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Comparison of the Biodistribution of ^{225}Ac and Radio-Lanthanides as Citrate Complexes

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Dedicated to Professor Dr. rer. nat. habil. Rudolf Münze on the occasion of his 60th birthday

A method for production of ^{225}Ac is described. Uranium oxide was irradiated with 650 MeV protons. The radium fraction was separated by precipitation with $BaSO_4$, the isotopically pure ^{225}Ac as daughter product was then isolated by means of cationexchange chromatography. The biological behaviour of ^{225}Ac as a potential candidate for radionuclide tumor therapy is compared with the behaviour of other Me^{3+}-citrate complexes. The actinium shows the faster blood clearance and highest liver uptake in rats and tumor-bearing mice. The tumor uptake is smaller in comparison to ytterbium as a representative for the heavy rare earth elements and slightly higher compared to promethium as representative for the light rare earth elements. In general the biodistribution of actinium is in good agreement with the radiums alteration of Me^{3+}-ions.

Eine Methode zur Erzeugung von ^{225}Ac wird beschrieben. Uranium Oxid wird mit 650 MeV Protonen bestrahlt. Die Radium-Fraktion wird durch Fällung mit $BaSO_4$ abgetrennt. Isotopenreines ^{225}Ac wird dann als Tochterprodukt mittels Kationenaustausch-Chromatografie isoliert. Das biologische Verhalten des ^{225}Ac als ein potentieller Kandidat für die Radionuklid Tumor Therapie wird verglichen mit dem Verhalten anderer Me^{3+}-Citrat Komplexe. Das Actinium zeigt die schnellere Blut-Clearance und die höchste Leber-Anreicherung in Ratten und tumortragenden Mäusen. Die Tumor-Aufnahme des Actiniums ist geringer im Vergleich zu Ytterbium als Vertreter der schweren Lanthanoide und geringfügig höher im Vergleich zu Prometium als Vertreter der leichten Lanthanoide. Allgemein entspricht die Bioverteilung des Actiniums den Erwartungen, die sich aus den Radien der Me^{3+}-Ionen ableiten läßt.

Keywords

actinium 225; citrates; ion exchange chromatography; isotope production; radionuclide kinetics; radiopharmaceuticals; rare earths; spallation; tissue distribution; uranium

1. Introduction

It is well accepted that Me^{3+}-citrate complexes (^{67}Ga, ^{111}In, some lanthanides) are useful tumor imaging agents [1]. Radioisotopes of rare earth elements administered as citrate complexes show a considerably high tumor uptake [1, 2]. In comparison to ^{67}Ga the ^{167}Tm (9.25 d, 208 keV gamma radiation) as a representative of the heavy rare earth elements shows the much faster blood clearance [3]. On the other hand the bone uptake of the heavy rare earth elements is much higher than for ^{67}Ga [3, 4]. A significant influence of complex ligand variation on the biokinetics of Me^{3+}-complexes could be observed [7]. In comparison to the citrate complex higher tumor/background ratios could be obtained for nitrilotriacetic acid (NTA) and hydoxyethylenediaminetriacetic acid (HEDTA) complexes [8]. On the basis of these results a new concept for radionuclide tumor therapy was evaluated [9]. The alpha-emitting ^{225}Ac seems to be a suitable candidate in this concept. It was the aim of this experiment, to obtain first information on the biological behaviour of ^{225}Ac in direct comparison with the lanthanides. As a standard for the heavy rare earth elements ^{169}Yb instead of our usual standard ^{167}Tm and ^{148m}Pm for the lighter rare earth elements were applied.

In this Experiment ^{225}Ac, ^{148m}Pm and ^{169}Yb in the chemical form of the corresponding citrate complexes were used. The ^{225}Ac as well as the ^{148m}Pm were produced via high energy proton-induced reaction on natural uranium. In this type of reaction the ^{225}Ra is produced as a spallation product. Simultaneously the neutron rich isotopes of the light rare earth elements are formed due to the high energy proton-induced fission reaction. Isotopically pure ^{225}Ac can be isolated from the radium fraction which will be obtained by coprecipitation with $BaSO_4$.

