HACETTEPE JOURNAL OF BIOLOGY AND CHEMISTRY
Volume 35, Issue 1, January 2007

Research Article

Hacettepe J. Biol. & Chem., 2007, 35 (1), 25-30

Investigation of Renal Histopathological Changes Due to HIV-RT Inhibitor 2-phenoxymethyl-5-chlorobenzimidazole Administration in Rats

Ayşegül Akbay Yarpuzlu[1], Yeşim Uğur[2], Ayşegül Nazikoğlu[2], Esin Asan[2], İlkay Yildiz[3]

[1]Ankara University, Faculty of Health Education and Institute of Biotechnology, Department of Health Education, Ankara, TURKEY

[2]Hacettepe University, Faculty of Medicine, Department of Histology and Embryology, Ankara, TURKEY

[3]Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Ankara, TURKEY

Abstract

2-phenoxymethyl-5-chlorobenzimidazole is a newly synthesized Human Immunodeficiency Virus- Reverse Transcriptase (HIV-RT) inhibitor. In a previous study it was found that this chemical entity was partially secreted and possibly metabolized in the liver of rats depending on histological data. This study was aimed to investigate the histological changes in the kidneys of rats receiving 2-phenoxymethyl-5-chlorobenzimidazole intramuscularly dissolved in dimethylsulfoxide (DMSO) injected as a single and consecutive 7 doses (5mg/kg) within 14 days with respect to the control group without any intervention. Additionally control experiments to observe the possible effects of the solvent DMSO were also designed. The results indicated that; there is minor toxicity in the renal tubular epithelia disrupting the reabsorbtion and secretion functions of the tubules as both DMSO and 2-phenoxymethyl-5-chlorobenzimidazole are hydrophobic and have capacity to distract membranous fractions of the cells.

Key Words: AIDS, HIV-RT inhibitor, 2-phenoxymethyl-5-chlorobenzimidazole, kidney, rat, renal tubules, dimethylsulfoxide

Introduction

2-Phenoxymethyl-5-chlorobenzimidazole is a substituted benzimidazole that has been synthesized by our team. Benzimidazoles and their substituted derivatives have been used as antimicrobials in the treatment of *Pneumocystis pneumonia* (1), *Helicobacter pylori* (2), in plant protection (3), as antivirals against cytomegalovirus (4), as well as antiparasitics against *Giardia lamblia* (5) and some Microsporidia species such as *Encephalitozoon hellem* and *Encephalitozoon intestinalis* (6) by binding to the cytoskeleton (7). Some have also been shown to be inhibitors of HIV-RT (8), and Adenosine Deaminase (ADA) (9).

Even though there are some studies with other substituted benzimidazoles; previously; neither LD50 studies, nor pharmacokinetics or pharmacodynamics studies have been done with 2-phenoxymethyl-5-chlorobenzimidazole and from a chemical perspective; the purity in solution with respect to any possible isomeric forms is also not investigated.

This study was intended to observe some fast and accumulative effects of 2-phenoxymethyl-5-chlorobenzimidazole administration that may lead to renal histological changes to be observed at light microscopic level.

Materials and Methods

Chemicals

The method of synthesis of 2-phenoxymethyl-5-chlorobenzimidazole compound which has been shown to have antimicrobial activity is as follows; 0.01 moles (1.42 grams) of 4-chloro-o-phenylene diamine was mixed with 0.01 moles (1.52 grams) of phenoxyacetic acid. The mixture is heated at 100°C for 5 hours in 15 ml 6N HCl under continious cooling. Water is added to the reaction mixture until cooling. The mixture is alkalinized with sodium bicarbonate and the precipitate is washed after filtration followed by a clean-up through product colon chromatography. The product is recrystallized from ethanol-water mixture.

Animals and Experimental Design

Wistar Swiss albino male rats (*Rattus rattus*) weighing 300-350 grams were obtained and housed in Experimental Animals Facility of Ankara University, Faculty of Medicine. The rats were housed in standart stainless steel cages. All animals were kept under the same environmental conditions with a room temperature of 20±2°C, 12-hour light-dark cycle, diet and water were allowed ad libitum. A total of 10 male rats were divided randomly into four groups. The first group consisted of 3 rats receiving intramuscular injections of 5 mg/kg 2-phenoxymethyl-5-chlorobenzimidazole dissolved in DMSO in a total volume of 0.5 ml, only once on day 1. The second group consisting of 4 rats receiving intramuscular injections of 5 mg/kg 2-phenoxymethyl-5-chlorobenzimidazole dissolved in DMSO, in seven consecutive injections on days 1, 3, 5, 7, 9, 11 and 13. The third group consisted of 2 rats receiving 0.5 ml DMSO alone, one of them receiving a single injection on day 1 and the other receiving seven consecutive injections on days 1, 3, 5, 7, 9, 11 and 13. Finally; the fourth experimental group had a single rat without any intervention. All animals were fed with standart rat chow. At the end of the treatment period, the animals were anaesthesized with ethanol and killed by decapitation Followed by partial excision of kidneys. The study started with 4 rats in each group but due to losses and deaths, the final numbers were as depicted.

Tissue Samples

Tissue samples were fixed in 2% glutaraldehyde-pfosphate buffered saline (PBS) solution overnight. After PBS wash, post-fixation was applied in 1% osmium tetroxide-buffer solution for 1.5 hour. Again after PBS wash samples were dehydrated. After being processed in propylene oxide for half an hour, they were left in araldite (ARALDITE CY212 Kit)- propylene oxide mixtures at ratios 1:3, 1:1, 3:1 for 0.5, 1 hours and overnight respectively. These samples were processed in pure araldite for an hour and embedded in beem capsules. They were left to polymerization in an incubator at 60°C for 48 hours. Semi-thin sections were cut and stained with methylene blue. Sections were examined under light microscope (Olympus BH-2) by three histologists working seperately and photographs were taken representing each group. Tables summarizing the findings of each investigator were constructed and uncoinciding results were evaluated together again until concensus was reached.

RESULTS

Fourth Group-Normal rat

Some renal tubular epithelial cells display vacuolar appearance within them. They also display a granular appearance. In some tubular cells only a few tiny vacuoles are seen whereas rarely other cells are filled up with these tiny vacuoles. Epithelial cells of collecting tubules are filled up with vacuoles. The apical surfaces of some of these cells are degenerated and seemed brush-like. In a very small number of proximal tubular epithelial cells there are large vacuoles bulging to lumen. Circulatory stasis is observed in both interstitium and glomeruli. Otherwise glomeruli are normal. The vacuoles are basicaly observed at apical surfaces.

Third Group-48th hours solvent group

Most of proximal tubular epithelial cells display medium-sized vacuoles and degeneration. Distal tubular epithelial cells also display medium-sized vacuolization and are degenerated especially at their apical surfaces. Epithelial cells of collecting tubules are enlarged, in medulla they display huge vacuoles. Glomeruli are normal.

Third Group-15th day solvent group

Hyalene material-like appearance is observed in some regions of proximal tubular epithelial cells. Proximal tubular epithelial cells are filled with tiny vacuoles. Distal tubular epithelial cells display large vacuoles. Epithelial cells of collecting tubules are filled up with huge vacuoles. Collecting tubules are degenerated. Glomeruli are normal.

First Group-48th hours chemical+solvent group

In two of the rats, proximal tubular epithelial vacuolization is not very prominent. Very rarely some of them display hyalene material-like appearance in them. They also display prominent granular appearance. Apical faces of distal tubular epithelial cells are degenerated and seem brush-like. In one of them, distal tubules are not much degenerated, their epithelial cells rarely display large vacuoles. Epithelial cells of collecting tubules seem better than distal tubular ones in one of these two rats. In the other rat, proximal tubules are strikingly degenerated. Proximal tubular epithelial cells display huge vacuoles. The granular appearance within the proximal tubular epithelial cells is converged to display a patchy hyalene material-like appearance. Distal tubular epithelial cells are also strikingly degenerated. Epithelial cells of collecting tubules too are strikingly degenerated and vacuolized. Glomeruli are normal. Circulatory stasis is observed. General structure of the organ is highly distorted.

