RADIOPHARMACEUTICAL POTENTIAL OF I-131 LABELLED DIAZEPAM

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Abstract

In this study, diazepam is a derivative of the 1.4 benzodiazepine family that the most widely used drug as anticonvulsant agent has been labeled with I-131, as a new radiopharmaceutical and its radiopharmaceutical potential has been determined.

Labeling of diazepam has been performed by iodogen method and optimum labeling conditions have been determined.

Optimum reaction conditions are 1 mg for iodogen amount; 1-5 mg for diazepam amount; 15-20 minutes for reaction time and room temperature for reaction temperature. Specific activity of labeled compound was 0.15 Ci/mmol level.

N-octanol/water ratio was found 1.9 for ^{131}IDZ (^{131}I labeled diazepam). In vivo experiments have been carried out to determine radiopharmaceutical potentials of labeled compound. Biodistribution studies on rats showed that ^{131}IDZ have accumulated in kidneys, liver, lungs and brain tissues.

Scintigraphic results taken with gamma camera on rabbits agree with biodistribution results of rats.

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INTRODUCTION

Since usage of radioisotopically labeled radiopharmaceuticals increase day by day, importance of the other disciplines that is related with this area have increased. Certainly developing of the Nuclear Medicine depends on the Radiopharmacy and Radiochemistry that most are the most related disciplines with this scientific area.

Since their introduction to human use as antianxiolytic and anticonvulsan in the 1950's, benzodiazepines have been the most widely prescribed drugs. They have antianxiolytic and anticonvulsant effects. Diazepam is the most used of them [1]. Fig.1 shows molecular structure of diazepam.

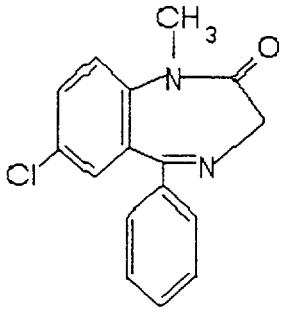

Figure-1: Molecular structure of diazepam.

Innis and coworkers reported that ^{125}I labeled Ro16-0154 has 10 times more affinity compared with fluoride derivative [2]. ^{123}I labeled Ro16-0154 have been proposed as a good benzodiazepine receptor imaging agent for SPECT. Additional iodinated derivative has higher lipophilicity. Octanol/water ratio is two times higher.

There are some reports about benzodiazepine receptor imaging agents in use for SPECT or PET [2-6]. Comparing two techniques can be useful. Iida and coworkers have been prepared 2' iododiazepam with ^{125}I by chemical synthesis and they have attached ^{11}C to N-1 position, ^{125}I to 2' position of the molecule. They showed that this ligand has 9 times more affinity comparison to diazepam [3].

Generally ^{3}H-diazepam and ^{3}H-flunitrazepam have been used as radioligands for benzodiazepine receptor studies. However working with ^{3}H-labeled compounds are confusing and time consuming. Liquid scintillation counting equipment is necessary for radioactivity measurements. Besides ^{125}I labeled ligands can be measured easily and more quantitatively by NaI(Tl) scintillation equipment. Furthermore ^{123}I-diazepam (2'^{123}IDZ) can be used for benzodiazepine receptor studies in Nuclear Medicine. It has been shown that both ^{125}I-Diazepam and ^{3}H-Diazepam bond to same receptors. According to these results ^{125}I-Diazepam can be used for benzodiazepine receptor studies. Saji and coworkers prepared ^{125}I labeled diazepam by chemical synthesis and reported that 2' position of diazepam in phenyl ring is the best position for iodine labeling. ^{125}I labeled benzodiazepines have higher receptor affinity and they are appropriate for SPECT studies [4] and they proposed that ^{125}I labeled diazepam can be an ideal receptor imaging radiopharmaceutical. Maziere and coworkers labeled flunitrazepam and diazepam with ^{11}C [5].

In this study, diazepam (7-chloro-1,3-dihydro-1-methyl-5-phenyl-phenyl-2H-1,4-Benzodiazepine-2-one) was labeled with iodogen method and its radiopharmaceutical potential was experimented on rabbits and rats.

EXPERIMENTAL

Diazepam was purchased commercially. Na^{131}I was obtained from Department of Nuclear Medicine. Iodogen was purchased from Sigma Co. All other chemicals were purchased from Merck and they were reactive grade.