2. Experimental

Radionuclides

A target consisting of 27 g U_3O_8 was irradiated with the internal 650 MeV proton beam of the Dubna Phasotron for 22 hours, the beam intensity was 1.5 to 2 µA. After a cooling period of three days the target was dissolved in 5 M HNO_3 and the uranium was separated from the spallation and fission-product mixture by means of anion exchange chromatography using a Dowex1 × 8 column. 110 mg of barium carrier were added to the uranium free solution. By adding sulfuric acid the $BaSO_4$-precipitate was formed collecting all radium and the earth alkaline elements. The gammaspectroscopic analysis demonstrated that the rare earth elements as well as the ^{225}Ac were collected in the precipitate in >95% yield. This effect is known from the classical work of *Hahn* [10]. The $BaSO_4$ was transformed into the carbonate. In this step no losses of ^{225}Ac and rare earth elements could be observed. The activity of ^{225}Ac was determined to be 3.0 MBq (EOB + 10 d).

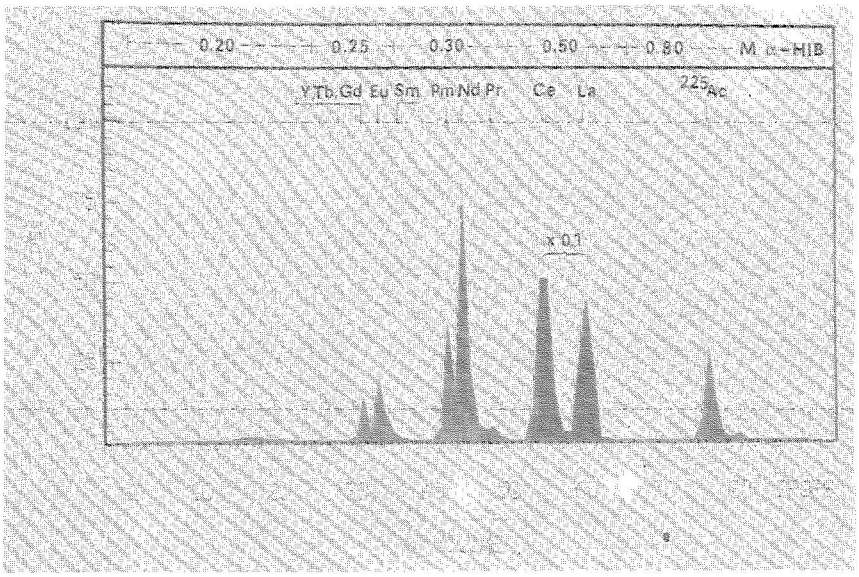

Fig. 1. Radio-chromatogram of the ^{225}Ac separation from a product mixture formed in high energy proton-induced spallation and fission reaction on natural uranium. A cation exchange column (3 · 70 mm²) filled with Aminex A5 was used. The gradient of α-HIB concentration (*p*H = 5) is given at the top of the figure. Single drop fractions were measured using a standard NaI(Tl)-scintillation counter one hour after elution to allow the gamma emitting daughter isotopes ^{221}Fr and ^{213}Bi to grow up. The second small peak to the right of the actinium main peak is induced due to the formation of ^{221}Fr daughter while eluting the column. For information on the separation of radium and barium from the target see text.

The $BaCO_3$ was shipped to the CINR Rossendorf and dissolved in 4 ml 1 M HCl. 3 mg of luthetium carrier were added and a $Lu(OH)_3$ precipitate was formed by adding an excess of concentrated NH_3 solution. After centrifugation all rare earth elements and the ^{225}Ac were collected in the precipitate. The ^{140}Ba and radium (mass numbers 225 and 223 could be identified) remain completely in solution. The hydroxide precipitate was washed with water and then dissolved in a few drops of 0.1 M HCl. A traditional cation exchange chromatography was performed using an Aminex A5 column (3 × 70 mm²) and α-hydroxy isobutyric acid (*p*H = 5) as elution agent. The chromatographical conditions are shown in Fig. 1. The main products were ^{141}Ce and ^{140}La, useful fractions of ^{148m}Pm, ^{147}Nd and others could be separated. A well-separated peak of ^{225}Ac was eluted using 0.8 M α-HIB. A total activity of 0.25 MBq of ^{225}Ac was obtained 45 d after EOB. The activity of the ^{148m}Pm and ^{147}Nd-fractions was approximately 0.12 MBq and 0.5 MBq at this time respectively.

