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Multitracer screening: Brain delivery of trace elements by eight different administration methods

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Abstract

Trace elements are closely associated with the normal functioning of the brain. Therefore, it is important to determine how trace elements enter, accumulate, and are retained in the brain. Using the multitracer technique, which allows simultaneous tracing of many elements and comparison of their behavior under identical experimental conditions, we examined the influence of different administration methods, i.e., intravenous (IV), intraperitoneal (IP), intramuscular (IM), subcutaneous (SC), intracutaneous (IC), intranasal (IN), peroral (PO), and percutaneous (PC) administration, on the uptake of trace elements. A multitracer solution containing 16 radionuclides (i.e., ^{7}Be, ^{46}Sc, ^{48}V, ^{51}Cr, ^{54}Mn, ^{59}Fe, ^{56}Co, ^{65}Zn, ^{74}As, ^{75}Se, ^{83}Rb, ^{85}Sr, ^{88}Y, ^{88}Zr, ^{95m}Tc, and ^{103}Ru) was used. The results indicated that the ^{83}Rb brain uptake rate with intranasal administration was approximately twice those obtained with the other administration methods. This result indicated that a portion of Rb was delivered into the brain circumventing the blood circulation and that delivery could be accomplished mainly by olfactory transport. Multitracer screening of trace element delivery revealed differences in brain uptake pathways among administration methods.

Introduction

To sustain normal functions, homeostasis of the brain is strictly maintained by the blood–brain barrier, which is composed of endothelial cells of the central capillary. This barrier protects the brain from harmful substances and regulates the exchange of biologically important substances (Pollay & Roberts 1980). The exchange of essential trace elements is also strictly regulated. Therefore, it is important to examine and discuss how they enter, accumulate, and are retained in the brain from the extracranial environment. The type of biological system used for trace-element delivery depends on the entry pathway of the elements. Therefore, it may alter the transport velocity and uptake rate of the elements. However, the influence of the delivery pathway during the transport and uptake of trace elements has not been examined in detail.

Simultaneous determination of a whole range of trace elements can be achieved by a multi-element method, such as the radioactive multitracer technique developed at RIKEN (Ambe *et al.* 1991a, b). This technique uses a large number of radioactive tracers produced from a metal target, such as Ag or Au foil, irradiated with high-energy heavy ions, which enables the simultaneous evaluation of behavior for a large number of elements under identical conditions. The technique has been applied successfully in various fields and has been shown to be effective in studying the metabolism of trace elements in biological systems (Ambe *et al.* 1995; Ambe 1996; Enomoto & Hirunuma 2001; Nayak 2001). Using the multitracer technique, Gouthu *et al.* studied the subcellular

Table 1. Specification of the multitracer solution in one administration (25 μl).

Tracer	Half-Life (days)	γ-Energy* (keV)	Intensity* (%)	Activity (kBq)	Mass (pg)	Concentration (nmol/ml)	Chemical form**
^{7}Be	53.1	477.6	10.5	2.64	0.20	1.16	Be^{2+}
^{46}Sc	83.8	1120.6	100	0.18	0.15	0.13	Sc^{3+}
^{48}V	16.0	983.5	100	0.33	0.05	0.04	VO^{2+}, VO_2^{2+}
^{51}Cr	27.7	320.1	9.9	0.82	0.24	0.19	Cr^{3+}
^{54}Mn	312.3	834.9	100	0.43	1.49	1.11	Mn^{2+}
^{56}Co	77.3	1238.3	67.6	0.12	0.11	0.08	Co^{2+}
^{59}Fe	44.5	1099.3	56.5	0.08	0.04	0.03	Fe^{3+}
^{65}Zn	244.3	1115.6	50.6	0.43	1.41	0.87	Zn^{2+}
^{74}As	17.8	595.8	59	0.33	0.09	0.05	AsO_3^-
^{75}Se	119.8	264.7	58.9	0.87	1.62	0.87	SeO_3^{2-}
^{83}Rb	86.2	552.6	16	2.45	3.63	1.75	Rb^+
^{85}Sr	64.8	514	96	3.19	3.65	1.72	Sr^{2+}
^{88}Y	106.7	1836.1	99.2	0.72	1.39	0.63	Y^{3+}
^{88}Zr	83.4	392.9	100	0.97	1.48	0.67	ZrO^{2+}
^{95m}Tc	61.0	204	63.2	0.17	0.20	0.08	TcO^{4-}
^{103}Ru	39.3	497	91	0.11	0.10	0.04	Ru^{3+}, Ru^{4+}

*The energy and intensity used for γ-ray spectrometry are shown.**Amano *et al.* (1996); Yanagiya *et al.* (2000).

distribution of trace elements in soybean (*Glycine max* Merr.) and cucumber (*Cucumis sativus* L.), and the translocation of plant-absorbed radioactive tracers with growth in soybean (Gouthu *et al.* 1999). Monitoring the uptake and translocation of tracers in plants should indicate how these elements enter the plant system and reach the edible parts. Ozaki *et al.* also applied the multitracer technique to examine the uptake mechanism of yttrium and rare earth elements by autumn fern (*Dryopteris erythrosora*) (Ozaki *et al.* 2002). Yanagiya *et al.* reported the reduction of Cd cytotoxicity by Mn. They established a cadmium-resistant cell line from immortalized metallothionein-null mouse cells, and found that these cells exhibited a marked decrease in Cd uptake. To investigate the mechanism of altered uptake of Cd, incorporation of various metals was determined simultaneously using the multitracer technique (Yanagiya *et al.* 2000). Nabekura *et al.* compared uptake of the multitracer in the lenses of normal and hereditary cataract UPL rats to investigate the mechanisms of trace element transport during cataract development (Nabekura *et al.* 2003).

Radioactive tracers in the multitracer are salt- and carrier-free, and are extremely useful for animal experiments because the *in vivo* behavior of elements can be traced without toxic effects on animals (Amano *et al.* 1996a, b; Enomoto *et al.* 1996; Yanaga *et al.* 1996; Hirunuma *et al.* 1997). In fact, using the multitracer technique, several researchers have examined the distribution behavior of trace elements in the brain, using intravenous, intraperitoneal, or peroral administration. Hirate *et al.* investigated the distribution of trace elements in the brain of mice with epileptic seizures by intravenous injection and discussed the alterations in brain homeostasis of Zn, Co, and Se associated with susceptibility, development, and termination of epileptic seizures (Hirate *et al.* 2002). Amano and Enomoto examined the brain regional uptake of trace elements in mice during aging following intraperitoneal injection and demonstrated the functional degenerative process of the blood–brain barrier (Amano & Enomoto 2001). To obtain better understanding of the functions of elements in the brain, it is necessary to examine the influence of different types of administration on brain uptake.

It is necessary to investigate several administration methods to analyze the brain uptake behavior of trace elements, to determine if indeed the brain uptake of trace elements is dependent on the entry pathway. Therefore, we examined the influence of method of administration on the uptake of trace elements. In the present study, the multitracer technique was applied to a massive screening study of the uptake behavior of trace elements using eight different administration methods.

Materials and methods

Preparation of multitracer solution

A multitracer was obtained from an Ag foil target irradiated with the 135 MeV/nucleon ^{14}N beam from the RIKEN Ring Cyclotron. Chemical separation was performed according to the method described previously (Ambe *et al*. 1991a, b, 1995). After cooling, the Ag target, which contained various kinds of radioisotopes, was dissolved in (1:1) HNO_3. The Ag was precipitated as AgCl with concentrated HCl, and the AgCl was filtered out completely. The filtrate was evaporated to dryness under reduced pressure and was finally dissolved in 0.9% NaCl solution as the multitracer solution for the animal experiments (pH 6–7). All chemicals used were of reagent grade and were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The Ag foil was 250 μm thick, measured 15×15 mm^2, and were of high purity (99.99%). The multitracer solution contained tracers of various elements: ^{7}Be, ^{46}Sc, ^{48}V, ^{51}Cr, ^{54}Mn, ^{59}Fe, ^{56}Co, ^{65}Zn, ^{74}As, ^{75}Se, ^{83}Rb, ^{85}Sr, ^{95m}Tc, ^{88}Y, ^{88}Zr, and ^{103}Ru. The chemical species of these elements were as follows: Be^{2+}, Sc^{3+},

Table 2a. Uptake rates (%dose/g) of multitracers in blood, plasma, and brain following administration by 8 different methods.

