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Drug Distribution and Biliary Excretion Pattern of a Cyclic Somatostatin Analog: A Comparison of ^{14}C Labeled Drug and a ^{131}I Iodinated Drug Analog

Larry J. Caldwell[1,4], Alan Parr[2], Robert M. Beihn[3], Bushra J. Agha[2], Arthur R. Mlodozeniec[1], Michael Jay[2], and George A. Digenis[2,3]

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Abstract: A cyclic somatostatin analog was compared to an iodinated analog of the same compound with respect to organ distribution and biliary excretion in the rat. The cyclic hexapeptide was radiolabeled with either ^{14}C or ^{131}I (tyrosine). Organ distribution of the iodinated compound as a function of time was nearly identical to that observed for the non-iodinated compound. Results indicated a rapid uptake by the liver and subsequent rapid excretion of the intact peptide in bile. Activity in other organs examined tended to fall off in a manner similar to the activity in blood with sequential samples. Because of the similarity in the *in vivo* behavior of the two compounds, the iodinated analog was deemed a suitable model for less invasive distribution studies, and was further examined in the dog using external gamma scintigraphy. In the unanesthetized dog the iodine activity was rapidly taken up by liver and collected in the gallbladder, thus exhibiting a similar rapid excretion pattern to that observed in the rat.

There has been an increasing interest in recent years concerning somatostatin and compounds which display somatostatin-like activity. This is primarily because of the powerful effects which somatostatin exerts on many diverse organ systems within the mammalian body, and the potential of using this type of compound to treat a variety of disease states.

One cyclic hexapeptide analog of somatostatin[5], [cyclo(L-*N*-methylalanyl-L-tyrosyl-D-tryptophanyl-L-lysyl-L-valyl-L-phenylalanyl)], A, displays resistance to enzymic degradation and at the same time is far more potent than somatostatin in eliciting certain physiological responses (1). This compound is in fact so resistant to degradation *in vivo*, that after i.v. injection, most of the compound is excreted intact in the feces. This implies a remarkable resistance to enzymic attack in blood, within the liver, and within the intestinal tract itself.

During the process of trying to better understand the *in vivo* disposition of A, it became desirable to find a suitable means to non-invasively monitor the fate of this compound after i.v. injection. The objective of the work described herein was to determine the feasibility of using an iodinated analog of A as a model compound to study the disposition of such polypeptides using external gamma scintigraphy (2). In this case we compared the organ distribution in the rat of ^{14}C-A to a ^{131}I-iodinated analog at various times after i.v. injection. Subsequently, the biliary excretion profile of both compounds was more carefully examined in the rat. Finally, the liver uptake and biliary excretion of the iodinated analog was quantitated in unanesthetized dogs using external gamma scintigraphy as a non-invasive technique.

Experimental

Preparation of iodinated drug analog

The cyclic hexapeptide A was radioiodinated [^{131}I] by a modification (3) of the procedure of Hunter and Greenword (4). $Na^{131}I$ was used as the source of the radionuclide and chloramine-T as the oxidizing agent. In a typical experiment the hexapeptide (5 µl, 1 mg/ml H_2O) was added to a test tube containing 50 µl of sodium phosphate buffer (pH 7.6), $Na^{131}I$ (10 µl, 1 mCi) was added followed by Chloramine-T (10 µl, 0.5 mg/ml H_2O) and the reaction mixture was gently shaken for 20 sec. To stop the reaction, sodium thiosulfate was added and gently mixed at room temperature. Bovine serum albumin (BSA) was added to the reaction mixture before subjecting the latter to Sephadex filtration.

[1] INTERx Research Corporation (Subsidiary of Merck & Co., Inc.), Lawrence, Kansas 66044
[2] College of Pharmacy, University of Kentucky, Lexington, Kentucky 40536
[3] Department of Nuclear Medicine, University of Kentucky Medical Center, Lexington, Kentucky 40536
[4] Correspondence to be addressed to Larry J. Caldwell, INTERx Research Corporation, 2201 West 21st Street, Lawrence, Kansas 66044

[5] Merck Sharp & Dohme Research Laboratories

The iodinated A was purified by column chromatography (medium-mesh Sephadex G-25, 30 × 1 cm) using 0.1 % BSA in 0.5 % (v/v) acetic acid as the eluent, and flow rates of 1 ml/min. One hundred and twenty 1-ml fractions were collected and counted for radioactivity in a gamma counter (Packard Model 9012 Auto-Gamma Scintillation Spectrometer) to give three major peaks in the reconstituted radiochromatogram. Fractions 30–60 co-chromatographed with the ^{125}I-iodinated hexapeptide which was previously identified by a radioimmuno assay (3). Thin layer chromatography (silica gel) of ^{131}I-iodinated A in two different solvents (EtoAc/Pyridine/HOAc/H_2O, 10:5:1:3 and $CHCl_3$/MeOH/concNH_3, 60:30:5) indicated no contamination of the product with free Na^{131}I. The isolated product displayed a specific activity of ca. 40 μCi/μg.

Rat organ distribution study

Female, Sprague Dawley rats, 175–200 g, were anesthetized with ethyl carbamate and both external jugular veins were cannulated using PE 50 tubing. Thirty rats were divided into two groups of 15/group. The first group received a combination dose i.v. consisting of ca. 1 μCi ^{14}C-A[6] (40 μg) and ca. 1 μCi ^{131}I-A (tyrosine) (25 ng). The second group received ca. 1 μCi ^{131}I-iodinated compound, diluted with 40 μg unlabeled A. Within each of the two groups, subgroups of 5 rats were serially killed at 10, 20, and 30 min, with subsequent organ removal of blood, liver, small intestine, spleen, pancreas, kidneys, lungs, and thyroid. Blood activity for the entire blood volume was calculated from a 1 ml sample. All organs were counted for ^{131}I activity immediately. The combined dose group samples were retained at −20 °C for 4 months before the samples were combusted and ^{14}C activity determined.

Rat biliary excretion study

Male, Sprague Dawley rats, 200–250 g, were anesthetized with ethyl carbamate. Bile duct cannulation was carried out through a ventral laparotomy with PE-10 tubing. Labeled compound (1 μCi), either ^{14}C-A or the ^{131}I-iodinated analog was injected i.v. in the tail vein. Bile fractions were collected at frequent intervals and plotted as percent cumulative radioactivity excretion, using the total activity excreted within the entire 90 min sampling time as 100 %.

In vivo disposition of the iodinated analog in dogs

Beagle dogs, 9–12 kg were outfitted with Pavlov-type restrainers and positioned laterally in front of a Searle LFOV gamma camera. The iodinated analog of A diluted with 1 mg non-radioactive A was injected i.v. in the foreleg, and the ^{131}I activity was followed by the camera. Data were collected in one minute intervals and were subsequently examined and processed with the aid of a conventional computer system for this type of camera. The activity of the injected ^{131}I analog was ca. 80 μCi (ca. 2 μg). Use of the computer allowed areas of interest to be selected and quantitated. The areas examined were liver (including gallbladder), and gallbladder alone, as being representative of liver uptake and biliary excretion. Results were presented graphically as counts per minute against time within the selected areas, in accordance with the techniques described by Digenis (2).

Results and Discussion

The results of the organ distribution profile of A (^{14}C) and the iodinated analog following i.v. administration are presented in Table I. It is evident that both compounds exhibit an enormous affinity for liver extraction from blood and rapid biliary excretion, if one assumes that the high activity in the small intestine is associated with the excreted bile.

Overall, there is no apparent differ-

Table I. Organ Distribution Comparison of (^{14}C)-[Cyclo(L-*N*-methylalanyl-L-tyrosyl-D-tryptophanyl-L-lysyl-L-valyl-L-phenylalanyl)] and Its ^{131}I-Tyrosine Iodinated Analog in the Rat

		Total Activity Found in Whole Organs at Successive Killing Times (Percent of Dose Administered ± SD, n = 5)		
Organ	Killing Time After Dosing (min)	Combination Dose ^{14}C plus ^{131}I % ^{14}C Activity	Combination Dose ^{14}C plus ^{131}I % ^{131}I Activity	Single Label Dose ^{131}I % ^{131}I Activity
blood	10	4.9 ± 1.0	6.7 ± 1.7	4.9 ± 1.1
	20	2.0 ± 1.0	3.0 ± 0.8	3.0 ± 0.6
	30	1.8 ± 0.7	2.8 ± 1.1	1.7 ± 0.4
liver	10	21.1 ± 9.5	28.8 ± 5.2	26.7 ± 4.0
	20	7.1 ± 2.2	10.9 ± 2.5	12.1 ± 0.8
	30	5.1 ± 0.6*	9.9 ± 1.3*	7.9 ± 1.3
small intestine	10	20.0 ± 10.5*	45.0 ± 21.8*	47.6 ± 14.1*
	20	79.3 ± 32.4	70.9 ± 7.0	63.0 ± 6.6
	30	91.1 ± 27.9	75.5 ± 9.7	72.8 ± 5.7*
spleen	10	0.10 ± 0.01	0.15 ± 0.03	0.14 ± 0.02
	20	0.13 ± 0.01	0.09 ± 0.02	0.08 ± 0.01
	30	0.04 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
pancreas	10	0.26 ± 0.05	0.47 ± 0.13	0.59 ± 0.23
	20	0.24 ± 0.09	0.36 ± 0.20	0.46 ± 0.36
	30	0.20 ± 0.04	0.21 ± 0.05	0.22 ± 0.11
kidneys	10	2.6 ± 0.5*	2.8 ± 0.5	2.9 ± 0.6
	20	1.3 ± 0.4	1.3 ± 0.4	2.3 ± 1.1
	30	1.1 ± 0.2	1.1 ± 0.2	1.0 ± 0.3
lungs	10	0.68 ± 0.04	0.92 ± 0.09	0.73 ± 0.13
	20	0.35 ± 0.12	0.50 ± 0.17	0.44 ± 0.13
	30	0.23 ± 0.06	0.27 ± 0.13	0.32 ± 0.05
thyroid	10	0.040 ± 0.006	0.089 ± 0.013	0.081 ± 0.014
	20	0.031 ± 0.012	0.108 ± 0.011	0.090 ± 0.016
	30	0.016 ± 0.009	0.091 ± 0.014	0.106 ± 0.010

* N = 4 instead of 5

[6] ^{14}C-A (D-tryptophan methylene ^{14}C), synthesized by Dr. Robert L. Ellsworth, MSDRL, Rahway, NJ.

ence between the parent compound A and its iodinated analog. With the exception of the thyroid, the ratio of ^{131}I to ^{14}C in all tissues sampled was similar to the ^{131}I:^{14}C ratio in blood when the variability of the observed numbers is considered. It was decided to examine the thyroid as an indicator of the *in vivo* integrity of the ^{131}I label on the tyrosine residue. If the label were to dissociate *in vivo,* then the thyroid should sequester the label as iodide. The results suggest that a very small percentage of the label may dissociate, but the relative amount is practically insignificant in relation to the total dose.

These initial experiments were deemed promising because of such similar behavior between the iodinated drug analog and the parent A. However, it was decided to directly confirm that A and ^{131}I-A were eliminated by biliary excretion in the rat before proceeding with the scintigraphic studies in the dog.

Figure 1 presents the cumulative biliary excretion in single rats for both ^{14}C-A and the iodinated analog of A for a period of 90 minutes after i.v. injection. The data indicate that indeed both ^{131}I-A and ^{14}C-A are rapidly excreted in bile. Furthermore, when the ^{131}I-labeled compound was compared by TLC (silica gel G-F plate, chloroform/methanol/58 % ammonium hydroxide – 60:30:5) to a bile fraction containing the excreted ^{131}I label, the radioactive label displayed the same RF in each case, confirming that iodinated A, like A itself, is excreted unchanged.

There was some indication that ^{131}I-A was generally excreted more rapidly than ^{14}C-A, at least in the first few minutes after i.v. administration (Table I). Although this trend was reversed in the single animals in which the bile duct was cannulated, the initial biliary excretion rates fall within the range of variability observed in the organ distribution study.

In Figure 2, the ascending portion of the curve represents the typical pattern of ^{131}I activity accumulation in the region of the gallbladder after i.v. injection of ^{131}I-A in the dog. This profile is strikingly similar to the biliary excretion profile observed in the rat for ^{131}I-A, but in this case has been observed using an unanesthetized animal of a different species.

