Prevention of enteral radiocesium absorption by hexacyanoferrates(II) in piglets

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Summary. The efficacy of different hexacyanoferrates(II) in preventing the enteral absorption of ^{134}Cs was studied in piglets. As compared to the controls, oral application of ^{134}Cs together with $KFe[Fe(CN)_6]$, $NH_4Fe[Fe(CN)_6]$, or $Fe_4[Fe(CN)_6]_3$ resulted in a strong reduction of the ^{134}Cs-uptake by more than 97%. The decrease in enteral absorption depends on the dose of administered hexacyanoferrate(II), whereas differences between the compounds under study were small. The biological half-life of ^{134}Cs in non-hexacyanoferrate(II) treated piglets was 21.6 ± 3.3 days (mean $\pm$ SD).
Key words. Hexacyanoferrate(II); ^{134}Cs; cesium absorption; biological half-life; piglet.

The Chernobyl disaster resulted in Europe-wide contamination with the potentially hazardous radionuclides ^{134}Cs and ^{137}Cs. The use of hexacyanoferrates(II) for the prevention of the enteral uptake of radiocesium or enhancement of radiocesium excretion in animals and humans was studied in some detail two decades ago[1–4]. However, little is conclusively known about the comparative effects of different hexacyanoferrates(II). Early in vivo studies gave only conflicting results. Nigrovic studied the action of several hexacyanoferrates in rats and found decreasing effectivity in the series Fe(III)HCF > