♦) pH 1.2 and in phosphate buffer (■) pH 7.4

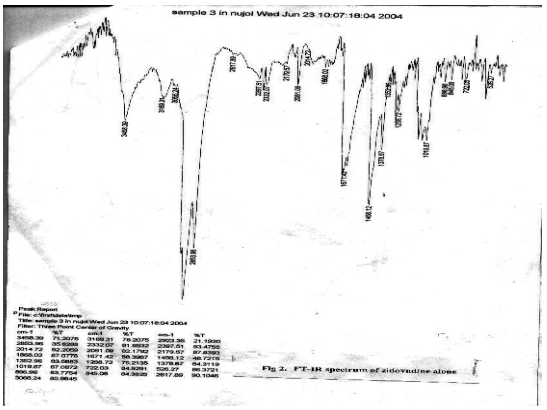

Fig 2. FT-IR spectrum of zidovudine alone

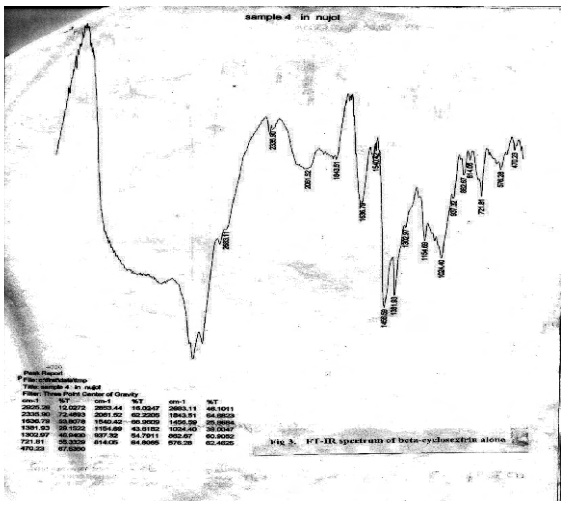

Fig 3. FT-IR spectrum of beta-cyclodextrin alone

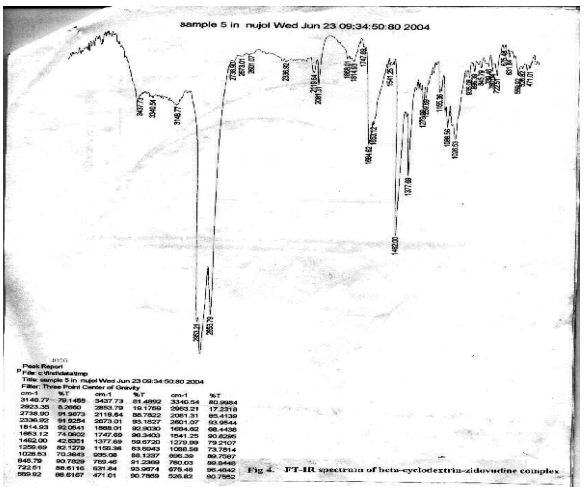

Fig 4. FT-IR spectrum of beta-cyclodextrin-zidovudine complex

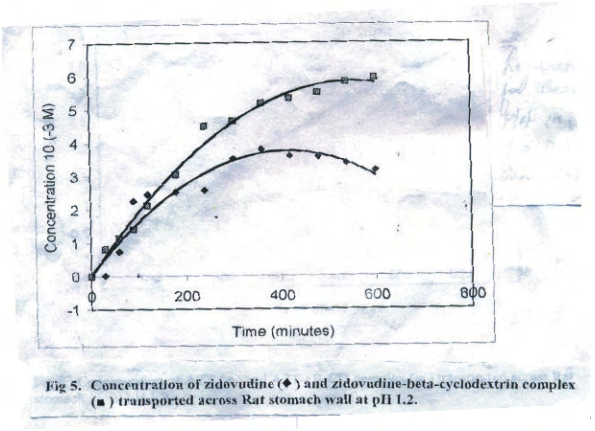

Fig 5. Concentration of zidovudine (♦) and zidovudine-beta-cyclodextrin complex (■) transported across Rat stomach wall at pH 1.2.

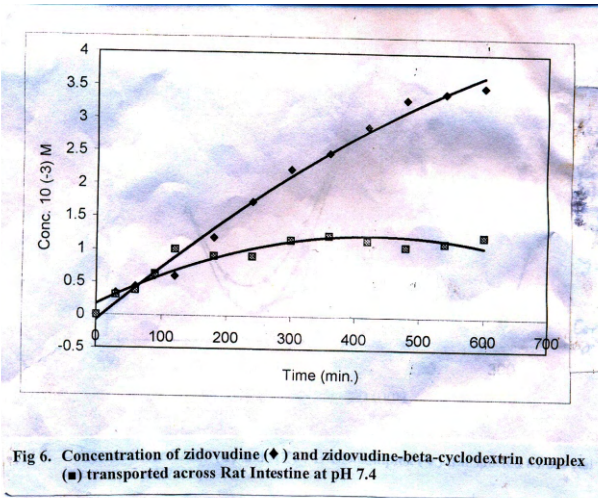

Fig 6. Concentration of zidovudine (♦) and zidovudine-beta-cyclodextrin complex (■) transported across Rat Intestine at pH 7.4

