Iodine-131- Labeled Diphosphonates for the Palliative Treatment of Bone Metastases: I. Organ Distribution and Kinetics of I-131 BDP3 in Rats

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I-131-labeled α-amino-(4-hydroxybenzylidene)-diphosphonate (BDP3) was examined in Sprague-Dawley rats for its bone affinity and other biokinetical characteristics. Iodine-131 BDP3 exhibited high bone affinity, with rapid blood clearance and renal excretion. Total-body retention measurements revealed an effective half-life of the activity of $t_{1/2}$ = 169 hr. Similar kinetics were found in rat femurs, with $t_{1/2}$ = 158 hr. Rats bearing osteosarcomas showed an enhanced uptake with a slower clearance ($t_{1/2}$ = 177 hr). The amount of I-131 BDP3 excreted in urine agreed with the total body retention values. Fifty percent was excreted in the urine, as unchanged I-131 BDP3, by 24 hr after application. Almost no activity was found in the feces of the rats. LD_{50} measurements in rats amounted to 64 mg/kg. Because of these biokinetical characteristics and the simple labeling procedure, I-131 BDP3 is suggested as a radiopharmaceutical for the palliative treatment of pain that often accompanies disseminated bone metastases.

J Nucl Med 25: 1356–1361, 1984

Intractable pain often accompanies advanced bone metastases originating from various primary carcinomas. This kind of pain syndrome has been treated with osteotropic beta-emitting radionuclides such as Sr-89 (*1*,*2*). This is a relatively long-lived nuclide, with a physical half-life of 50.5 days, however, and because of radiation protection laws, special care must therefore be taken with the disposal of excretions from patients treated with Sr-89. Recently Y-90 was introduced for treating pain. Despite its desirably short physical half-life (64.5 hr), the high liver uptake (~10%) and the production of the Y-90 EDTA chelate remained a problem (*3*). In contrast to these cations, P-32-labeled phosphorus compounds have been applied as osteotropic anions. Along with the palliative response, bone-marrow depression was observed with [^{32}P]phosphate, and to a lesser degree with [^{32}P] HEDP (*4–6*). Moreover, [^{32}P] HEDP is difficult to synthesize. The complexing of the beta-emitting Re-186 with osteotropic diphosphonate ligands has also been suggested for this kind of pain treatment (*7*,*8*). However, high kidney uptake and Re-186 diphosphonate complex formation proved to be unfavorable. In response to these disadvantages, a search for an aryl-substituted diphosphonate that would easily accept the I-131 label was initiated. Iodine-131 was selected as a beta source, because it has become well established in nuclear medical therapy, and no health problems would arise in connection with the excreted activity. α-Amino-(3-[I-131]iodo-4-hydroxybenzylidene)-diphosphonate (I-131 BDP3) proved to exhibit this feature and its high bone affinity was expected to provide a palliative response in patients with disseminated bone metastases. This paper describes the biodistribution and pharmacokinetic characteristics of I-131 BDP3 in rats.

Received Dec. 30, 1983; revision accepted July 23, 1984.

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MATERIAL AND METHODS

α-Amino-(4-hydroxybenzylidene)-diphosphonate (BDP3), synthesized by a route recently described (*9*), was labeled with I-131 by electrophilic aromatic substitution in the presence of IO_3^- and 1 *N* HCl. The structural formula is shown in Fig. 1. Thus, 20 μl of

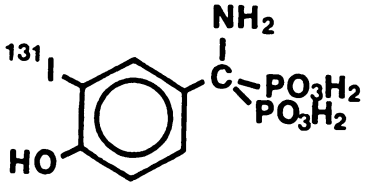

FIG. 1. Structural formula of I-131 BDP3

BDP3 (14.5 mg/ml 1 *N* HCl) were mixed with 5 μl KIO_3 (10.9 mg/ml H_2O) and 20 μl $Na^{131}I$ (800 mCi/ml in 0.02 *N* NaOH). After a 15-min reaction time at room temperature, the mixture was neutralized with 1 *N* NaOH and treated with 10 μl $Na_2S_2O_5$ (9.7 mg/ml H_2O) to reduce unreacted iodate. The reaction was performed in a closed vessel in order to prevent air contamination with volatile activity. Unreacted radioiodide was then removed by filtration over AgCl. About 95% radiochemical yield was generally achieved after sterile filtration. The specific activity of the product was 50 mCi/mg diphosphonate.