Equipment: Labeling yields were determined by ITLC (Instant Thin Layer Chromatography) and electrophoresis methods. Procedure was the same with Unak and coworker's study [7]. R_f values were shown in table-I.

Preparing Iodogen Coated Tubes: Iodogen coated tubes were prepared as described earlier [6,7].

Labeling Procedure: Diazepam was labeled described earlier by Yurt et al. appling Iodogen method [6]. Two different solvent systems were used to solve diazepam. Solvent

1. Propylene glycol mixture (propylene glycol 40%, sodium benzoate 5%, ethyl alcohol 1.5%, v/v) Solvent 2. 50% HCl (v/v). Specific activity of labeled compound was 0.15 Ci/mmol level for solvent-1.

Preparation of Iodinated Diazepam in Inactive Conditions: Diazepam was iodinated in inactive conditions using iodogen method. The same procedure was applied with inactively iodinated chlorodiazopoxide [7]. This sample was used to take GC-MS and ^{1}H NMR spectra.

Measurement of the Octanol/Water Partition Coefficient: Lipophilicity was determined according to a previously reported method [8]. A 50 µl aliquot of radioiodinated sample was mixed with 3 ml each of 1-octanol and 0.1 M phosphate buffer (pH 7.4) in a test tube. The tube was cortexed (3x1 min), incubated for 1 hr at room temperature, and then centrifuged for 5 minutes. The 0.5 ml aliquots of each phase were removed and counted in a well-type NaI scintillation counter.

Scintigraphic Study in Rabbits: ^{131}I labeled diazepam with 160 mCi/mmol specific activity was sterilized by passing through a 0.22 µm membrane filter. Then it was injected from ear vein of male rabbits. Static perspectives were taken by Sophy DX 124x124 Gamma Camera.

Biodistribution Study in Rats: ^{131}I labeled diazepam was passed through from 0.22 µm membrane filter and it was administered intraperitoneally to male rats. Three rats were used for each point of the experiment. Injections were administered intraperitoneally. The rats were killed by decapitation after ether narcotization in an ether atmosphere and their organs were removed and weighted at different times. Their activities were counted by a well-type NaI(Tl) scintillation detector of multi-channel analyzer after marking 364 keV γ photons of ^{131}I. Total injected activities were supposed relatively 100 and percentage activities of organs per gram were calculated.

RESULTS AND DISCUSSION

Seventy-nine per cent yield were obtained in control experiments for strongly acidic conditions. It is known that iodide oxidizes in strongly acidic medium [9]. Iodide might enter a substitution reaction at this condition. On the other hand, if diazapam is waited for

Table -I Rf values of ^{131}IDZ and ^{131}IDHydrolysis product in different developing medium.

ITLC1:[n-Buthanol-Water-Acetic Acid (4/2/1)].

ITLC2:[Isopropyl Alcohol-n-Buthanol-0.2NAmmonium hydroxide (2/1/1)].

Developing medium	ITLC1	ITLC2
	Rf	Rf
^{131}IHydrolysis product	0.33 - 0.50	0.43 - 0.57
^{131}IDZ	0.91 - 1.00	0.88 - 1.00

Table-II Some of the molecular fragments peaksof iododiazepam at Mass Spectrum.

fragments	m/z
$M^+ + H^+$	412
$(M^+ + H^+) - CH_3$	396
$M^+ - C= O$	383
A grubu(C_{13} H_8 Cl I)	326
$A^+ + C= O$	354
$A^+ + CH_3$	341
$A^+ - Cl$	291
Ph - 1	203

Table-III Per cent of injected activity per gram for several organs of rats.