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References

Ammar, H.O.; Ghorab, M.; El Hahhas, S.A.; Omar, S.M. and Ghorab M.M. (1995): Improvement of some pharmaceutical properties of drugs by cyclodextrin complexation. Part 4: Chlorpromazine Hydrochloride. *Pharmazie* 50, 805-808

Ammar, H.O.; El Nahhas, S.A. and Ghorab, M.M. (1996): Improvement of some pharmaceutical properties of drugs by cyclodextrin complexation. Part 6: Ampicillin. *Pharmazie* 51, 568-570

Attwood, J.L.; Davies, J.E.D. and MacNicol, A. (1984): Industrial Applications of Cyclodextrins. In "Inclusion compounds" vol.3. Academic Press, p349-360.

Bruistein, S.M.; Reiss, P.; Lorelinger, A.E.; van Sureten, P.; Sehuurman, R. Boucher, C.A.; Weverling, G.J.; Huisman, J.G. (1998): Cellular proviral HIV type I DNA load persist after long term RT-inhibitor therapy in HIV type-I infected persons. AIDS-*Res. Hum. Retroviruses* 10 , 1053-58.

Fasamade, A.A. (1993): Trends in Pharmaceutical Analysis and Validation of Analytical Methods. In " Towards Better Quality Assurance of Drugs and Foods. Proceedings of a national workshop, 23 -27th March (Olaniyi, A.A Ed), Omitade Press, Ibadan, p 73-95.

Fletcher, C.V.; Acosta, E.P.; Henry, K.; Page, L.M.; Gross, C.R.; Kawle, S.P.; Remmel, R.P.; Erice, A. and Balfour, H.H. (1998): Concentration-controlled Zidovudine therapy. *Clin. Pharmacol . Ther.* 64(3), 331-338.

Ghorab, M.; Abdul-Salam, H.M.; El-Sayed, M.A. and Mekhel, M.M. (2004): Tablet formulation containing Meloxicam and beta-cyclodextrin: Mechanical characterization and bioavailability evalustion. *AAPS PharmSci Tech.* 5(4), e59.

Jadwign, M. (1998): Analytical studies of photodegradation of inclusion complexes of Nimodipine with beta- , gamma-cyclodextrin and methyl-beta-cyclodextrin and hydroxypropyl-beta-cyclodextrin. *Drug Dev. and Ind. Pharm.* 24(2),197-200.

Kralova, K.; Sersen, F. and Devinsky, F. (1995): Interactions of N-(1-methyldeoxy)dimethylamine-N-oxide with beta-cyclodextrin and their effects on the biological activity of the surfactant. *Ceska. Slov. Farm* 44(5), 238-241.

Merck Index 12th Ed. (1996)

Notermans, D.W.; Goudsmit, J.; Danner, S.A; De Wolf, F.; Perelson, A.S. and Mitter, J. (1998): Rate of HIV-I decline following antiretroviral therapy is related to viral load at baseline and regimen. *AIDS*, 12 (12), 1483-90.

Onah, J.O. (2005): Spectrophotometric determination of amitryptilline by the method of charge-transfer complexation with chloranilic acid. Global *J. Pure Appl. Sci.* 11(2), 237-240.

Odzemir, N. and Ordu, S. (1998): Improvement of dissolution properties of furosemide by complextion with beta-cyclodextrin. *Drug Dev. Ind. Pharm.* 24(1), 19-25.

Tambussi, G.; Boeri, E.J.; Carreva, P.; Gianotti, N. and Lazzarin, A. (1998): Prevention of mutation associated to resistance with nucleoside analogs in a cohot of naïve HIV-1 positive subjects during the period 1984 to 1997. *J. boil Regul. Homeost. Agents.* 12 (1-2 suppl.), 32-34.

Uekama, K. (1981): Pharmaceutical applications of cyclodextrin complexations. *J. Pharm. Soc. Japan* 101,857-873.

Uekama, K.; Otagiri, M.; Irie, T.; Seo, H.; and Tsuruoka, M. (1985): Improvement of dissolution and absorption characteristics of phenytoin by water soluble beta-cyclodextrin-epichlorohydrin polymer. *Intl. J. Pharm.* 23, 35-42.

Van Deneen, L.L.M. (1969): In "Regulatory Functions of Biological membranes" Elsevier, Amsterdam. P72-86.

Weverling, G.T.; Lange, J.M.; Jurriaans, S.; Prins, J.M.; Lukashov, V.V.; Nortemans, D.W. (1998): Alternative multidrug regimen provides improved suppression of HIV-I replication over triple therapy. *AIDS* (1), F117-22.