After a grow-up period of 3 weeks a second separation was performed. 3 mg luthetium carrier was added to the barium–radium stock solution, which was acidified by adding the required amount of HCl. The hydroxide precipitate contained ^{225}Ac and ^{140}La formed only from the decay of the corresponding mother isotopes. The corresponding radio-chromatogram is shown in Fig. 2, demonstrating, that the first Me^{3+}/Me^{2+}-separation was almost complete.

Both ^{225}Ac preparations were used in animal experiments, the first preparation in normal rats, and the second sample in tumor-bearing mice.

Since our usual reference radionuclide ^{167}Tm was not available this time, we had to replace it by ^{169}Yb as a representative of the heavy rare earth

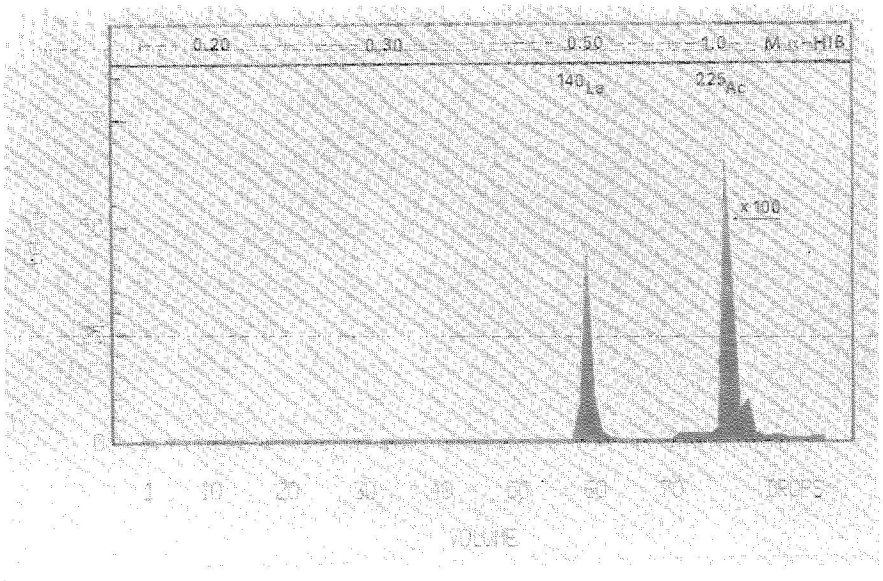

Fig. 2. Second radio-chromatogram obtained after three weeks grow-up period. From the radium–barium stock solution a second hydroxide precipitate was obtained using 3 mg of luthetium carrier. Since all Me^{3+}-ions were separated in the first hydroxide precipitate (see Fig. 1) only the daughter nuclides from the radioactive decay of radium barium isotopes could be detected.

elements in order to simulate the behaviour of thulium. The ^{169}Yb used in this experiment is commercially available from our (ROTOP) routine isotope production.

A mixture of the three radioisotopes was prepared with an activity ratio of ^{225}Ac:^{169}Yb:^{148}Pm = 1:3:0.3. The injection solution was isotonic Na-citrate, the *p*H was adjusted to 6.5.

Animals

Male normal Wistar rats (weight 445 g) and tumor-bearing female C_3H SPF mice (weight 25 g) were used. The mixture of citrate complexes was injected into the rats i.v. through a catheter. In the case of the tumor-bearing mice the preparation was injected intraperitoneally (i.p.). In all cases the animals were sacrificed 5 h p.i. From former studies we learned that 5 hours are sufficient to establish near equilibrium conditions in radioactivity distribution for rare earth citrate complexes in animals. The total injected volume per animal was 0.1 ml, the total injected amount of ^{225}Ac was 40 kBq (1 μCi).

Measurement

The contents of all three radioisotopes in a number of organs were measured using a high resolution Ge(Li)-spectrometer. This measurement was performed one day after organ sampling for establishing the radioactive decay equilibrium of the decay chain for ^{225}Ac. The nuclear data used in this experiment are summarized in Tab. 1. The usual data acquisition time was 0.5–2 hours because of the low radioactivity applied per animal. Therefore only the most important and significant organs could be measured.