In the dog, the clearance of the ^{131}I activity by the liver was much more rapid than indicated by the gallbladder accumulation curve, but some time was required for the activity to collect or concentrate at the gallbladder site. Interestingly, at these dosage levels, the activity which accumulated in gallbladder did not normally spill over into the small intestine for many hours, indicating biliary retention, possibly drug induced. To confirm that the region of interest was indeed gallbladder, a series of i.v. CCK (Kinevac® Squibb octapeptide) injections were administered. The stepwise drop in activity seen in Fig. 2 in response to these injections of the CCK analog, confirmed that the selected area was representative of gallbladder. Fig. 3 shows the activity profile in the region of the small intestine. The stepwise increase in activity in response to CCK challenges mirrors the pattern seen in

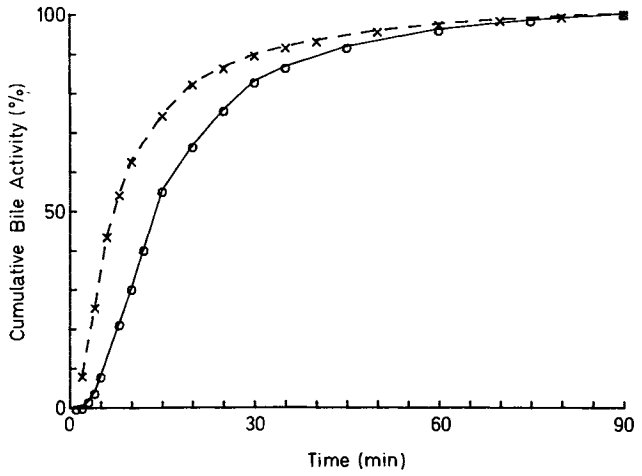

Fig. 1 Biliary excretion profile in the rat of activity after i.v. injection of ^{14}C-(tryptophan)A (x) and ^{131}I-(tyrosine)A (o). The total activity excreted within 90 minutes is defined as 100 % of the dose adminstered.

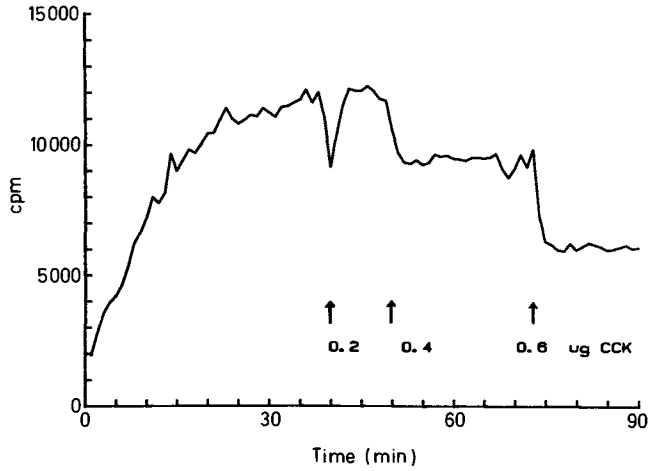

Fig. 2 Activity profile of the gallbladder region in dog after i.v. injection of ^{131}I-(tyrosine)A. Arrows indicate i.v. bolus of a CCK analog (Kinevac®).

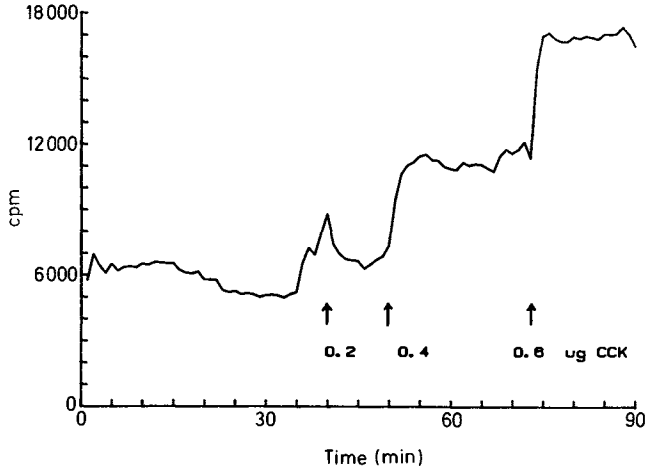

Fig. 3 Activity profile of the intestinal region in dog after i.v. injection of ^{131}I-(tyrosine)A. Arrows indicate i.v. bolus of a CCK analog (Kinevac®).

Fig. 2 and confirms that gallbladder emptying was the mechanism involved.

In conclusion, the feasibility and practicality of using iodinated analogs of tyrosine-containing small peptides to study *in vivo* drug disposition has been demonstrated. The modes of clearance and biliary excretion are similar for both A and ^{131}I-A in the rat, and ^{131}I-A behaves similarly in both rat and dog.