Photopenic I-BDP3 was synthesized and found to be identical with I-131 BDP3. This was proved by HPLC, chemical analysis, and spectroscopic methods. The data will be published elsewhere.

The biodistribution was studied in male Sprague-Dawley rats weighing about 400 g. Fifteen μCi were injected into a tail vein. The organs were removed after 4, 24, 96, 192, and 384 hr, for six rats for each time interval. Uptakes of I-131 BDP3 in the various organs were calculated as a function of time, as % dose/g— that is, (% dose/g) × animal weight in grams. The values in Table 1 are given as means with standard deviations.

The use of the whole femurs to monitor bone uptake suffers from the disadvantage of including contributions of other tissues. Cartilage, bone marrow, and possibly bone tissues differing in activity in the various parts of the femur contribute to the total femur uptake. Pieces of the cleaned marrow-free femoral diaphysis were therefore measured also to represent typical bone tissue, since it generally appears in bone scintigrams with the most homogeneous density of activity.

The bone retention of I-131 BDP3 was measured in four healthy and three osteosarcoma-bearing Sprague-Dawley (S-D) rats in a total-body animal counter. These animals weighed ~280 g. Constant radiation geometry and a standard of activity guaranteed reproducible results.

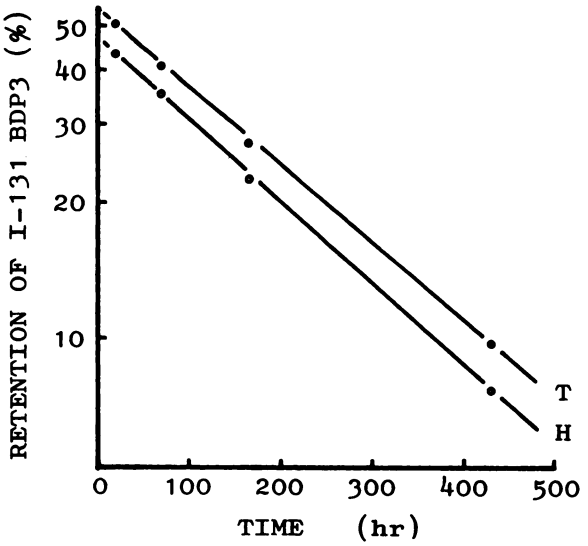

FIG. 2. Effective total-body retention of I-131 BDP3 in healthy rat (H) and rat bearing osteosarcoma growing subcutaneously (T). Effective half-lives in these animals were 165 hr (H) and 173 hr (T). Correlation coefficients were r = 0.9998 for both curves.

The kinetics of I-131 BDP3 in the femurs of S-D rats was also quantified by scintigraphy with a pinhole collimator. Four anterior images of the femurs of anesthetized S-D rats were taken over a period of about 500 hr. Three animals weighing ~400 g were subjected to these measurements. The constancy of the target geometry during scintigraphy, and comparison with images of standard activities, showed the results to be reproducible. The influence of the intensity distribution, which decreases from the center of the pinhole image, was thereby eliminated.

The effective half-lives were calculated by linear regression fitting of the experimental data to a straight line in a semilogarithmic plot. Examples of fitted curves are shown in Figs. 2 and 3. The results are summarized as means with standard deviations.

TABLE 1. TISSUE DISTRIBUTION OF I-131 BDP3 IN RATS

Time (hr)	F. diaphys.	Femur	B. marrow	Blood	Heart	Lung	Spleen	Liver	Kidney
4	313* (85)	254 (53)	8 (5)	6 (3)	3 (1)	14 (8)	8 (5)	9 (5)	79 (43)
24	251 (65)	245 (37)	3 (2)	1 (0)	1 (1)	7 (9)	7 (6)	6 (3)	53 (35)
96	281 (59)	256 (75)	1 (1)	0	0	1 (1)	7 (4)	8 (2)	43 (32)
192	245 (72)	220 (77)	0	0	0	1 (1)	9 (4)	10 (4)	30 (20)
384	223 (52)	201 (48)	0	0	0	1 (1)	9 (4)	9 (3)	25 (22)

* In % g dose/g, mean and (s.d.) for 6 rats.