Time (min.)	Brain	Liver	Lung	Kidney	Heart	Blood	Brain/Blood
10	0.04 (0.01)*	0.07 (0.02)	0.09 (0.03)	0.12 (0.08)	0.07 (0.07)	0.12 (0.05)	0.32 (0.04)
20	0.06 (0.01)	0.13 (0.01)	0.13 (0.01)	0.42 (0.14)	0.11 (0.01)	0.16 (0.01)	0.36 (0.01)
40	0.15 (0.01)	0.50 (0.09)	0.46 (0.02)	0.91 (0.06)	0.68 (0.16)	0.41 (0.07)	0.37 (0.07)
60	0.19 (0.06)	0.63 (0.10)	0.53 (0.12)	1.40 (0.26)	0.40 (0.08)	0.45 (0.01)	0.42 (0.12)
120	0.11 (0.01)	0.27 (0.06)	0.23 (0.10)	0.55 (0.19)	0.46 (0.35)	0.24 (0.10)	0.55 (0.24)

* Standard deviation

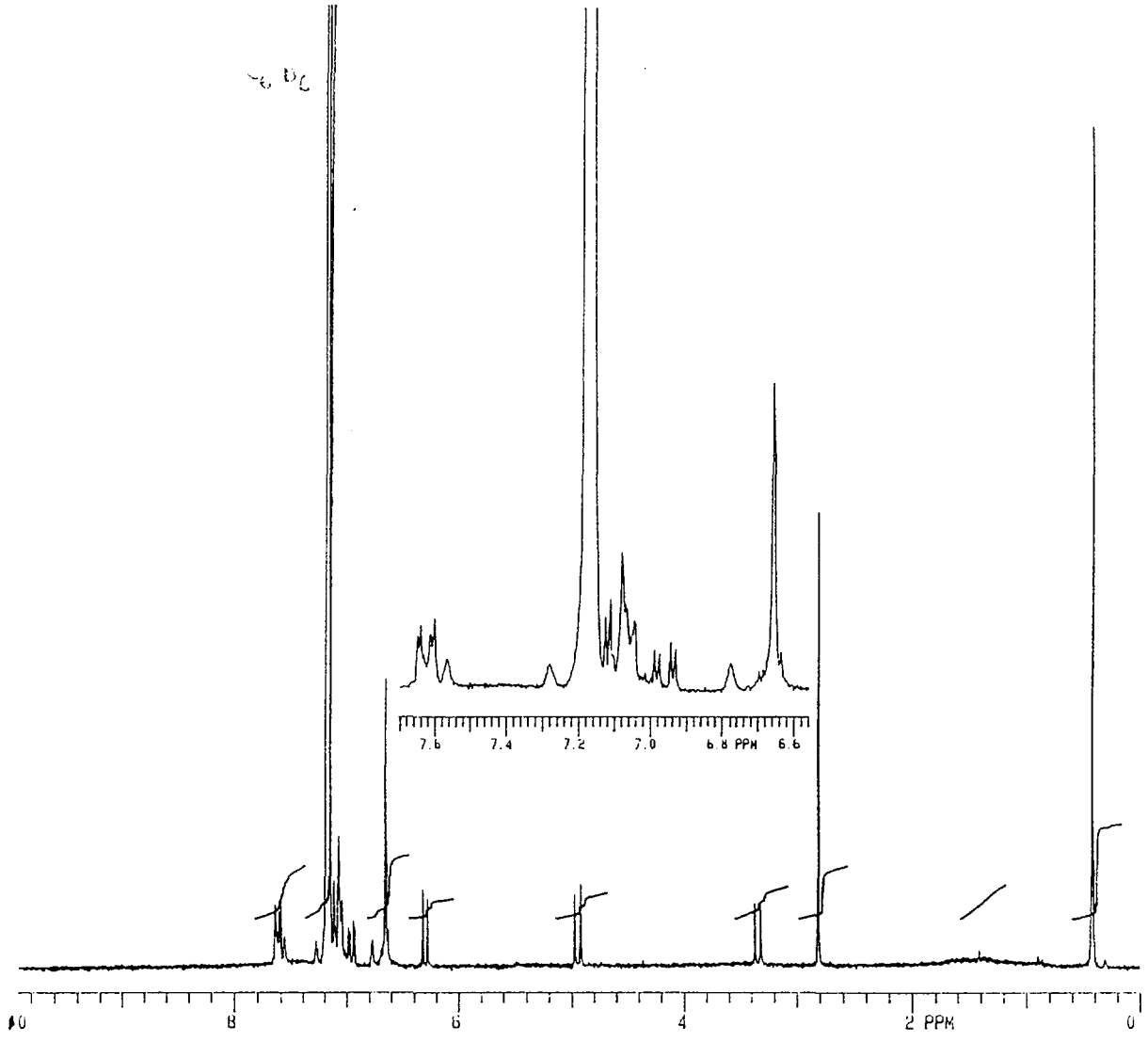

Figure-2: ^1H NMR Spectrum iodo derivative of diazepam.