Tab. 1. Nuclear Data used in this experiment taken from [6]

nuclide	$t_{1/2}$	Ey	abundancy
225-Ac	10.0 d	daughter radiation	
221-Fr	4.8 min	217.6 keV	12.5%
213-Bi	45.65 min	440.0 keV	27.3%
148m-Pm	41.3 d	550.2 keV	91.0%
		629.9 keV	87.0%
		725.7 keV	32.0%
169-Yb	30.7 d	130.5 keV	11.5%
		177.2 keV	22.0%
		198.0 keV	36.0%
		307.7 keV	11.1%

3. Results and discussion

The results of these experiments are summarized in Tab. 2. In order to illustrate the differences in the biodistribution of the three trace elements used we demonstrate the main characterizing segments from the organ measurements. A marked change in the isotopic composition can be seen (Fig. 3).

Tab. 2. Organ-distribution and ratios of radioactivity concentration of ^{225}Ac, ^{148}Pm and ^{169}Yb injected as citrate complexes in Wistar rats (upper part of the Tab.) and tumor-bearing mice (lower part of the Tab.) 5 h p.i.
Animals: Wistar rats, male, 440 g each i.v. injection tumor-bearing mice, female C_3H strain, 25 g each, i.p. injection, mama adenocarcinoma implanted
(*) urine data expressed in [%] only

animal	organ	[%/g tissue]			ratio	
		Ac	Pm	Yb	Ac/Yb	Pm/Yb
rats	blood	0.06	0.07	0.30	0.20	0.23
	liver	5.7	5.2	0.88	6.5	5.9
	femur	1.18	1.26	2.12	0.55	0.60
	urine (*)	2.0	10.9	11.8	0.17	0.93
mice	blood	1.2	0.7	4.0	0.29	0.18
	liver	38.7	30.9	5.3	7.3	5.8
	femur	16.8	16.3	23.4	0.72	0.70
	tumor	3.54	2.56	5.38	0.62	0.51
	urine (*)	0.4	5.1	5.4	0.05	0.7