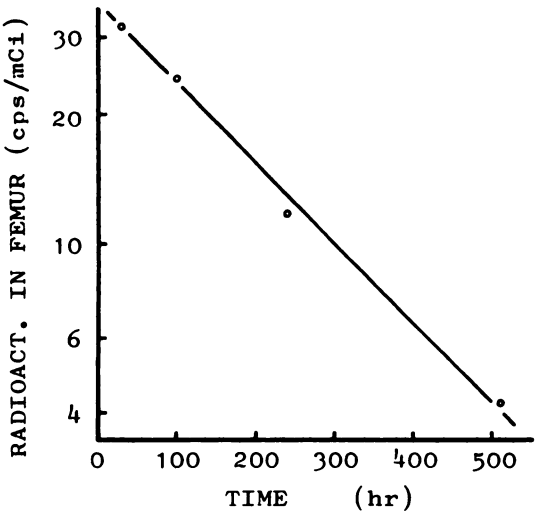

FIG. 3. Effective bone retention of I-131 BDP3 in rat femur. Effective half-life in this example was 164 hr, and correlation coefficient r = 0.9974.

The excretion studies used a metabolism cage. As summarized in Table 2, the activity excreted during various time intervals was collected and counted. The cumulative values were expressed in % of the injected dose for five S-D rats.

RESULTS

Table 1 summarizes the concentrations of I-131 BDP3 in nine selected tissues of male S-D rats. The animals showed the expected high uptake only in the femoral diaphysis and the whole femur. At 4 hr after injection, maximum tracer concentration was achieved in the bone compartment. Values for the femoral diaphysis were highest, with whole femur a close second. Blood clearance was rapid, bone-marrow uptake was low, and the kidneys retained a comparatively small amount of the activity, which was released with time. The other organs showed negligible uptake.

The excretion of the activity after the administration of I-131 BDP3 in S-D rats was measured as a function of time. The results in Table 2 indicate rapid urinary excretion. At 24 hr the cumulated urinary activity amounted to 50.3 ± 1.5%. On the other hand, almost no activity was found in the feces of the rat. Thin-layer chromatography showed that the activity of the 10- and 24-hr urines consisted of unchanged I-131 BDP3.

According to the data in Tables 1 and 2, the bone appeared to contain the bulk of the activity. The total-body retention of I-131 BDP3 in rats was therefore considered equivalent to the retention in the bone compartment.

Figure 2 shows the clearance of the activity from two rats as a function of time. The upper curve (T) was derived from a rat bearing a subcutaneously transplanted osteosarcoma, about 1.5 cm in diameter, while the lower curve was obtained from a healthy rat (H). Both rats were males of identical age, weighing 242 g (H) and 300 g (T). The 24-hr retention values were 47.9 ± 0.8% (H, five animals) and 55.7 ± 0.7% (T, three animals), both corrected for physical decay. The effective half-lives were estimated by linear regression from the curves through the experimental data for the clearance values in the 20- to 430-hr time span. The calculations yielded 168.9 ± 3.8 hr (H, four animals) and 177.3 ± 4.5 hr (T, three animals).

Similar values were obtained by quantification of sequential scintigrams of rat femurs over a time period of about 500 hr. Figure 3 shows the decay of the activity in the femur of one of the animals as a function of time. The effective half-life was 158.4 ± 7.0 hr (three animals).

The acute intravenous toxicity of the starting material (BDP3) was measured in S-D rats. The LD_{50} was 64 mg/kg.

DISCUSSION

The findings suggest the use of the new I-131 BDP3 in the palliative treatment of the pain syndrome that often accompanies disseminated bone metastases. For this purpose it appeared attractive to use I-131 as a beta source, since contemporary therapy wards are equipped for the radioiodine therapy of thyroid malignancies. Convenient radioactive waste disposal, a simple labeling procedure, and advantageous pharmacokinetical characteristics, discussed below, represent essential prerequisites for the clinical applicability of I-131 BDP3.