a few days in acid solution, hydrolyses occur. For this reason two different products have been obtained for control studies (table-1). In the case of using of iodogen method 84% yield has been obtained for solvent-2 and 64% for solvent-1. Although the yield is less, solvent-1 was preferred to protect hydrolysis of diazepam. Optimum reaction conditions are 1 mg for iodogen amount; 1-5 mg for diazepam amount; 15-20 minutes for reaction time and room temperature for reaction temperature. On the other hand this product was stable at refrigerated temperature for a few days.

^1H NMR spectrum of iodo derivative of diazepam in C_6D_6 is shown in fig. 2. Molecular fragments of GC-MS are given in fig.3 and table-II. δ Values are 2.8 ppm for

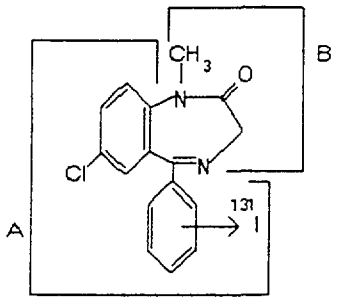

Figure-3: Molecular fragments of GC-MS.

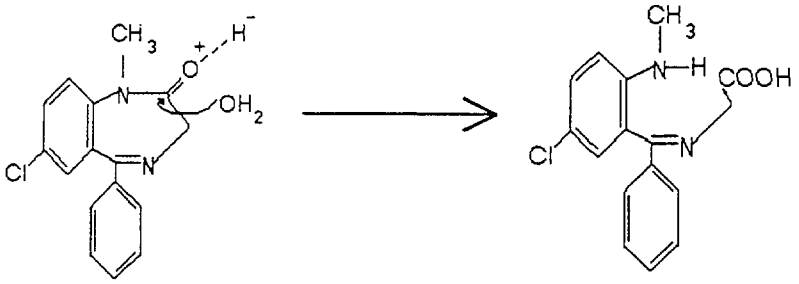

Figure-4: Hydrolysis mechanism of diazepam in acidic medium.

N-CH_3 protons, 4.97 for two doublets, 3.37 for 3rd position aliphatic C-H protons, 6.32-7.1 for phenyl ring protons. ^1H NMR results indicate that iodine may substitute with phenyl ring hydrogen by electrophilic substitution. GC-MS spectrum shows that diazepam hydrolysis in acidic medium as shown in fig. 4. Table-II shows the molecular fragments according to A fragmentation.

Fig. 5 shows the biodistribution of radioactivity for several organs in rabbits. Counts per pixel/background of brain were 1.9 after five minutes from the injection then this value remained as 1.9. The highest accumulation occurs in liver within 30 minutes after injection. Then a very rapid decline takes place. Elimination is slower for other organs like brain and kidneys.

Table-III shows percentage of injected activity per gram for several organs of rats. Uptake in heart and lungs is higher than brain after ten minutes from the injection. This result agrees with Iida and colloborates [3]. Maximum brain uptake was reached at 60th minutes. Innis and coworkers were obtained maximum brain uptake at 70th minutes at