Radionuclide	Time interval	^{7}Be			^{46}Sc		
Administration method		Blood	Plasma	Brain	Blood	Plasma	Brain
IV	2 min	5.618 ± 0.365	11.897 ± 2.350	0.026 ± 0.025	38.26 ± 5.70	73.56 ± 4.77	0.242 ± 0.090
	30 min	0.458 ± 0.119	0.603 ± 0.156	0.026 ± 0.019	32.68 ± 1.25	61.30 ± 1.43	0.175 ± 0.046
	3 h	0.183 ± 0.069	0.107 ± 0.044	0.024 ± 0.002	15.98 ± 2.15	28.63 ± 6.31	0.285 ± 0.074
	24 h	0.090 ± 0.004	0.013 ± 0.010	0.032 ± 0.009	2.72 ± 0.21	5.00 ± 0.13	0.540 ± 0.059
IP	5 min	1.287 ± 1.148	2.496 ± 2.233	0.008 ± 0.002	2.37 ± 2.28	4.02 ± 3.95	0.268 ± 0.059
	30 min	0.658 ± 0.603	1.194 ± 1.100	0.015 ± 0.010	2.91 ± 2.55	6.64 ± 5.87	0.090 ± 0.020
	3 h	0.124 ± 0.086	0.059 ± 0.091	0.019 ± 0.010	3.61 ± 5.25	7.70 ± 11.27	0.117 ± 0.056
	24 h	0.170 ± 0.035	0.010 ± 0.008	0.033 ± 0.042	2.60 ± 0.72	5.13 ± 1.43	0.272 ± 0.077
IM	5 min	0.491 ± 0.104	0.799 ± 0.353	0.007 ± 0.002	0.15 ± 0.07	0.29 ± 0.18	0.041 ± 0.013
	30 min	0.820 ± 0.171	1.001 ± 0.286	0.006 ± 0.003	1.01 ± 0.55	1.48 ± 0.47	0.045 ± 0.014
	3 h	0.292 ± 0.077	0.169 ± 0.075	0.021 ± 0.005	1.92 ± 0.46	3.74 ± 0.83	0.038 ± 0.015
	24 h	0.155 ± 0.058	0.028 ± 0.040	0.009 ± 0.005	1.30 ± 0.25	2.34 ± 0.20	0.092 ± 0.010
SC	30 min	0.610 ± 0.488	1.169 ± 1.002	0.017 ± 0.022	0.65 ± 0.77	1.21 ± 1.47	0.121 ± 0.027
	3 h	0.107 ± 0.089	0.159 ± 0.048	0.009 ± 0.006	1.91 ± 1.61	3.37 ± 2.45	0.116 ± 0.055
	24 h	0.097 ± 0.071	0.024 ± 0.009	0.037 ± 0.027	1.29 ± 0.67	2.23 ± 0.76	0.132 ± 0.095
IC	30 min	0.211 ± 0.068	0.310 ± 0.111	0.003 ± 0.003	0.09 ± 0.03	0.23 ± 0.07	0.006 ± 0.008
	3 h	0.162 ± 0.010	0.165 ± 0.012	0.007 ± 0.003	0.79 ± 0.07	1.44 ± 0.20	0.012 ± 0.006
	24 h	0.124 ± 0.026	0.014 ± 0.002	0.014 ± 0.007	1.23 ± 0.10	2.29 ± 0.30	0.041 ± 0.008
IN	30 min	0.081 ± 0.060	0.124 ± 0.105	0.008 ± 0.004	0.09 ± 0.09	0.14 ± 0.04	0.048 ± 0.002
	90 min	0.069 ± 0.033	0.064 ± 0.015	0.004 ± 0.005	0.10 ± 0.08	0.27 ± 0.11	0.029 ± 0.012
	3 h	0.058 ± 0.032	0.046 ± 0.043	0.006 ± 0.002	0.13 ± 0.08	0.31 ± 0.15	0.025 ± 0.005
	24 h	0.046 ± 0.038	0.017 ± 0.013	0.009 ± 0.007	0.08 ± 0.01	0.12 ± 0.07	0.041 ± 0.013
PO	30 min	0.039 ± 0.011	0.006 ± 0.005	0.043 ± 0.027	0.28 ± 0.13	0.20 ± 0.02	0.313 ± 0.026
	90 min	0.020 ± 0.022	0.037 ± 0.038	0.034 ± 0.026	0.31 ± 0.10	0.22 ± 0.18	0.247 ± 0.062
	3 h	0.028 ± 0.011	0.009 ± 0.001	0.028 ± 0.022	0.24 ± 0.17	0.29 ± 0.10	0.166 ± 0.067
	24 h	0.031 ± 0.024	0.015 ± 0.015	0.061 ± 0.082	0.39 ± 0.24	0.44 ± 0.13	0.106 ± 0.021
PC	3 h	0.030 ± 0.022	0.015 ± 0.010	0.017 ± 0.016	0.16 ± 0.05	0.24 ± 0.08	0.113 ± 0.011
	24 h	0.003 ± 0.003	0.021 ± 0.007	0.006 ± 0.004	0.13 ± 0.06	0.14 ± 0.05	0.095 ± 0.011

Abbreviations used: IV, intravenous; IP, intraperitoneal; IM, intramusuclar; SC, subcutaneous; IC, intracutaneous; IN, intranasal; PO, peroral; PC, percutaneous administration. Data are presented as mean ± SD of three mice.

Table 2b. Uptake rates (%dose/g) of multitracers in blood, plasma, and brain following administration by 8 different methods.

Radionuclide	Time interval	^{48}V			^{51}Cr		
Administration method		Blood	Plasma	Brain	Blood	Plasma	Brain
IV	2 min	7.03 ± 1.60	11.55 ± 1.22	0.020 ± 0.027	14.38 ± 1.53	32.63 ± 0.25	0.059 ± 0.042
	30 min	4.10 ± 1.60	5.89 ± 2.04	0.023 ± 0.020	10.23 ± 1.58	20.62 ± 3.65	0.095 ± 0.018
	3 h	3.13 ± 0.36	5.22 ± 0.81	0.060 ± 0.031	6.98 ± 0.93	13.83 ± 2.20	0.088 ± 0.052
	24 h	0.56 ± 0.11	1.27 ± 0.09	0.026 ± 0.016	1.46 ± 0.30	3.05 ± 0.61	0.044 ± 0.030
IP	5 min	4.77 ± 4.37	5.52 ± 5.13	0.010 ± 0.005	1.22 ± 1.04	2.66 ± 2.32	0.017 ± 0.023
	30 min	2.10 ± 1.82	2.52 ± 2.28	0.009 ± 0.005	2.76 ± 2.58	5.56 ± 5.17	0.017 ± 0.015
	3 h	1.66 ± 2.00	2.11 ± 3.41	0.018 ± 0.014	2.85 ± 3.91	4.69 ± 7.25	0.023 ± 0.022
	24 h	0.87 ± 0.30	1.48 ± 0.91	0.027 ± 0.025	1.21 ± 0.23	2.22 ± 0.45	0.018 ± 0.012
IM	5 min	2.42 ± 0.47	2.97 ± 1.41	0.005 ± 0.004	1.74 ± 0.69	3.01 ± 0.74	0.010 ± 0.004
	30 min	3.51 ± 1.09	4.93 ± 1.87	.0010 ± 0.010	4.29 ± 0.88	6.73 ± 0.46	0.012 ± 0.014
	3 h	2.10 ± 0.65	3.38 ± 1.30	0.023 ± 0.019	4.08 ± 0.59	6.64 ± 0.72	0.023 ± 0.008
	24 h	0.56 ± 0.40	0.99 ± 0.71	0.014 ± 0.013	1.14 ± 0.28	1.91 ± 0.24	0.026 ± 0.032
SC	30 min	7.95 ± 0.48	10.29 ± 1.24	0.036 ± 0.034	3.46 ± 1.74	5.40 ± 3.10	0.064 ± 0.047
	3 h	2.57 ± 0.70	3.48 ± 0.09	0.007 ± 0.006	3.09 ± 1.40	4.75 ± 1.46	0.019 ± 0.010
	24 h	1.19 ± 0.36	2.09 ± 0.47	0.073 ± 0.054	1.84 ± 0.46	2.85 ± 0.49	0.122 ± 0.123
IC	30 min	3.23 ± 1.44	3.81 ± 1.99	0.013 ± 0.011	1.20 ± 0.44	2.19 ± 0.75	0.011 ± 0.009
	3 h	2.30 ± 0.49	2.88 ± 1.04	0.017 ± 0.013	2.29 ± 0.35	3.94 ± 0.88	0.005 ± 0.003
	24 h	1.02 ± 0.24	1.29 ± 0.22	0.038 ± 0.015	1.46 ± 0.31	2.17 ± 0.32	0.010 ± 0.009
IN	30 min	0.89 ± 0.25	1.42 ± 0.40	0.026 ± 0.009	0.18 ± 0.10	0.49 ± 0.17	0.034 ± 0.014
	90 min	0.36 ± 0.08	0.29 ± 0.24	0.004 ± 0.003	0.34 ± 0.30	0.33 ± 0.11	0.012 ± 0.010
	3 h	0.80 ± 0.21	0.84 ± 0.54	0.007 ± 0.008	0.77 ± 0.18	0.86 ± 0.33	0.011 ± 0.009
	24 h	0.41 ± 0.22	0.58 ± 0.03	0.011 ± 0.004	0.08 ± 0.05	0.15 ± 0.03	0.046 ± 0.069
PO	30 min	0.03 ± 0.03	0.04 ± 0.04	0.010 ± 0.008	0.03 ± 0.03	0.06 ± 0.04	0.034 ± 0.025
	90 min	0.01 ± 0.02	0.01 ± 0.02	0.012 ± 0.010	0.08 ± 0.04	0.05 ± 0.03	0.037 ± 0.012
	3 h	0.09 ± 0.04	0.08 ± 0.06	0.010 ± 0.014	0.08 ± 0.02	0.11 ± 0.03	0.010 ± 0.007
	24 h	0.05 ± 0.06	0.04 ± 0.05	0.019 ± 0.009	0.06 ± 0.02	0.06 ± 0.03	0.069 ± 0.008
PC	3 h	0.78 ± 0.30	1.00 ± 0.44	0.012 ± 0.006	0.46 ± 0.37	0.64 ± 0.38	0.027 ± 0.019
	24 h	0.15 ± 0.13	0.32 ± 0.27	0.005 ± 0.006	0.08 ± 0.07	0.12 ± 0.11	0.013 ± 0.010

