LABELING OF CHLORODIAZEPOXIDE WITH I-131 AND BIODISTRIBUTION STUDIES ON RATS

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ABSTRACT

Chlordiazepoxide (CDZ) was labeled with I-131 and investigated its radiopharmaceutical potential as a benzodiazepine receptor agent. Iodogen was used as iodination agent. Labeling yields were determined by radio-ITLC (Instant Thin Layer Chromatography) and Paper Electrophoresis. Labeling yield was approximately 90%. Purification were performed by Sep-Pak C-18 plus. Biodistribution studies were carried out by Albino Wistar rats. I-131 labeled Chlordiazepoxide which is the 62.2 GBq/mmol specific activity was administered by tail vein into the rats. Rats were sacrificed by ether narcotization at certain time intervals and the organs were removed. The brains were dissected into their components. Their activities were counted by a gamma counter. The activity per gram tissue was calculated and time activity curves were generated.

INTRODUCTION

Benzodiazepine derivatives have been largely used in Medicine since 1960. It has been found to have anti-anxiety, sedative, muscle relaxant and anti convulsant activities. Benzodiazepines are the most commonly prescribed tranquilizers and almost 30% of all the hospitalized patients throughout the world receive a prescribed for a BD each year[1,2,11,12]. There are a set of benzodiazepine derivative drugs have already been used. Chlordiazepoxide (7-Chloro-2-(methyl amino)-5-phenyl-3H-1.4 benzodiazepine 4-oxide) has been mostly used and the oldest one after diazepam. Chlordiazepoxide has been synthesized by Steinback and coworkers in Roche Laboratory in 1955[2]. Food and Drug Administration has approved it with Librium name[3].

1,4 Benzodiazepine derivatives and their radiolabeled derivatives have been prepared for the in vivo imaging and quantitation of benzodiazepine GABA receptor sites in the human brain[10]. Increasing in the use of SPECT and PET in clinical diagnosis depend on developing and presentation of new radiopharmaceuticals to clinicians. Radiolabeling of agonist of antagonist compounds related the brain receptors and using of them in SPECT and PET studies may provide more knowledge about brain chemistry. Iomazenil and flumazenil are the most common benzodiazepine antagonists and diazepam and flunitrazepam are the agonist compounds[4,5,16,17]. Iomazenil has been labeled with ^{123}I [5]. On the other hand, Flunitrazepam has been labeled with C-11[6]. Saji and coworkers have synthesized ^{125}I labeled benzodiazepine and they obtained 2'-^{125}I-diazepam[7]. Diazepam and chlordiazepoxide have also been labeled with ^{131}I by iodogen method [8,9,13].

The purpose of this work is to determine of chlordiazepoxide that is a benzodiazepine receptor agent might be an efficiently brain radiopharmaceutical.

MATERIALS AND METHODS

Chlordiazepoxide was obtained from Center for Drug Research Development and Pharmacokinetic Applications of Ege University. $Na^{131}I$ was taken from Department of Nuclear Medicine. Iodogen was purchased from Sigma Co. All other chemicals were purchased from Merck and they were reactive grade.

ITLC (Instant Thin Layer Chromatography) was done with a Sigma ITLC chamber supply using cellulose coated plastic sheets (Merck 5565). Merck 20x20 cm cellulose coated plastic sheets with a thickness of 0.1 mm were cut into smaller 10x1.5 cm sheets and were used as ITLC support material. Two different solvent systems were used: Mixture I:[N-Butanol-Water-Acetic Acid (4/2/1) (v/v)]. Mixture II: :[Isopropyl Alcohol-n-Butanol-0.2 N Ammonium hydroxide (2/1/1)].(v/v). ITLC sheets were coated with cello-bands after development and were cut into 0.5 cm sections. They were counted by a Cd(Te) detector equipped with RAD 501 single-channel analyzer. ITLC chromatograms were obtained from these counts. In the end, R_f values and labeling yields were found.

Electrophoresis Conditions: Electrophoresis was performed with a Gelman Electrophoresis Chamber supply using cellulose acetate strips. Cathode and anode poles and application points were indicated on cellulose acetate strips and these strips were moistened by buffer solution [n-Butanol-Water-Acetic Acid (4/2/1)]. They were placed in electrophoresis chamber after the samples set on the strips. Standing time and applied voltage for two hours and 250 volts. Developed strips were dried and cut into one cm pieces. They were counted by a Cd(Te) detector equipped with RAD 501 single-channel analyzer. Electrophoresis diagrams were obtained from these counts.

Preparing Iodogen Coated Tubes: Milligram amount of iodogen was dissolved in CH_2Cl_2 and transferred to closed tubes. CH_2Cl_2 was evaporated by air flow and iodogen was deposited on the walls of glass tubes as a thin film. These tubes were stored at 0°C until use.

Labeling Procedure: One mg chlordiazepoxide was dissolved in 50 µL 50 % HCl solution (v/v) and volume was adjusted to one ml by distilled water. Chlordiazepoxide solution was added to iodogen coated reaction tube, then approximately 37-74 MBq (1-2 mCi) carrier-free $Na^{131}I$ was added. The mixture was separated from the tube at the end of the reaction after 15 minutes and 0.2 N Na_2SO_3 solution (100 µL) was added to reduce non-incorporated iodine. Labeled product was purified by Sep-Pak ® Plus C18 (Waters). Labeling efficiencies were determined by ITLC and paper electrophoresis. Labeling efficiency was higher than 90%. Specific activity was approximately 62.2 GBq/mmol.

Biodistribution Studies on Rats: ^{131}I labeled chlordiazepoxide has been neutralized and sterilized by passing through a 0.22 µm membrane filter after serum physiological was added to

make up it isotonic. Then it has been injected from tail vein of male Albino Wistar Rats which were 24 weeks age and 150-200 g weight. 3-5 rats were used for each point of the experiments. The rats were sacrificed after narcotization in an ether atmosphere after certain times and their organs were removed. The brains were excised quickly, washed into cold saline solution and dissected into their components. Their activities were counted by a Cd(Te) detector equipped with RAD 501 single-channel analyzer.

RESULTS AND DISCUSSION

Figure-1 shows the biodistribution in percentage of injected radioactivity per gram of tissue for some selected organs by time. The highest accumulation occurs in brain, muscle, thyroid, stomach, kidneys, pancreas, lungs, spleen within 90 minutes. This result agree with the reports explaining the CDZ is a muscle relaxant drug [12]. Radioactivity was cleared from all organs except brain and stomach in 3 hours. Considerable amount of radioactivity was seen in thyroid. This result agrees with other reports related with some other BZ receptor ligands[4].

The blood, the brain radioactivities and the brain/blood ratio are plotted in figure-4. The highest value in the blood is reached within 20 minutes after injection. The decrease of radioactivity in the blood is faster than brain and comparable to other organs. After 45 minutes brain activity is maximum while nearly all activity is eliminated from the blood. The elimination from brain is considerably slower and the brain has the highest activity after stomach 180 minutes after injection. Brain to blood ratio is the highest in 1 hour and reaches 25.6. These results agree with Schubiger and Hasler who reported Pharmacological and animal data of Iomazenil which is a benzodiazepine partial inverse agonist[4]. Percentage of injected radioactivity per gram tissue was 1.00 in hippocampus in five minutes then it decreased while radioactivity increasing in medulla pons, mid-brain and hypothalamus. It is known that benzodiazepine derivatives affect to GABA receptors in the CNS and GABA has a widespread distribution with high concentrations in the hypothalamus, hypocampus and basal ganglia of the brain[14]. Benzodiazepines are GABA uptake inhibitors although the significance of this action with respect to their clinical effectiveness is not known. Therefore radiolabeled benzodiazepine derivatives like CDZ may be a useful tool for PET and SPECT studies related to GABA receptor system investigations. The usefulness of the BZ's (diazepam, chlorodiazepoksit, nitrazepam) may be related to their GABA-enhancing actions in the CNS [14].

These in vivo results in rat suggest that I-131-labeled CDZ and I-123 derivatives shows promise as a selective high affinity of the brain BZ receptor.

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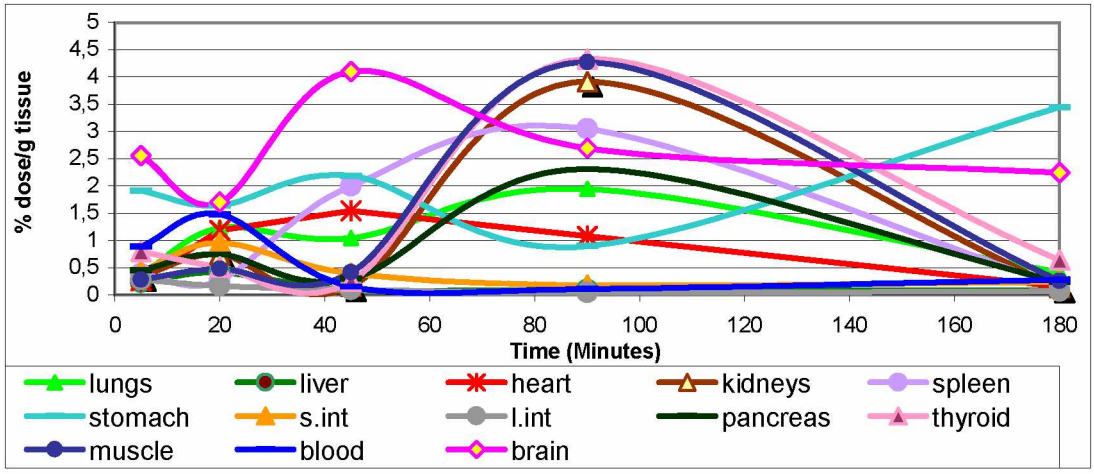

Figure-1 Biodistribution of ^{131}I CDZ in rat.