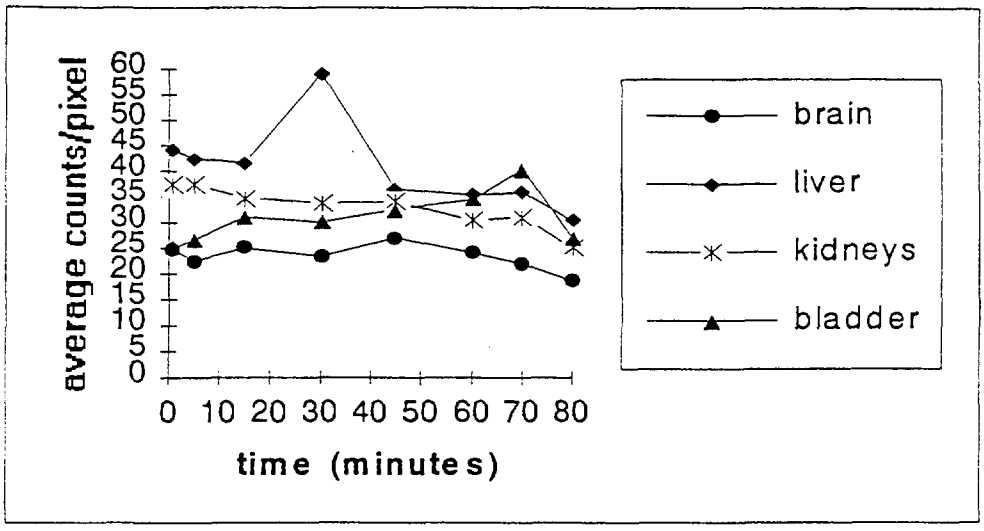

Figure-5 Biodistribution of radioactivity for several organs in rabbits.

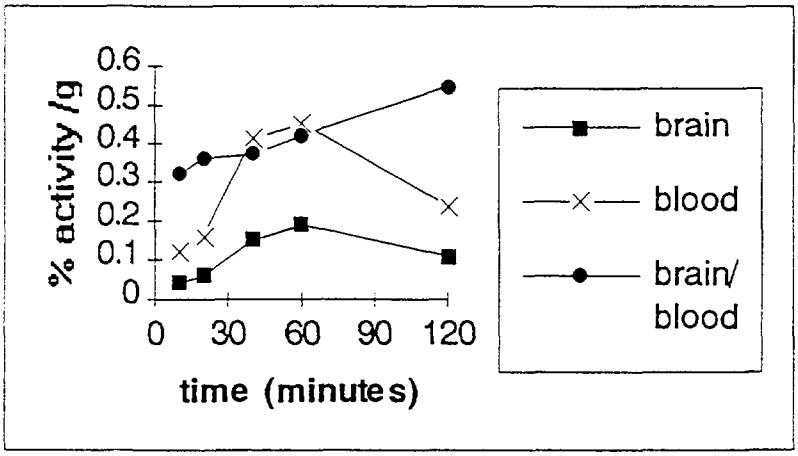

Figure- 6 Blood and brain radioactivities and brain/blood ratio in rats.

monkeys with ^{123}I-Ro-16-0154 [2]. Another study carried out with rats with ^{123}I Ro 16-0154. Maximum uptake was obtained 10 minutes after intravenous injection [10]. As a result of intravenous administration migration of radiopharmaceutical becomes faster. Percentage of injected activity in kidneys was 1.4 after one hour and 0.55 after two hours. This means that 2/3 of activity clears from kidneys in one hour. Clearance rate is 1/2 in lungs and it is higher in kidneys than lungs. Clearance rate is not very high for other organs (<1/2). If distribution depends on blood perfusion high uptake organs should be clear rapidly. It has been shown that uptake in ^{11}C-2'Iododiazepam doesn't depend on only

blood perfusion. Clearance rate is not also very high in this work. Obtained results agree with Iida [3]. For this reason we can say that distribution doesn't depend on only blood perfusion. Activity decrease in blood is faster than brain. This result agrees with Beer and coworkers [10]. The elimination from brain is considerably slower and reaches 0.19% per g organ after 1 hour and 0.11 at two hours. Highest activity is seen in liver (0.63) after kidneys. This rate decreases to 0.27 after two hours. Diazepam desmethylates in liver and elimination is slow. Decreasing rate is low because of the demetabolization in liver.

Figure 6 shows blood and brain radioactivities and brain/blood ratio in rats. The highest value in the blood and the brain is reached within 60 minutes after injection. The decrease of radioactivity in the blood is faster than in the brain. A good uptake in brain indicates that this radiopharmaceutical might be a good SPECT agent. Increasing brain/blood ratio by time shows ^{131}I-iododiazepam is cleared rapidly from blood and brain uptake is high. This result agrees with Iida [3] and Saji [4].

Lipophilicity value was found 1.9. It is known that brain uptake is fast and high related with the high n-octanol/water ratio [4]. On the other hand, Kung and coworkers reported that lipid soluble and neutral compounds can freely cross the blood-brain barrier [11].

As a result, I-131 labeled diazepam shows a potential and can be used to obtain SPECT images of brain.

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