Abbreviations used: IV, intravenous; IP, intraperitoneal; IM, intramusuclar; SC, subcutaneous; IC, intracutaneous; IN, intranasal; PO, peroral; PC, percutaneous administration. Data are presented as mean ± SD of three mice.

VO^{2+}, VO_2^{2+}, Cr^{3+}, Mn^{2+}, Fe^{3+}, Co^{2+}, Zn^{2+}, AsO_3^-, SeO_3^{2-}, Rb^+, Sr^{2+}, Y^{3+}, ZrO^{2+}, TcO_4^-, Ru^{3+}, and Ru^{4+} (Amano *et al.*1996a, b; Yanagiya *et al.* 2000). The half-life, energy of measured γ-rays, activity, and calculated mass of the tracers contained in an administered volume of the multitracer solution are listed in Table 1. Although the impurities of the target and chemical contaminants from the reagents were not checked, the multitracer solution was prepared essentially under carrier-free conditions and no carriers were added during the subsequent screening experiments. Using ICP-MS, the mass of stable isotopes in 25 μl of the multitracer solution were estimated at levels less than the calculated mass shown in Table 1. Each isotope in the multitracer behaved independently *in vivo* and showed the characteristic biobehavior of the element.

Animal experiments

All animal experiments were carried out in compliance with the guidelines for the care and use of laboratory animals as approved by the Committee on Animal Experimentation of Kanazawa University.

Table 2c. Uptake rates (%dose/g) of multitracers in blood, plasma, and brain following administration by 8 different methods.

Radionuclide	Time interval	^{54}Mn			^{56}Co		
Administration method		Blood	Plasma	Brain	Blood	Plasma	Brain
IV	2 min	3.30 ± 0.89	5.13 ± 1.45	0.425 ± 0.039	5.57 ± 0.43	10.04 ± 0.67	0.173 ± 0.040
	30 min	1.59 ± 1.16	2.20 ± 1.82	0.316 ± 0.041	3.18 ± 0.77	4.41 ± 0.67	0.184 ± 0.065
	3 h	2.57 ± 1.29	4.41 ± 2.47	0.325 ± 0.047	2.06 ± 0.52	2.76 ± 0.43	0.124 ± 0.018
	24 h	0.88 ± 0.48	1.69 ± 0.86	0.445 ± 0.080	0.54 ± 0.23	0.87 ± 0.30	0.239 ± 0.067
IP	5 min	2.96 ± 2.81	2.97 ± 2.91	0.038 ± 0.036	1.48 ± 1.15	2.43 ± 2.02	0.071 ± 0.025
	30 min	0.77 ± 0.69	0.95 ± 0.84	0.033 ± 0.027	2.00 ± 1.71	5.03 ± 4.25	0.106 ± 0.016
	3 h	3.59 ± 2.66	2.11 ± 3.18	0.064 ± 0.052	0.97 ± 1.21	2.23 ± 2.89	0.076 ± 0.022
	24 h	1.50 ± 0.99	2.50 ± 2.01	0.252 ± 0.208	0.94 ± 0.26	2.14 ± 0.43	0.113 ± 0.031
IM	5 min	2.07 ± 0.77	2.87 ± 1.50	0.156 ± 0.004	2.73 ± 0.40	5.67 ± 0.84	0.060 ± 0.044
	30 min	0.71 ± 0.53	0.78 ± 0.68	0.259 ± 0.005	2.81 ± 0.35	4.84 ± 0.35	0.097 ± 0.011
	3 h	2.29 ± 1.25	3.99 ± 2.49	0.262 ± 0.058	2.13 ± 0.77	4.05 ± 0.82	0.085 ± 0.040
	24 h	0.66 ± 0.72	1.14 ± 1.42	0.335 ± 0.051	0.59 ± 0.23	1.42 ± 0.48	0.074 ± 0.020
SC	30 min	0.84 ± 0.24	1.02 ± 0.97	0.330 ± 0.093	2.77 ± 0.81	5.11 ± 1.81	0.152 ± 0.045
	3 h	1.74 ± 0.63	2.69 ± 1.59	0.314 ± 0.094	2.13 ± 0.38	3.92 ± 0.11	0.123 ± 0.098
	24 h	0.81 ± 0.24	1.24 ± 0.57	0.384 ± 0.098	1.04 ± 0.40	1.56 ± 0.33	0.187 ± 0.030
IC	30 min	0.54 ± 0.11	0.39 ± 0.08	0.304 ± 0.023	2.42 ± 0.23	4.34 ± 0.39	0.065 ± 0.023
	3 h	1.03 ± 0.31	1.05 ± 0.01	0.254 ± 0.166	2.07 ± 0.25	3.87 ± 0.21	0.062 ± 0.031
	24 h	0.60 ± 0.17	0.54 ± 0.15	0.461 ± 0.078	1.23 ± 0.16	2.18 ± 0.19	0.076 ± 0.013
IN	30 min	0.96 ± 0.14	0.76 ± 0.16	0.081 ± 0.041	0.95 ± 0.30	1.61 ± 0.39	0.044 ± 0.015
	90 min	0.52 ± 0.30	0.36 ± 0.52	0.023 ± 0.006	0.52 ± 0.19	0.83 ± 0.16	0.045 ± 0.007
	3 h	1.51 ± 0.10	1.22 ± 1.14	0.026 ± 0.017	0.78 ± 0.28	1.20 ± 0.18	0.067 ± 0.015
	24 h	1.43 ± 0.96	2.10 ± 0.30	0.139 ± 0.057	0.38 ± 0.30	0.59 ± 0.01	0.029 ± 0.011
PO	30 min	0.31 ± 0.12	0.12 ± 0.14	0.025 ± 0.016	0.33 ± 0.14	0.40 ± 0.16	0.089 ± 0.080
	90 min	0.48 ± 0.35	0.60 ± 0.24	0.010 ± 0.008	0.15 ± 0.07	0.40 ± 0.05	0.129 ± 0.083
	3 h	1.73 ± 1.17	1.83 ± 0.31	0.025 ± 0.003	0.33 ± 0.03	0.35 ± 0.33	0.216 ± 0.055
	24 h	1.88 ± 0.60	1.41 ± 0.95	0.096 ± 0.067	0.28 ± 0.06	0.21 ± 0.19	0.164 ± 0.102
PC	3 h	0.82 ± 0.40	0.94 ± 0.41	0.198 ± 0.251	0.94 ± 0.27	1.68 ± 0.35	0.193 ± 0.085
	24 h	0.71 ± 0.30	0.81 ± 0.13	0.069 ± 0.043	0.37 ± 0.23	0.51 ± 0.32	0.073 ± 0.068

Abbreviations used: IV, intravenous; IP, intraperitoneal; IM, intramusuclar; SC, subcutaneous; IC, intracutaneous; IN, intranasal; PO, peroral; PC, percutaneous administration. Data are presented as mean ± SD of three mice.