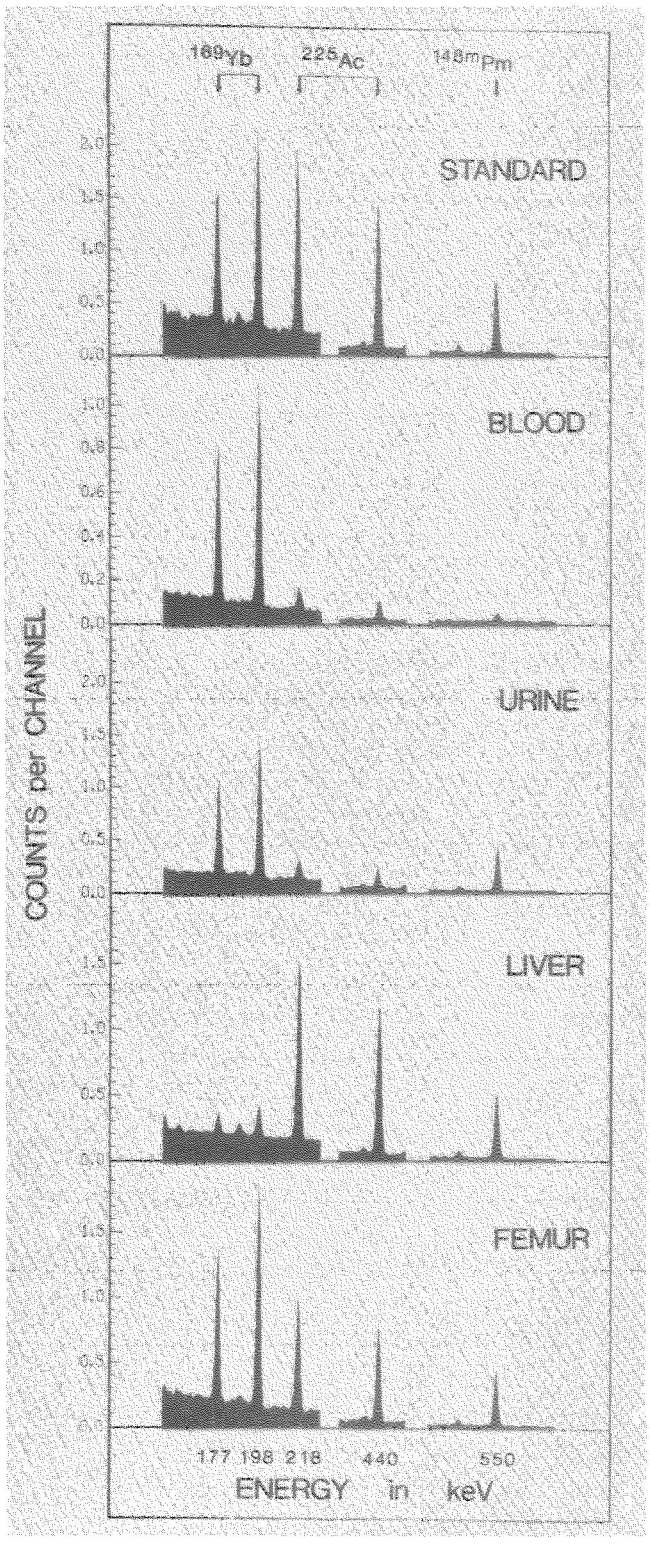

Fig. 3. Most important segments of gamma ray spectra obtained in gamma-spectroscopic measurements of the organ samples of the Wistar rats. The differences in the intensity of the gamma lines illustrate the sensitivity in this simultaneous study.

For the rats, the expected behaviour for Yb-citrate could be confirmed: fast blood clearance, high bone uptake, low liver uptake and the urine excretion. The bone uptake seemed to be smaller (by a factor of 2), but note that the animals used in these experiments were of double the weight compared to the young Wistar rats (weight 220 g) used in the former study [2].

The behaviour of promethium is in good agreement with the former simultaneous study [2]: faster blood clearance, significant higher liver uptake and decreased bone uptake of ^{148m}Pm compared to the heavy rare earth elements.

The behaviour of actinium is very different from the behaviour of ytterbium. The blood clearance is much faster and the liver uptake significantly higher. The urine excretion is negligibly small. The absolute liver uptake for this kinds of animals was 70%!

For the tumor-bearing mice (in contrast to the rats) the radioactivity preparation was injected not i.v. but i.p. The biodistribution of ytterbium corresponds well with our former results: high tumor uptake (5%/g), similar liver uptake (5%/g) and high bone uptake (23%/g). However, the ytterbium concentration in blood (4%/g) seemed to be rather high at 5 hours p.i., the urine excretion was accordingly low. This behaviour is not in agreement with our former studies, where i.v. injection was applied.

The behaviour of promethium is in all parameters in good agreement with our former study: high liver uptake, reduced tumor and bone uptake compared to ytterbium

For actinium a similar behaviour can be seen. The liver uptake is higher compared to promethium, but the bone uptake is of the same order and tumor uptake is increased compared to promethium. The urine excretion is negligibly small.

4. Conclusion

A first conclusion from the results is, that the biological behaviour of actinium, applied as citrate complex, is very different from the behaviour of the heavy rare earth elements such as thulium or ytterbium and also yttrium [4]. On the other hand the behaviour is more comparable to the biokinetics of the light rare earth elements. This result is in good agreement with the radius alteration of the Me^{3+}-ions.

The observed high liver uptake of ^{225}Ac and the lighter lanthanides seemed to be caused by the well known lower stability of citrate and other complexes of these elements resulting in an increased formation of hydrolized species. The observed behaviour suggests a common mechanism of the lanthanide biodistribution without any particular interaction of a single element of the lanthanide group [2]. The ionic radius of Ac^{3+} is 1.12 Å, which is much higher than for Pm^{3+} (0.968 Å) [5]. Consequently the liver uptake should be even higher for actinium compared to promethium. On the other hand a decreased tumor uptake should be expected. We conclude that the change in application technique is the reason for the increased tumoral and femural accumulation of actinium for the tumor-bearing mice.

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