Second Group-15th day chemical+solvent group (Figure 1a, 1b,1c)

In one of the rats, apical regions of proximal tubular epithelial cells display a few tiny vacuoles. They also display granular appearance. Rarely, hyalene material-like appearance is also seen. Apical surfaces of some distal tubular epithelial cells are strikingly degenerated but this is not disseminatedly seen, vacuoles are not detected. Apical surfaces of collecting tubule epithelial cells are also prominently degenerated but vacuoles are not seen again. Circulatory stasis is observed. In the worst case, proximal tubular epithelial cels are filled up with medium-sized vacuoles. They are degenerated. All collecting tubule epithelial cells are vacuolated. The structure of the organ is wholly distorted. There is circulatory stasis. In another rat, proximal tubular epithelial cells seem better, they have rare tiny vacuoles but they have a flu appearance as if all of them mixed up together. Distal tubular epithelial cells are degenerated and vacuolated. Collecting tubule epithelial cells are highly degenerated and vacuolated. Their vacuoles are huge. Circulatory stasis is seen. In the last rat, in proximal tubular epithelial cells, there are both tiny and large vacuoles but they are not much in number. The pathology seen in proximal tubular epithelial cells is not disseminated. Apical surfaces of distal tubular epithelial cells are degenerated and they are vacuolated.

Collecting tubules are wholly degenerated and vacuolated. There is again circulatory stasis. Glomeruli are normal in each case.

Other figures can be supplied by the authors on demand.

Discussion

The kidneys are a paired organ system located in the retroperitoneal space. Nephrons are the functional renal units. A nephron consists structurally of (1) a glomerulus, a spherical epithelial space invaginated by a capillary tuft that connects the afferent and efferent arterioles and (2) a

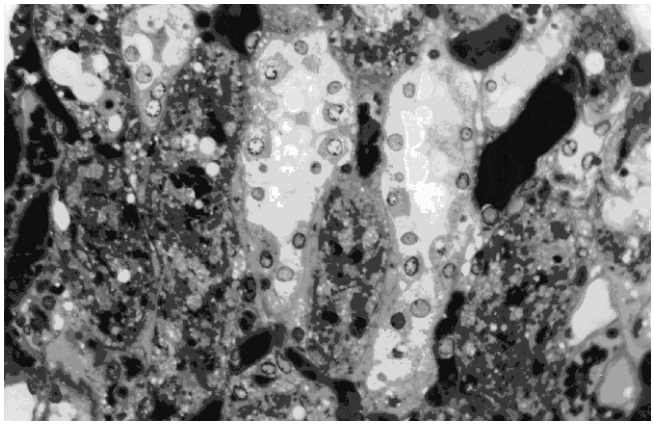

Figure 1a. Kidney section from rats receiving intramuscular injections of 5 mg/kg 2-phenoxymethyl-5-chlorobenzimidazole dissolved in dimethylsulfoxide, in seven consecutive injections, 40x magnification.

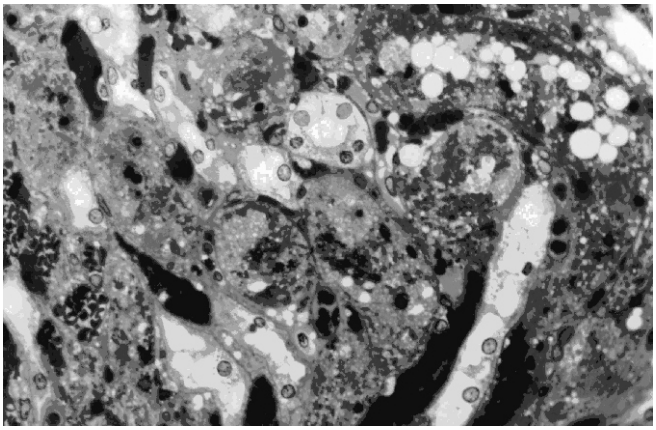

Figure 1b. Kidney section from rats receiving intramuscular injections of 5 mg/kg 2-phenoxymethyl-5-chlorobenzimidazole dissolved in dimethylsulfoxide, in seven consecutive injections, 40x magnification.

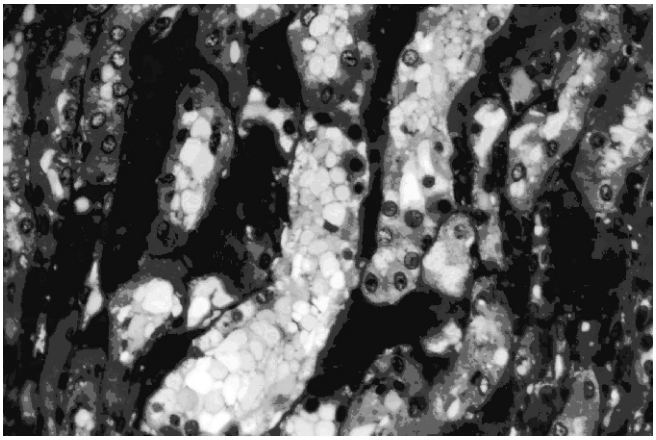

Figure 1c. Kidney section from rats receiving intramuscular injections of 5 mg/kg 2-phenoxymethyl-5-chlorobenzimidazole dissolved in dimethylsulfoxide, in seven consecutive injections, 40x magnification.

tubule of epithelial cells, continious with the glomerular epithelial space and ultimately leading to collecting ducts that empty into the renal pelvis. The functions of the kidneys may be characterized as excretory, regulatory and endocrine. The excretory function serves to rid the body of most of the undesirable end products of metabolism as well as any excess of inorganic substances ingested in the diet. Thus the other important role is to maintain constant optimal chemical composition of the blood and thereby to maintain constant optimal chemical composition of the interstitial and intracellular fluids throughout the body. Homeostasis, the maintenance of the internal milieu, largely depends on he efficient operation of the reabsorptive and secretory mechanisms of the kidneys. The kidneys must therefore be viewed not only as excretory organs but also regulatory organs.

2-phenoxymethyl-5-chlorobenzimidazole is known to be a hydrophobic substance and thus in this experiment is injected to the animals in DMSO dissolved form. DMSO, however is a lipophilic substance and has capacity the interact chemico-biologically with the lipid fractions in the biological membranes. The primary functions of the renal tubules is reabsorbtion of the material filtrated at the glomeruli. The distal tubules and the collecting ducts have also been assigned some secretory capacity. The results of this study indicated that the number of vacuoles have gradually increased in the tubuli in the solvent and drug groups due to possible excretion of 2-phenoxymethyl-5-chlorobenzimidazole by the kidneys. However with the present data it is not possible to predict if the primary mechanism of excretion is secretion of glomerular filtration and if glomerular filtration is the case if there is any reabsorbtion of 2-phenoxymethyl-5-chlorobenzimidazole or its possible metabolites from the tubules. To gain more insight an electron microscopical investigation is planned as a later stage.

The luminal surfaces of the tubuli have also been observed to be distorted due possibly to either lipid

Solubilizing and membrane disrupting capacity of either DMSO or mostly 2-phenoxymethyl-5-chlorobenzimidazole as seen in the long-term experimental group.

The increased intraepithelial granular appearance may indicate a renal intraepithelial metabolism for 2-phenoxymethyl-5-chlorobenzimidazole and a final hyalene membrane accumulation may appear due to toxic metabolite accumulation within the renal tubular cells and disruption of the overall cell biology.

The metabolic by-products of 2-phenoxymethyl-5-chlorobenzimidazole in the body after absorbtion by the vascular system on an intramuscular injection is obscure. However in a previous study (unpublished data) where the histological changes in the liver at light microscopical level was observed, a minor infiltration of the hepatic parenchyma by inflammatory cells and vacuolar appearance in the secretory epithelia around the bile cannaliculi of the liver supports the idea that there is some hepatic excretion and minor hepatotoxicity as well as possible detoxification due to the appearance of hyaline-like material accumulation within the hepatocytes.

With these results, we may suggest that there is minor toxicity to kidneys by parenteral 2-phenoxymethyl-5-chlorobenzimidazole administration and studies to increase the hydrophilicity (water-solubility), to detect toxic metabolites and their pharmacology as well as pharmacodynamics and pharmacokinetics studies should be planned.

The authors are currently investigating the histopathological changes in the cardiac and brain tissues of the same experimental design and planning for funding and collaboration for immune-electron microscopy studies.

References

1. Fishman JA. Treatment of Infection Due to Pneumocystis carini. Antimicrobial Agents and Chemotherapy. 1998; 42;6:1309-1314.
2. Hof H. Critical Annotations to the Use of Azole Antifungals for Plant Protection. Antimicrobial Agents Chemother. 2001; 45;11:2987-2990.
3. Chiba N, von Zanten SJ, Sinclair P, Ferguson RA, Escobedo S, Grace E. Treating Helicobacter pylori infection in primary care patients with uninvestigated dyspepsia: the Canadian adult dyspepsia empiric treatment-Helicobacter pylori positive (CADET-Hp) randomised controlled trial. BMJ 2002,324;7344:1012.
4. Buerger I, Reefschlaeger J, Bender W, Eckenberg P, Popp A, Weber O, Graeger S, Klenk HD, Ruebsamen,Waigmann H, Hallenberger S. A Novel Nonnucleoside Inhibitor Specifically Targets Cytomegalovirus DNA Maturation via the UL89 and UL56 Gene Products. J. Virol.2001,75;19:9077-9086.
5. Adam RD. Biology of Giardia lamblia. Clin Microbiol Rev. 2001,14;3:447-475.
6. Ridoux O, Drancourt M. In vitro Susceptibilies of the Microsporidia Encephalitozoon cuniculi, Encephalitozoon hellem and Encephalitozoon intestinalis to Albendozole and its sulfoxide and sulfone Metabolites. Antimicrob Agents Chemother. 1998,42 ;12: 3301-3303.
7. Morrissette NS. Sibley LD. Cytoskeleton to Amplicomplexon Parasites. Microbiol Mol Biol Rev. 2002,66;1:21-38.
8. Akbay A, Ören İ, Temiz-Arpacı Ö, Akı-Şener E, Yalçın İ. Synthesis and HIV-1 Reverse transcriptase Inhibitor Activity of Some 2,5,6-Substituted Benzoxazole, Benzimidazole, Benzthiazole and Oxazolo (4,5-b) pyridine Derivatives. ArzneimForschDrugRes. 2003,53;4:266-71.
9. Yarpuzlu Akbay A, Yıldız İ, Temiz-Arpacı Ö, Karagenç N, Aki-Şener E, Yalçın İ. Adenosine Deaminase Inhibitor Activity of Some Benzoxazole, Benzimidazole and Oxazolo (4,5-b) pyridine Derivatives. XIII. Biyoteknoloji Kongresi Bildiriler Kitabı, 25-29 Ağustos 2003 Çanakkale sf.21-26.

10. Arterburn LM, Zurlo J, Yager JD, Overton RM, Heifetz AH. A morphological study of differentiated hepatocytes in vitro. Hepatology. 1995, 22 ;1:175-187,1995.
11. Associated Intestinal Tract Organs, Part 25 Liver, pages:575-591 in Histology for Pathologists, edited by Sternberg SS. Second edition, Lippincott-Raven Publishers 1997, Philadelphia.