Eighty-four 8-week-old ICR mice (purchased from Charles River Japan, Inc., Yokohama, Japan), weighing 32.8 ± 1.7 g, were divided into eight groups, and administered the multitracer solution by eight different methods: intravenous (IV), intraperitoneal (IP), intramuscular (IM), subcutaneous (SC), intracutaneous (IC), intranasal (IN), peroral (PO), and percutaneous (PC) administration. The administration–dissection time interval differed across the methods. In the IV group, the mice were sacrificed at intervals of 2 min, 30 min, 3 h, and 24 h following administration; in the IP and IM groups, 5 min, 30 min, 3 h, and 24 h; in the SC and IC groups, 30 min, 3 h, and 24 h; in the IN and PO groups, 30 min, 90 min, 3 h, and 24 h; and in the PC group, 3 h and 24 h (n = 3 mice/time point). The volume of solution administered was also different across the methods: the IV, IP, and PO groups were administered 0.1 ml of the multitracer solution, while the other groups received 25 μl.

The detailed procedures of IN, PO, and PC administration were as follows. In the IN group, the mice were first anesthetized with thiopental sodium (Ravonal; Tanabe Seiyaku Co., Ltd., Osaka, Japan) in the supine position. The multitracer

Table 2d. Uptake rates (%dose/g) of multitracers in blood, plasma, and brain following administration by 8 different methods.

Radionuclide	Time interval	^{59}Fe			^{65}Zn		
Administration method		Blood	Plasma	Brain	Blood	Plasma	Brain
IV	2 min	27.06 ± 1.08	62.23 ± 0.34	0.109 ± 0.084	10.86 ± 0.80	19.89 ± 2.50	0.376 ± 0.016
	30 min	19.31 ± 5.24	39.14 ± 9.48	0.140 ± 0.115	1.17 ± 0.37	1.80 ± 0.54	0.614 ± 0.059
	3 h	7.07 ± 0.47	8.27 ± 2.87	0.102 ± 0.102	0.98 ± 0.08	0.96 ± 0.10	0.680 ± 0.126
	24 h	12.53 ± 0.91	0.35 ± 0.26	0.239 ± 0.212	0.77 ± 0.05	0.32 ± 0.06	1.053 ± 0.106
IP	5 min	0.39 ± 0.37	1.61 ± 1.21	0.027 ± 0.020	0.45 ± 0.36	1.30 ± 1.08	0.054 ± 0.029
	30 min	3.85 ± 3.38	5.51 ± 4.80	0.039 ± 0.022	0.91 ± 0.75	1.63 ± 1.37	0.136 ± 0.108
	3 h	2.57 ± 3.48	2.52 ± 3.70	0.065 ± 0.048	0.41 ± 0.26	0.33 ± 0.21	0.194 ± 0.138
	24 h	11.03 ± 1.97	0.27 ± 0.17	0.109 ± 0.083	0.73 ± 0.26	0.29 ± 0.03	0.869 ± 0.161
IM	5 min	0.21 ± 0.18	0.32 ± 0.42	0.030 ± 0.041	2.44 ± 0.33	4.32 ± 1.95	0.064 ± 0.027
	30 min	1.52 ± 0.25	2.26 ± 0.90	0.059 ± 0.042	2.14 ± 0.25	3.30 ± 0.51	0.298 ± 0.050
	3 h	2.33 ± 0.72	2.78 ± 0.26	0.049 ± 0.034	0.79 ± 0.18	0.84 ± 0.21	0.495 ± 0.078
	24 h	4.84 ± 0.83	0.40 ± 0.08	0.051 ± 0.019	0.78 ± 0.03	0.39 ± 0.10	0.947 ± 0.064
SC	30 min	0.97 ± 0.83	2.20 ± 3.75	0.070 ± 0.062	1.31 ± 0.53	2.77 ± 0.45	0.156 ± 0.007
	3 h	2.44 ± 2.45	3.37 ± 2.57	0.146 ± 0.130	0.61 ± 0.14	0.65 ± 0.24	0.395 ± 0.064
	24 h	4.30 ± 2.25	0.18 ± 0.10	0.072 ± 0.043	0.69 ± 0.07	0.30 ± 0.03	0.762 ± 0.099
IC	30 min	0.23 ± 0.09	0.60 ± 0.20	0.011 ± 0.008	1.13 ± 0.14	1.95 ± 0.29	0.152 ± 0.027
	3 h	1.05 ± 0.22	1.64 ± 0.05	0.026 ± 0.021	0.75 ± 0.05	0.83 ± 0.14	0.323 ± 0.222
	24 h	2.82 ± 0.46	0.23 ± 0.05	0.071 ± 0.034	0.67 ± 0.04	0.28 ± 0.00	0.911 ± 0.019
IN	30 min	0.34 ± 0.36	0.44 ± 0.40	0.023 ± 0.009	0.37 ± 0.04	0.55 ± 0.09	0.075 ± 0.028
	90 min	0.46 ± 0.25	0.39 ± 0.05	0.040 ± 0.033	0.28 ± 0.04	0.37 ± 0.06	0.044 ± 0.011
	3 h	0.53 ± 0.57	0.77 ± 0.22	0.041 ± 0.032	0.26 ± 0.06	0.37 ± 0.07	0.077 ± 0.009
	24 h	0.85 ± 0.20	0.18 ± 0.16	0.065 ± 0.028	0.28 ± 0.05	0.17 ± 0.04	0.279 ± 0.018
PO	30 min	1.15 ± 1.02	2.04 ± 1.92	0.118 ± 0.062	0.02 ± 0.01	0.05 ± 0.05	0.031 ± 0.033
	90 min	0.68 ± 0.44	0.98 ± 0.61	0.110 ± 0.097	0.03 ± 0.04	0.03 ± 0.01	0.021 ± 0.032
	3 h	1.71 ± 1.89	2.73 ± 2.43	0.227 ± 0.110	0.03 ± 0.02	0.09 ± 0.05	0.050 ± 0.046
	24 h	5.77 ± 7.89	0.14 ± 0.17	0.356 ± 0.152	0.13 ± 0.12	0.07 ± 0.08	0.160 ± 0.186
PC	3 h	1.42 ± 1.33	1.88 ± 1.51	0.038 ± 0.008	0.13 ± 0.09	0.21 ± 0.10	0.186 ± 0.223
	24 h	0.80 ± 0.23	0.08 ± 0.02	0.033 ± 0.025	0.09 ± 0.04	0.06 ± 0.01	0.119 ± 0.028

Abbreviations used: IV, intravenous; IP, intraperitoneal; IM, intramusuclar; SC, subcutaneous; IC, intracutaneous; IN, intranasal; PO, peroral; PC, percutaneous administration. Data are presented as mean ± SD of three mice.

solution was instilled slowly into the nostrils of 12 mice using a micropipette. A total of 25 μl was administered over 5 min with a break of 2 min between instillations on the right and left sides (time intervals for distribution were measured immediately after the start of administration). In the PO group, the mice were not allowed to eat but were provided tap water *ad libitum* for 12 h prior to administration. Then, the multitracer solution was introduced perorally into the stomach using a sonde. I the PC group, to examine percutaneous absorption of the multitracer, the dorsal fur of the mice was shaved taking care not to scratch their skin one day before the experiment. Each mouse was anesthetized with thiopental sodium in the prone position during the examination. The multitracer solution was applied slowly onto the dorsal skin and air-dried. Then, Vaseline (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was applied to the same part of the skin. The mice were housed individually to avoid radioactivity cross-contamination until sacrifice. For the other administration methods, the multitracer was injected into appropriate parts of the body.

Table 2e. Uptake rates (%dose/g) of multitracers in blood, plasma, and brain following administration by 8 different methods.

Radionuclide	Time interval	^{74}As			^{75}Se		
Administration method		Blood	Plasma	Brain	Blood	Plasma	Brain
IV	2 min	4.33 ± 0.87	9.10 ± 1.07	0.073 ± 0.018	7.91 ± 2.52	10.66 ± 3.72	0.094 ± 0.016
	30 min	1.38 ± 0.55	1.39 ± 0.43	0.215 ± 0.066	4.43 ± 0.59	7.41 ± 1.58	0.087 ± 0.023
	3 h	0.24 ± 0.05	0.28 ± 0.11	0.368 ± 0.152	6.81 ± 0.47	12.03 ± 1.81	0.102 ± 0.020
	24 h	0.16 ± 0.10	0.02 ± 0.01	0.060 ± 0.033	2.87 ± 0.45	5.01 ± 1.08	0.198 ± 0.037
IP	5 min	3.02 ± 2.51	4.73 ± 3.82	0.020 ± 0.016	2.39 ± 2.23	3.56 ± 3.27	0.028 ± 0.027
	30 min	0.76 ± 0.68	1.01 ± 0.95	0.063 ± 0.055	1.68 ± 1.43	2.34 ± 1.98	0.033 ± 0.029
	3 h	0.20 ± 0.03	0.10 ± 0.06	0.375 ± 0.131	5.09 ± 0.84	9.02 ± 1.80	0.082 ± 0.052
	24 h	0.03 ± 0.01	0.02 ± 0.01	0.021 ± 0.013	2.93 ± 0.59	5.11 ± 1.11	0.184 ± 0.027
IM	5 min	1.22 ± 0.25	2.44 ± 0.68	0.017 ± 0.015	6.99 ± 0.31	9.65 ± 1.01	0.043 ± 0.009
	30 min	0.89 ± 0.20	0.86 ± 0.13	0.084 ± 0.019	2.98 ± 0.24	3.34 ± 0.38	0.062 ± 0.003
	3 h	0.17 ± 0.14	0.29 ± 0.08	0.143 ± 0.050	6.69 ± 0.71	11.79 ± 1.28	0.106 ± 0.031
	24 h	0.04 ± 0.02	0.01 ± 0.01	0.019 ± 0.016	2.86 ± 0.77	4.72 ± 1.22	0.163 ± 0.018
SC	30 min	1.49 ± 0.43	2.12 ± 0.12	0.145 ± 0.056	1.88 ± 0.25	2.23 ± 0.79	0.082 ± 0.019
	3 h	0.28 ± 0.09	0.17 ± 0.03	0.223 ± 0.032	5.76 ± 0.87	9.98 ± 2.26	0.099 ± 0.021
	24 h	0.24 ± 0.36	0.06 ± 0.04	0.078 ± 0.054	2.66 ± 0.23	4.04 ± 0.53	0.139 ± 0.013
IC	30 min	0.96 ± 0.11	0.90 ± 0.09	0.058 ± 0.020	2.43 ± 0.21	2.51 ± 0.44	0.068 ± 0.006
	3 h	0.23 ± 0.10	0.15 ± 0.05	0.217 ± 0.045	5.80 ± 0.30	9.86 ± 0.46	0.081 ± 0.042
	24 h	0.02 ± 0.01	0.03 ± 0.01	0.019 ± 0.015	2.71 ± 0.56	4.34 ± 0.71	0.165 ± 0.009
IN	30 min	0.92 ± 0.22	0.76 ± 0.29	0.085 ± 0.037	0.64 ± 0.20	0.81 ± 0.25	0.050 ± 0.018
	90 min	0.44 ± 0.14	0.28 ± 0.23	0.029 ± 0.009	0.88 ± 0.04	1.18 ± 0.06	0.026 ± 0.009
	3 h	0.63 ± 0.13	0.41 ± 0.17	0.071 ± 0.043	1.93 ± 0.23	3.18 ± 0.56	0.031 ± 0.003
	24 h	0.05 ± 0.05	0.02 ± 0.01	0.031 ± 0.005	2.62 ± 0.36	4.59 ± 0.69	0.098 ± 0.017
PO	30 min	1.28 ± 0.31	0.66 ± 0.41	0.172 ± 0.057	0.29 ± 0.08	0.45 ± 0.23	0.011 ± 0.004
	90 min	0.65 ± 0.44	0.47 ± 0.29	0.322 ± 0.104	1.67 ± 0.85	3.14 ± 1.47	0.018 ± 0.005
	3 h	0.29 ± 0.12	0.16 ± 0.11	0.462 ± 0.075	3.03 ± 0.91	5.93 ± 1.81	0.023 ± 0.011
	24 h	0.04 ± 0.04	0.02 ± 0.01	0.088 ± 0.089	2.31 ± 0.50	3.88 ± 0.49	0.113 ± 0.015
PC	3 h	0.56 ± 0.23	0.48 ± 0.10	0.353 ± 0.123	2.66 ± 0.94	4.80 ± 1.76	0.041 ± 0.034
	24 h	0.06 ± 0.01	0.06 ± 0.03	0.034 ± 0.036	2.27 ± 0.65	4.07 ± 1.08	0.101 ± 0.018

Abbreviations used: IV, intravenous; IP, intraperitoneal; IM, intramusuclar; SC, subcutaneous; IC, intracutaneous; IN, intranasal; PO, peroral; PC, percutaneous administration. Data are presented as mean ± SD of three mice.

Radioactivity measurement and data analysis

The mice were dissected under ether anesthesia. Approximately 0.9 ml of blood was obtained from the right ventricle of the heart using a heparinized syringe for each animal. One-third of the blood sample was kept as whole blood, and the remainder was centrifuged. The supernatant was carefully obtained as the plasma sample. All the animals were perfused transcardially with physiological saline immediately after blood collection, and their brains were removed. After immediate weighing and lyophilization, the amount of radioactivity in each sample was measured by γ-ray spectrometry using high-purity Ge detectors. The uptake rates of each radioactive element into the blood, plasma, and brain are given as percentages relative to the administered dose per wet weight (%dose/g). The mean ± standard deviation for three mice was determined for each condition.

Results

Table 2 shows the uptake rates of ^{7}Be, ^{46}Sc, ^{48}V, ^{51}Cr, ^{54}Mn, ^{59}Fe, ^{56}Co, ^{65}Zn, ^{74}As, ^{75}Se, ^{83}Rb,

Table 2f. Uptake rates (%dose/g) of multitracers in blood, plasma, and brain following administration by 8 different methods.

Radionuclide	Time interval	^{83}Rb			^{85}Sr		
Administration method		Blood	Plasma	Brain	Blood	Plasma	Brain
IV	2 min	0.58 ± 0.22	0.35 ± 0.07	0.101 ± 0.014	5.068 ± 0.371	10.350 ± 0.814	0.074 ± 0.006
	30 min	0.32 ± 0.13	0.14 ± 0.03	0.171 ± 0.037	1.756 ± 0.268	3.270 ± 0.506	0.142 ± 0.018
	3 h	0.57 ± 0.10	0.12 ± 0.01	0.431 ± 0.021	0.412 ± 0.070	0.822 ± 0.047	0.220 ± 0.013
	24 h	1.06 ± 0.14	0.15 ± 0.03	1.193 ± 0.066	0.053 ± 0.015	0.102 ± 0.015	0.049 ± 0.012
IP	5 min	0.14 ± 0.05	0.17 ± 0.01	0.020 ± 0.015	3.045 ± 2.612	2.486 ± 2.103	0.046 ± 0.038
	30 min	0.19 ± 0.08	0.07 ± 0.06	0.051 ± 0.028	1.229 ± 1.058	2.033 ± 1.752	0.091 ± 0.077
	3 h	0.56 ± 0.08	0.14 ± 0.08	0.386 ± 0.058	0.461 ± 0.390	0.838 ± 1.099	0.105 ± 0.120
	24 h	1.00 ± 0.03	0.16 ± 0.02	1.150 ± 0.174	0.067 ± 0.013	0.287 ± 0.094	0.055 ± 0.015
IM	5 min	0.51 ± 0.10	0.49 ± 0.07	0.037 ± 0.007	3.049 ± 0.147	5.464 ± 0.572	0.023 ± 0.001
	30 min	0.25 ± 0.01	0.16 ± 0.01	0.094 ± 0.012	1.830 ± 0.087	3.048 ± 0.254	0.101 ± 0.005
	3 h	0.57 ± 0.07	0.15 ± 0.06	0.324 ± 0.087	0.450 ± 0.042	0.632 ± 0.144	0.173 ± 0.033
	24 h	1.16 ± 0.20	0.12 ± 0.03	0.985 ± 0.140	0.065 ± 0.018	0.110 ± 0.021	0.044 ± 0.007
SC	30 min	0.29 ± 0.17	0.62 ± 0.81	0.086 ± 0.005	2.739 ± 0.518	5.967 ± 0.415	0.137 ± 0.008
	3 h	0.52 ± 0.07	0.09 ± 0.03	0.325 ± 0.041	0.480 ± 0.043	0.917 ± 0.086	0.195 ± 0.003
	24 h	1.17 ± 0.11	0.17 ± 0.04	1.073 ± 0.074	0.104 ± 0.027	0.162 ± 0.019	0.062 ± 0.008
IC	30 min	0.22 ± 0.03	0.08 ± 0.04	0.070 ± 0.003	1.943 ± 0.477	3.220 ± 1.135	0.090 ± 0.033
	3 h	0.47 ± 0.03	0.10 ± 0.01	0.305 ± 0.013	0.358 ± 0.068	0.566 ± 0.142	0.141 ± 0.060
	24 h	1.14 ± 0.06	0.13 ± 0.01	1.058 ± 0.050	0.070 ± 0.004	0.120 ± 0.035	0.052 ± 0.013
IN	30 min	0.33 ± 0.03	0.24 ± 0.07	0.150 ± 0.030	0.896 ± 0.181	0.786 ± 0.305	0.081 ± 0.046
	90 min	0.42 ± 0.04	0.11 ± 0.08	0.208 ± 0.044	0.555 ± 0.027	0.591 ± 0.142	0.044 ± 0.001
	3 h	0.51 ± 0.09	0.10 ± 0.01	0.474 ± 0.037	0.435 ± 0.043	0.414 ± 0.034	0.074 ± 0.017
	24 h	1.05 ± 0.07	0.11 ± 0.04	2.002 ± 0.477	0.065 ± 0.031	0.285 ± 0.019	0.045 ± 0.006
PO	30 min	0.34 ± 0.10	0.30 ± 0.10	0.094 ± 0.015	0.137 ± 0.032	0.219 ± 0.131	0.010 ± 0.005
	90 min	0.36 ± 0.06	0.16 ± 0.03	0.193 ± 0.027	0.063 ± 0.010	0.107 ± 0.027	0.011 ± 0.003
	3 h	0.59 ± 0.09	0.23 ± 0.07	0.395 ± 0.073	0.085 ± 0.052	0.144 ± 0.096	0.034 ± 0.017
	24 h	1.10 ± 0.01	0.11 ± 0.01	1.197 ± 0.083	0.028 ± 0.025	0.059 ± 0.031	0.020 ± 0.017
PC	3 h	0.44 ± 0.09	0.10 ± 0.03	0.291 ± 0.062	0.267 ± 0.127	0.441 ± 0.206	0.126 ± 0.097
	24 h	1.18 ± 0.18	0.14 ± 0.02	1.152 ± 0.251	0.026 ± 0.013	0.040 ± 0.020	0.019 ± 0.011

Abbreviations used: IV, intravenous; IP, intraperitoneal; IM, intramusuclar; SC, subcutaneous; IC, intracutaneous; IN, intranasal; PO, peroral; PC, percutaneous administration. Data are presented as mean ± SD of three mice.

^{85}Sr, ^{95m}Tc, ^{88}Y, ^{88}Zr, and ^{103}Ru into the blood, plasma, and brain within 24 h after administration.

The ^{7}Be blood and plasma uptake rates decreased with time in all but the IM and PO groups. The ^{7}Be brain uptake rates were quite low and were almost the same in all groups (Table 2a). ^{48}V uptake behavior was similar to that of ^{7}Be, except for high blood and plasma uptake rates in the SC group 30 min after administration. For ^{48}V, the plasma uptake rate was higher than the blood uptake rate. The brain uptake rates were very low for all administration methods (Table 2b). ^{88}Y uptake behavior was also similar to ^{7}Be uptake behavior. ^{88}Y was hardly taken up into the blood in the PO and PC groups. ^{88}Zr showed the same tendency as ^{88}Y. However, the blood and plasma uptake rates of ^{88}Zr were higher than those of ^{88}Y (Table 2g). In contrast, ^{85}Sr was taken up into the brain for 3 h, and then washed out almost completely at 24 h in the IV, IP, IM, SC, and IC groups. The ^{85}Sr blood and plasma uptake rates decreased with time and these elements were washed out at 24 h (Table 2f).

Table 2g. Uptake rates (%dose/g) of multitracers in blood, plasma, and brain following administration by 8 different methods.

Radionuclide	Time interval	^{88}Y			^{88}Zr		
Administration method		Blood	Plasma	Brain	Blood	Plasma	Brain
IV	2 min	13.557 ± 1.010	26.800 ± 2.100	0.027 ± 0.007	15.603 ± 0.476	31.370 ± 1.858	0.029 ± 0.009
	30 min	4.715 ± 0.793	9.264 ± 2.276	0.008 ± 0.003	10.981 ± 0.882	20.850 ± 1.930	0.021 ± 0.013
	3 h	1.080 ± 0.415	1.922 ± 0.487	0.033 ± 0.010	7.104 ± 0.635	13.171 ± 1.511	0.029 ± 0.010
	24 h	0.084 ± 0.040	0.188 ± 0.102	0.017 ± 0.007	0.800 ± 0.077	1.596 ± 0.144	0.025 ± 0.014
IP	5 min	0.975 ± 0.910	2.099 ± 2.066	0.004 ± 0.003	1.514 ± 1.320	3.061 ± 2.690	0.002 ± 0.001
	30 min	1.882 ± 1.718	3.457 ± 3.208	0.009 ± 0.002	2.312 ± 2.387	4.589 ± 4.806	0.005 ± 0.004
	3 h	0.555 ± 0.860	1.234 ± 1.771	0.007 ± 0.010	2.692 ± 3.745	5.258 ± 7.936	0.006 ± 0.007
	24 h	0.259 ± 0.121	0.533 ± 0.395	0.031 ± 0.030	2.005 ± 0.076	3.946 ± 0.189	0.025 ± 0.018
IM	5 min	0.347 ± 0.137	0.634 ± 0.325	0.0017 ± 0.0004	0.407 ± 0.070	0.776 ± 0.157	0.002 ± 0.001
	30 min	2.298 ± 1.031	3.915 ± 1.747	0.002 ± 0.003	2.579 ± 0.673	4.259 ± 0.764	0.002 ± 0.002
	3 h	1.020 ± 0.224	1.883 ± 0.757	0.020 ± 0.016	3.331 ± 0.776	6.179 ± 1.401	0.008 ± 0.007
	24 h	0.164 ± 0.054	0.384 ± 0.135	0.0125 ± 0.0004	1.120 ± 0.302	2.076 ± 0.407	0.014 ± 0.002
SC	30 min	1.145 ± 0.843	2.376 ± 1.974	0.004 ± 0.001	1.747 ± 1.327	3.398 ± 2.931	0.005 ± 0.005
	3 h	0.831 ± 0.234	1.610 ± 0.324	0.008 ± 0.004	2.657 ± 0.671	4.758 ± 1.317	0.006 ± 0.002
	24 h	0.248 ± 0.067	0.410 ± 0.237	0.007 ± 0.005	1.664 ± 0.453	2.784 ± 0.863	0.020 ± 0.015
IC	30 min	0.264 ± 0.093	0.511 ± 0.173	0.001 ± 0.001	0.397 ± 0.154	0.760 ± 0.266	0.001 ± 0.001
	3 h	0.825 ± 0.231	1.733 ± 0.485	0.002 ± 0.001	2.515 ± 0.445	4.752 ± 1.123	0.004 ± 0.002
	24 h	0.191 ± 0.047	0.466 ± 0.067	0.016 ± 0.011	1.590 ± 0.357	2.862 ± 0.441	0.013 ± 0.008
IN	30 min	0.053 ± 0.028	0.082 ± 0.093	0.002 ± 0.001	0.201 ± 0.090	0.317 ± 0.180	0.0080 ± 0.0002
	90 min	0.044 ± 0.045	0.101 ± 0.096	0.004 ± 0.003	0.182 ± 0.101	0.258 ± 0.133	0.002 ± 0.002
	3 h	0.042 ± 0.046	0.116 ± 0.072	0.002 ± 0.001	0.265 ± 0.173	0.427 ± 0.289	0.003 ± 0.002
	24 h	0.005 ± 0.003	0.011 ± 0.006	0.026 ± 0.009	0.063 ± 0.017	0.112 ± 0.042	0.003 ± 0.001
PO	30 min	0.008 ± 0.007	0.010 ± 0.010	0.006 ± 0.001	0.006 ± 0.002	0.006 ± 0.002	0.008 ± 0.006
	90 min	0.011 ± 0.008	0.004 ± 0.004	0.012 ± 0.008	0.009 ± 0.005	0.013 ± 0.005	0.008 ± 0.007
	3 h	0.005 ± 0.005	0.006 ± 0.002	0.011 ± 0.007	0.013 ± 0.011	0.034 ± 0.018	0.006 ± 0.002
	24 h	0.005 ± 0.006	0.002 ± 0.002	0.003 ± 0.001	0.017 ± 0.012	0.016 ± 0.010	0.012 ± 0.010
PC	3 h	0.002 ± 0.003	0.013 ± 0.010	0.004 ± 0.004	0.046 ± 0.039	0.081 ± 0.036	0.005 ± 0.007
	24 h	0.003 ± 0.004	0.003 ± 0.002	0.0012 ± 0.0002	0.010 ± 0.007	0.030 ± 0.026	0.003 ± 0.002

Abbreviations used: IV, intravenous; IP, intraperitoneal; IM, intramusuclar; SC, subcutaneous; IC, intracutaneous; IN, intranasal; PO, peroral; PC, percutaneous administration. Data are presented as mean ± SD of three mice.

The uptake rates of ^{46}Sc were higher than those of ^{7}Be in most of the samples examined. The blood and plasma uptake rates were the highest in the IV group but decreased with time while the brain uptake rates increased. For the other seven methods, the blood, plasma, and brain uptake rates were very low. However, unlike those of ^{7}Be, increases in the blood and plasma uptake levels of ^{46}Sc were observed not only in the IM group but also in the IP, SC, and IC groups. The ^{46}Sc brain uptake rates of the PO and PC groups were higher than those of the IM, IC, and IN groups at 24 h (Table 2a). The ^{51}Cr brain uptake rates were very low in all groups (Table 2b). The ^{51}Cr blood and plasma uptake rates of the IV, IP, IM, SC, and IC groups were higher than those of the IN, PO, and PC groups. The blood and plasma uptake rates of ^{54}Mn were low in all groups. However, the ^{54}Mn brain uptake rates were high in the IV, IM, SC, and IC groups. The brain uptake in the IN group increased from 3 to 24 h after administration (Table 2c). For ^{56}Co, the brain uptake rates of the IV, SC, and PO groups were higher than those of the other groups (Table 2c).

Table 2h. Uptake rates (%dose/g) of multitracers in blood, plasma, and brain following administration by 8 different methods.

Radionuclide	Time interval	^{95m}Tc			^{103}Ru		
Administration method		Blood	Plasma	Brain	Blood	Plasma	Brain
IV	2 min	8.18 ± 0.62	8.52 ± 1.07	0.054 ± 0.026	5.16 ± 0.43	9.79 ± 1.40	0.070 ± 0.022
	30 min	3.96 ± 0.58	4.27 ± 0.69	0.022 ± 0.008	2.35 ± 0.51	4.62 ± 0.18	0.042 ± 0.064
	3 h	1.41 ± 0.36	1.39 ± 0.36	0.023 ± 0.008	1.84 ± 0.33	3.58 ± 0.79	0.036 ± 0.011
	24 h	0.10 ± 0.04	0.03 ± 0.01	0.046 ± 0.046	1.09 ± 0.11	1.78 ± 0.24	0.039 ± 0.046
IP	5 min	6.92 ± 0.63	7.96 ± 0.66	0.040 ± 0.027	2.24 ± 1.37	4.30 ± 2.25	0.020 ± 0.023
	30 min	3.01 ± 2.39	3.10 ± 2.47	0.006 ± 0.003	1.53 ± 1.31	2.55 ± 2.21	0.021 ± 0.021
	3 h	1.67 ± 0.56	1.44 ± 0.60	0.024 ± 0.017	1.25 ± 0.96	2.03 ± 2.16	0.021 ± 0.029
	24 h	0.07 ± 0.08	0.04 ± 0.01	0.004 ± 0.005	1.25 ± 0.19	1.98 ± 0.67	0.032 ± 0.025
IM	5 min	6.27 ± 0.30	6.20 ± 0.45	0.019 ± 0.007	2.17 ± 0.26	3.79 ± 0.19	0.028 ± 0.009
	30 min	4.07 ± 0.61	4.00 ± 0.23	0.008 ± 0.004	1.63 ± 0.34	3.01 ± 0.48	0.010 ± 0.013
	3 h	1.90 ± 0.44	1.84 ± 0.31	0.012 ± 0.012	1.57 ± 0.29	2.96 ± 0.28	0.030 ± 0.009
	24 h	0.02 ± 0.01	0.07 ± 0.02	0.008 ± 0.002	0.78 ± 0.05	1.34 ± 0.31	0.034 ± 0.028
SC	30 min	4.62 ± 0.30	4.82 ± 0.53	0.023 ± 0.026	1.76 ± 0.69	3.61 ± 1.42	0.055 ± 0.011
	3 h	1.49 ± 0.51	1.43 ± 0.28	0.007 ± 0.007	1.66 ± 0.40	2.61 ± 0.60	0.023 ± 0.015
	24 h	0.04 ± 0.03	0.04 ± 0.03	0.046 ± 0.026	1.22 ± 0.34	1.62 ± 0.02	0.094 ± 0.057
IC	30 min	3.93 ± 0.29	3.99 ± 0.07	0.021 ± 0.007	1.09 ± 0.13	1.78 ± 0.15	0.021 ± 0.013
	3 h	1.51 ± 0.10	1.53 ± 0.07	0.004 ± 0.004	1.27 ± 0.03	2.16 ± 0.12	0.013 ± 0.007
	24 h	0.02 ± 0.02	0.04 ± 0.01	0.012 ± 0.002	1.06 ± 0.19	1.70 ± 0.07	0.021 ± 0.009
IN	30 min	3.06 ± 0.56	2.96 ± 0.40	0.043 ± 0.023	0.65 ± 0.16	1.45 ± 0.32	0.049 ± 0.033
	90 min	2.19 ± 0.17	1.71 ± 0.31	0.010 ± 0.010	0.46 ± 0.06	0.67 ± 0.22	0.013 ± 0.004
	3 h	1.09 ± 0.07	1.05 ± 0.25	0.006 ± 0.003	0.58 ± 0.06	0.86 ± 0.17	0.012 ± 0.003
	24 h	0.12 ± 0.08	0.17 ± 0.06	0.020 ± 0.024	0.32 ± 0.12	0.53 ± 0.03	0.030 ± 0.040
PO	30 min	2.93 ± 0.37	2.37 ± 0.12	0.052 ± 0.041	0.90 ± 0.25	1.30 ± 0.55	0.044 ± 0.037
	90 min	2.34 ± 0.51	2.40 ± 0.51	0.056 ± 0.031	0.48 ± 0.27	0.90 ± 0.04	0.049 ± 0.036
	3 h	1.17 ± 0.20	1.12 ± 0.41	0.043 ± 0.020	0.45 ± 0.14	0.96 ± 0.35	0.128 ± 0.154
	24 h	0.08 ± 0.07	0.05 ± 0.03	0.044 ± 0.015	0.32 ± 0.28	0.37 ± 0.31	0.112 ± 0.067
PC	3 h	1.47 ± 0.43	1.51 ± 0.56	0.012 ± 0.010	0.95 ± 0.22	1.34 ± 0.35	0.034 ± 0.018
	24 h	0.08 ± 0.02	0.08 ± 0.02	0.006 ± 0.007	0.47 ± 0.19	0.70 ± 0.36	0.020 ± 0.020

Abbreviations used: IV, intravenous; IP, intraperitoneal; IM, intramusuclar; SC, subcutaneous; IC, intracutaneous; IN, intranasal; PO, peroral; PC, percutaneous administration. Data are presented as mean ± SD of three mice.