BONE AFFINITY

The organ distribution of I-131 BDP3, which was measured in male S-D rats through a period of 16 days, showed high uptake only in bony tissue (Table 1). The

TABLE 2. I-131 BDP3 EXCRETION IN RATS

Time (hr)	Urine	Feces
3	37.5*	—
10	46.2	—
22	49.2	0.3*
24	50.3	0.3
45	50.8	0.7

* In % of dose (cumulative) for 5 rats; corrected phys. decay.

TABLE 3. COMPARISON OF DISTRIBUTION OF Tc-99m DIPHOSPHONATES WITH I-131 BDP3 IN SPRAGUE-DAWLEY RATS

Time (hr)	Compound	F. diaphys.	Blood	Kidney
4	I-131 BDP3	313* (85)	6 (3)	79 (43)
	Tc-99m BDP3	193†	14	511
	Tc-99m MDP	296†	10	189
24	I-131 BDP3	251 (65)	1 (0)	53 (35)
	Tc-99m BDP3	150	5	452
	Tc-99m MDP	287	6	139

* In % g dose/g, as mean and (s.d.), 6 rats.
† In % g dose/g, as mean 3 rats.

values for total femur and femoral diaphysis peaked within the first 4 hr after administration, like those for the Tc-99m diphosphonate complexes, and then decreased slowly with time. Table 3 compares the 4- and 24-hr I-131 BDP3 uptakes in the femoral diaphysis with those for Tc-99m BDP3 and Tc-99m MDP, these having both been investigated recently, together with other BDP ligands, with respect to their in vivo characteristics (*10,11*). I-131 BDP3 and Tc-99m MDP proved to have about the same bone affinity. The somewhat lower uptake achieved with Tc-99m BDP3 can be explained by the alteration of the molecular structure of the ligand when complexed with Tc-99m, which obviously led to a less favorable binding characteristic. I-131 BDP3 represents a pure ligand, with uncompromised diphosphonate groups that have free valences for the hydroxyl apatite binding sites. Additionally, Tc-99m diphosphonate complex formation is known to yield a series of complex species with variable affinities for bone (*12,13*). Both effects may explain the different affinities.

The bone uptake of I-131 BDP3 was also estimated by the measurement of its total-body retention in S-D rats (Fig. 2). This method was chosen because bone tissue was the organ that contained almost the entire dose at 24 hr after application. The measurements were performed in healthy rats (H) and rats bearing a subcutaneously transplanted osteosarcoma (T). The latter was available as a tumor model, but it did not exhibit desirable characteristics as a lesion model (*10,11*). The reason was the rapid tumor growth and the resulting low content of calcified tissue that typically appears in bone metatstases. The osteosarcoma, measuring 1.5 cm in diameter, contributed at least an 8% enhancement to the 24-hr total-body retention of I-131 BDP3, although the tumor contained fewer binding sites than the femoral diaphysis—which, relative to other bony tissues, is far from the most avid area for osteotropic radiopharmaceuticals. This characteristic was proved with Tc-99m MDP and a series of Tc-99m benzylidene diphosphonate complexes (*10,11*).

Twenty-four hours after administration of I-131 BDP3, the total-body retentions, decay-corrected, amounted to 47.9 ± 0.8% and 55.7 ± 0.7% for healthy and osteosarcomatous rats, respectively. Compared with I-131 BDP3, less than 35% of Tc-99m MDP is reportedly retained within the same time period by dogs and humans (*14,15*).

The curves in Fig. 2—which illustrate the combined physical decay and biological clearance from a healthy and an osteosarcomatous animal—show a slight divergence. The effective half-lives, $t_{1/2}$ of the two animals were therefore different. The results from four H and three T rats (S-D) averaged to

$$H: \quad t_{1/2} = 168.9 \pm 3.8 \text{ hr}$$

$$T: \quad t_{1/2} = 177.3 \pm 4.5 \text{ hr}$$

Longer effective half-lives, which were also obtained in the metastatic bone lesions of pain-treated patients, parallel these findings. An explanation for the differing kinetic behavior of I-131 BDP3 in tumorous bone tissue cannot be given yet. In any case, the formation and/or liberation of hydroxyapatite binding sites—mediated by the enhanced osteoblastic and/or osteoclastic activity that goes along with enhanced bone turnover—must be the reason for this finding (*16*).