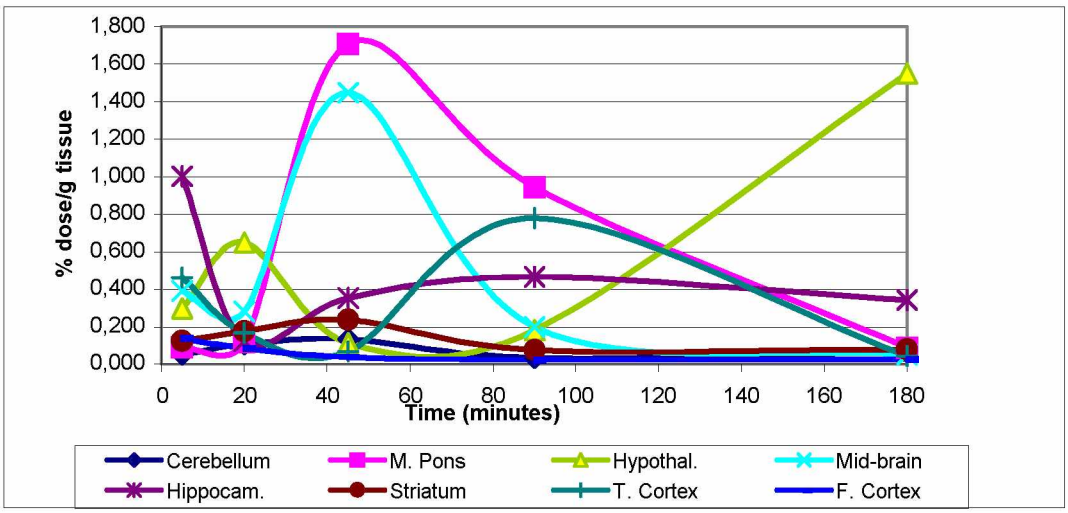

Figure-2 Biodistribution of ^{131}I CDZ in rat brain.

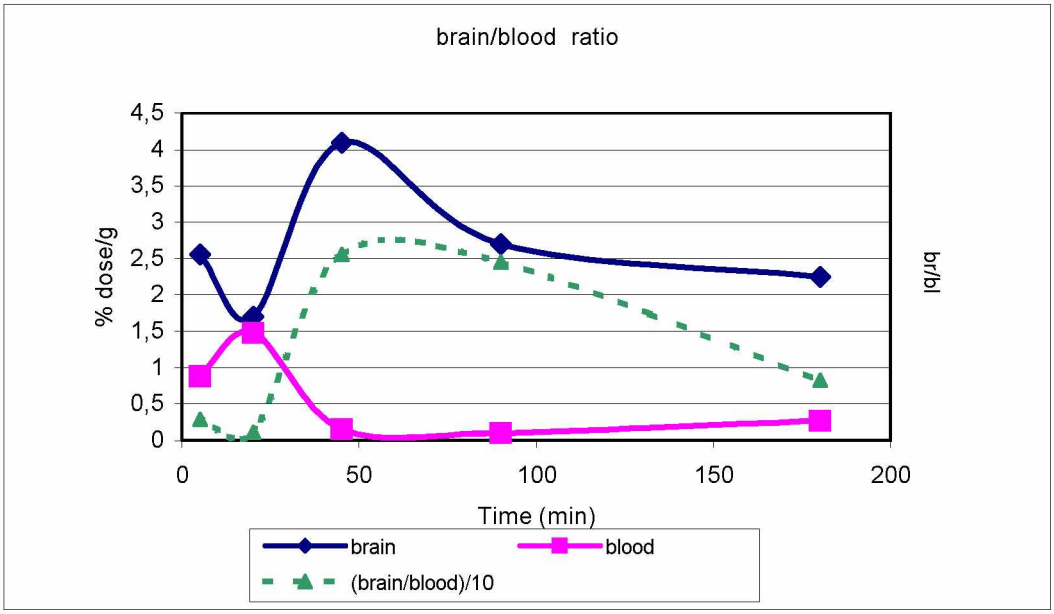

Figure 3 Percent of injected dose per gram tissue for the brain, the blood and brain/blood ratio.