^{59}Fe concentration in the blood increased with time and reached the highest levels at 24 h in all groups except the IV and PC groups. In comparison with the plasma uptake rate, the blood uptake rate was low until 3 h, although it exceeded the plasma uptake rate at 24 h. In contrast, the brain uptake rates were the highest in the PO group (Table 2d). ^{65}Zn concentrations in the blood and plasma decreased rapidly with time, while that in the brain increased. The ^{65}Zn brain uptake rates of the IV, IP, IM, SC, and IC groups were higher than those of the IN, PO, and PC groups. The ^{65}Zn brain uptake rate of the IN group increased slightly from 3 to 24 h after administration (Table 2d).

^{74}As was washed out of the blood at 24 h after administration in all groups. It was taken up into the brain for 3 h, and then washed out almost completely at 24 h (Table 2e). For ^{75}Se, the blood uptake rates were almost the same in all groups at 24 h. The plasma uptake rates were higher than the blood uptake rates, but were comparable in all groups. The brain uptake rates of the IV, IP, IM, SC, and IC groups were similar or slightly higher

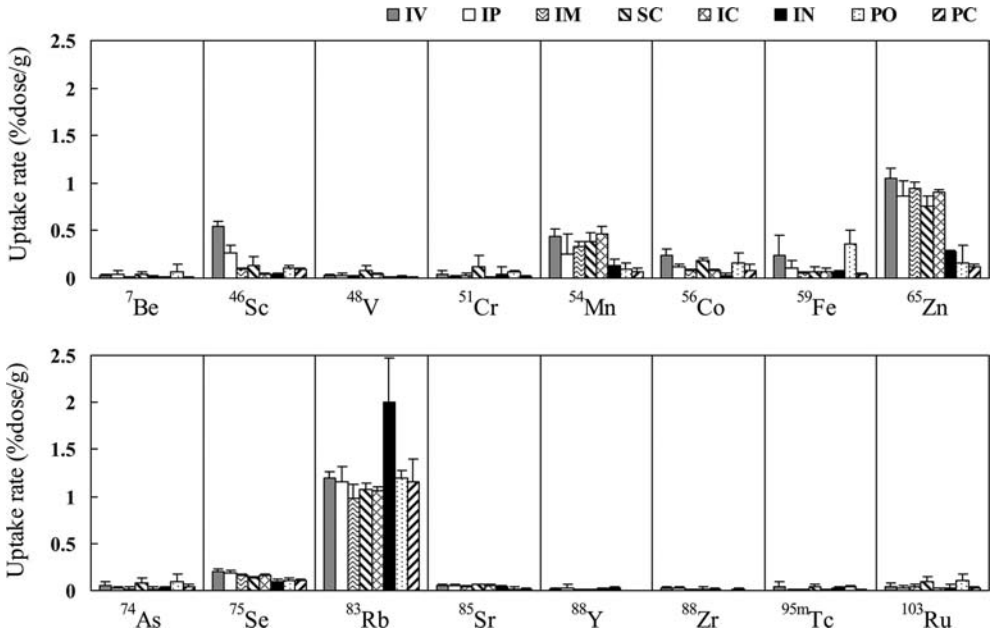

Figure 1. Brain uptake rates (%dose/g wet weight) of multitracer 24 h after administration. The uptake rate of intranasally administered Rb was the highest among those of all combinations of elements and administration methods examined. Abbreviations used: IV, intravenous; IP, intraperitoneal; IM, intramuscular; SC, subcutaneous; IC, intracutaneous; IN, intranasal; PO, peroral; PC, percutaneous administration. The errors are standard deviations for three samples.

than those of the IN, PO, and PC groups (Table 2e).

The behavior of ^{83}Rb uptake was of interest. In each method, ^{83}Rb showed the highest brain uptake rate among the 16 elements in the multitracer. Its blood and brain uptake rates increased with time and were comparable in all except the IN group. Twenty-four hours after administration, the ^{83}Rb brain uptake rate of the IN group was approximately twice those of the other groups, which was the highest brain uptake rate in all combinations of the 8 methods and 16 elements (Figure 1). In contrast, with all administration methods, the ^{83}Rb blood uptake rate increased and reached comparable levels. The ^{83}Rb plasma uptake rates were retained at almost the same levels, and were lower than the ^{83}Rb blood uptake rates (Table 2f).

The ^{95m}Tc blood uptake rates were high during the early periods after administration, and then decreased rapidly with time. The brain uptake rates of ^{95m}Tc were very low, similar to those of ^{88}Y and ^{88}Zr, and were not markedly different across the administration methods examined (Table 2g, h). The blood and plasma uptake rates of ^{103}Ru, were similar at 3 h in the IV, IP, SC, and IC groups. The ^{103}Ru brain uptake rates were very low at 24 h except in the PO group (Table 2h).

Discussion

The main findings of the present study can be summarized as follows: (1) Be, V, Cr, Y, Zr, Tc, and Ru did not enter the brain; (2) As and Sr entered the brain and were completely washed out within 24 h; (3) Sc, Co, and Fe entered the brain, yet their uptake rates differed across administration methods; and (4) Mn, Zn, Se, and Rb entered the brain and accumulated gradually within 24 h.

The delivery mechanisms of trace elements can be broadly classified into three patterns. The first is the uptake pattern of the IV group. As the solution entirely enters the blood circulation with IV injection, which is the most commonly used administration method, the IV uptake pattern is seen as the baseline for evaluation of the other methods. The second is the rapid accumulation pattern as seen in the IP, IM, SC, and IC methods. In these methods, trace elements were injected into the respective tissues using a hypodermic needle, and therefore can access the capillary circulation

directly and be distributed easily throughout the whole body. The last is the slow pattern as observed the IN, PO, and PC methods. In these administration methods, the trace elements must pass through several biological systems to reach the bloodstream, e.g., intranasally administered trace elements first spread in the nasal cavity, and are then absorbed by the nasal mucosa before coming into contact with the blood vessels. If the uptake pattern of an element is different from these three patterns, then a different delivery route for the element would be required.

Some elements showed unique uptake patterns. The ^{48}V blood concentrations were comparable between the IV and SC groups, although the mechanism underlying the V distribution is unclear. The Fe brain uptake rate of the PO group was relatively high as compared with the other groups. Hirunuma *et al.* reported that the liver uptake of Fe is similar to that of Sc because of their chemical similarity *in vivo*. These elements are usually trivalent in the blood; their ionic radii are 0.64 å for Fe^{3+} and 0.73 å for Sc^{3+} (Weast 1990; Hirunuma *et al.* 1999). In the present study, Fe and Sc showed similar brain uptake behavior in the PO group.

The ^{83}Rb uptake showed a more distinctive pattern. There was almost no difference in ^{83}Rb uptake pattern among all the methods examined except IN administration. This may have been because Rb is physiologically most similar to K and thus, when administered, it is distributed easily throughout the body in the same way as K (Love & Burch 1953; Meltzer 1991). Although there were no significant differences among blood and plasma uptake rates in all eight methods, the brain uptake rate for the IN method was approximately twice those of the other methods. These observations suggested that there is a direct transport route from the nasal cavity to the brain for Rb. One possible mechanism for this is olfactory transport, i.e., the axonal transport of olfactory sensory neurons from the nasal mucosa through the cribriform plate to the olfactory bulb (Mathison *et al.* 1998; Tjälve & Henriksson 1999). In addition, the uptake of ^{83}Rb in the brain was the highest among those of all tracers administered by the IN method (Fig. 1).

Mn and Zn showed high brain uptake rates close to that of Rb. Similar results were observed by Hirunuma *et al.* who determined the uptake and distribution of multitracers in normal and vitamin-D-overloaded rats (Hirunuma *et al.* 1999). The Mn and Zn brain uptakes were applicable to the three patterns as described above. In animals, ionic Mn, Co, and Zn have been shown to enter the brain *via* olfactory neurons (Brenneman *et al.* 2000; Persson *et al.*2003a, b). In the present study, the slight increases in the ^{54}Mn and ^{65}Zn brain uptake rates observed in the IN group at 24 h may have been associated with olfactory transport. Nevertheless, these metals were not taken up effectively. Therefore, we speculated that there is a specific direct olfactory transport route to the brain for Rb, an alkaline metal, and it is different from the known olfactory transport for Mn, Co, and Zn.

In conclusion, multitracer screening provided a clearer understanding of the delivery mechanism of 16 trace elements by 8 administration methods. The results of the present study indicated that there is a novel Rb-specific delivery route to the brain circumventing the blood–brain barrier.

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