The measurement of the kinetics of I-131 BDP3 may therefore be helpful for the diagnosis of metabolic bone disorders. Because of its longer half-life, I-131-labeled diphosphonates provide an additional parameter—namely the effective half-life—by comparison with Tc-99m diphosphonate complexes that have already been found useful in this respect (*15,17*). Since retention measurements in a total-body counter are sufficient to establish kinetic data, only small amounts of activity would be necessary.

URINARY EXCRETION

The total-body retention of I-131 BDP3 in the S-D rats agreed with the urinary excreted activity summarized in Table 2. About 50% (decay-corrected) of the injected activity was collected after 24 hr, whereas at that time negligible activity had appeared in the feces. At that time the distribution of the activity present intravascularly in the bone and kidney (urine) compartments was considered to be completed, because subsequent excretion was very small. The small amounts still appearing probably belong to radioactivity released metabolically from the bone compartment. The excreted activity was too low for chemical analysis. However, the first 10- and 24-hr urines contained unchanged I-131 BDP3, as was proved by thin-layer chromatography.

The fast excretion rate of I-131 BDP3 into the urine reduced renal uptake by an order of magnitude relative to the corresponding Tc-99m diphosphonates (Table 3). Thus no kidneys were observed scintigraphically after 4 hr. Moreover, as already mentioned, Tc-99m diphosphonate complex formation yields more or less kidney and complex species, which showed reciprocal affinity to kidney and bone (*12,13*). It appeared that some component complex fractions are not excreted at all and they probably become fixed in the renal parenchyma within the lifetime of Tc-99m.

BLOOD CLEARANCE AND UPTAKE IN OTHER ORGANS

The activity disappeared very rapidly from the blood pool. The distribution into the bone and urine compartments occurred somewhat faster than that achieved with Tc-99m diphosphonate complexes (Table 3). No return of activity into blood cells through some contribution of I-131 BDP3 to erythropoiesis could be observed. This was also verified by the measurements of the bone-marrow uptake, which paralleled the blood values (Table 1). This characteristic stands in contrast to [^{32}P]phosphate, which does participate in cell neogenesis. [^{32}P]HEDP, structurally related to the benzylidene diphosphonates, may also be considered as a potential [^{32}P]phosphate source. Although there is no indication so far for P-C cleavage reactions in vivo, the unchanged excretion of I-131 BDP3 in S-D rats within the first 24 hr supports this, whereas liberation of [P-32]phosphate is conceivable. Possible radioactive metabolites from I-131 BDP3 are $^{131}I^-$, I-131 benzoic acid or amide, and an I-131 benzylidenephosphonate. None of these participate in DNA formation.

The uptake of 0.1% of the injected activity in the thyroids of the S-D rats at 24 hr proved the metabolic stability of I-131 BDP3 regarding radioiodide liberation. Once bound to the bone tissue, I-131 BDP3 is safe against deiodination, for the thyroid activity was reduced to 0.05% after 16 days.

TOXICITY

The acute intravenous toxicity of the title compound (LD_{50} = 64 mg/kg rat) appeared to be rather low compared with methylene diphosphonate (LD_{50} = 45 mg/kg mouse). MDP is applied as a Tc-99m ligand in bone imaging in amounts of at least 1 mg/patient. Considering the easily obtained specific activity of 50 mCi I-131 per mg BDP3, an adequate safety factor can be maintained.

OUTLOOK ON CLINICAL APPLICATION

A disadvantage of the application of I-131 are the gamma photons. They are emitted from the bone compartment as long as I-131 BDP3 remains undecayed. They do bring, however, the advantage that imaging and absorbed dose calculations for normal and tumorous bone become possible by scintigraphy. Preliminary clinical studies have demonstrated a high affinity of I-131 BDP3 for bone metastases, and an extensive clinical trial with I-131 BDP3, including dosimetric calculations is currently in progress.

ACKNOWLEDGMENTS

The author thanks Dr. Worms (Henkel KGaA, Düsseldorf, FRG), who kindly provided a sample of BDP3 for comparative studies and toxicological measurements, and Dr. Berger (Deutsches Krebsforschungszentrum, Heidelberg, FRG) who made the osteosarcoma available. The author is also grateful to Dr. Gruner (Amersham Buchler, Braunschweig, FRG) for information about the toxicological actions of BDP3.

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