The advantages of non-invasive technology such as external gamma scintigraphy are many, including its applicability to human studies involving peptide drugs or drug candidates. The relatively straightforward process of preparing gamma-emitting iodinated peptide analogs, and performing the necessary control studies, may in many instances result in a convenient means to study the complex problems of polypeptide drug disposition and elimination. The unusual observation with the dog model that A may effect biliary retention is the subject of a follow-up study to be presented in another report.

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Effects of 5-Fluorouracil Prodrugs on the Central Nervous System in Mice and Rats

Katsuo Toide[1], Norio Unemi[1] and Tomio Segawa[2]

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Abstract: The effects on the central nervous system (CNS) of mice and rats were determined for the 5-fluorouracil prodrugs, 1-(2-tetrahydrofuranyl)-5-fluorouracil (FT), a combination of FT and uracil in a molar ratio of 1:4 (UFT), and 1-hexylcarbamoyl-5-fluorouracil (HCFU). Both FT and UFT failed to produce a significant prolongation of hexobarital sleeping time in mice, while HCFU, at the same dose levels, caused a significant ($P < 0.01$) prolongation of hexobarbital sleep. FT, UFT, and HCFU produced a slight suppression of coordinating ability in mice, but the effect of HCFU was more pronounced than that of FT and UFT. There were no significant changes in 5-hydroxytryptamine contents in the cerebral cortex and only small insignificant changes of dopamine contents in the corpus striatum by any of the drugs examined. Furthermore, HCFU was more potent than FT and UFT in potentiating the actions of ethanol. These results suggest that HCFU is more toxic to the CNS than are FT and UFT.

5-Fluorouracil (5-FU)[3] has been widely used for the treatment of cancer. In addition to its antitumor activity, however, 5-FU possesses various side effects such as gastrointestinal (GI) and hematological toxicity (1).

1-(2-Tetrahydrofuranyl)-5-fluorouracil (FT) (Fig. 1) was synthesized as a derivative of 5-FU by Hiller et al. (2) and is now commonly used as an oral antitumor agent in Japan. Because FT is slowly converted to 5-FU (3), its toxicities in bone marrow and GI tracts are lower than those of 5-FU (4). However, FT passes easily through blood brain barrier and produces occasionally side effects in the central nervous system (CNS), including lethargy, ataxia, confusion, dizziness, and hallucination (5, 6). 5-FU occasionally also causes reversible cerebellar ataxia (7).

It was found that coadministration of uracil with FT increased 5-FU level in tumor and blood, possibly because uracil inhibits the degradation of 5-FU formed from FT in the liver (8). There-

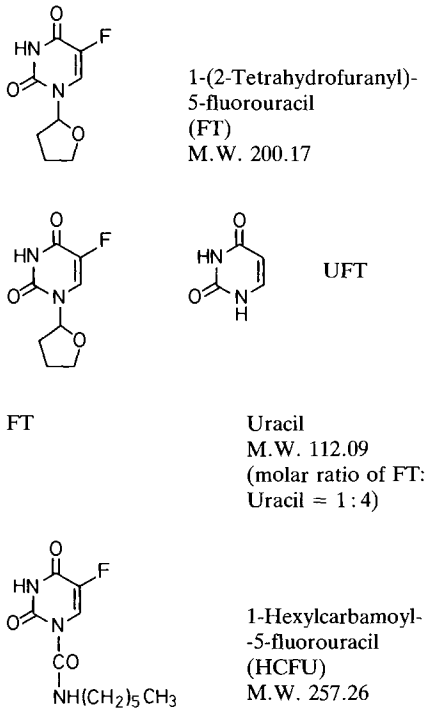

Fig. 1 Chemical structures of FT, UFT and HCFU.

[1] Division of Pharmacology of Research Laboratory, Taiho Pharmaceutical Co., Ltd., Ebisuno Hiraishi 224-2, Kawauchi Tokushima 771-01, Japan

[2] Department of Pharmacology, Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Kasumi 1-2-3, Minami-ku, Hiroshima 734, Japan

[3] Abbreviations
UFT: a fixed drug combination of 1-(2-tetrahydrofuranyl)-5-fluorouracil (FT) and uracil in a molar ratio of 1:4
FT: 1-(2-tetrahydrofuranyl)-5-fluorouracil
5-FU: 5-fluorouracil
HCFU: 1-hexylcarbamoyl-5-fluorouracil
5-HT: 5-hydroxytryptamine
DA: dopamine
p.o.: per os
i.p.: intraperitoneally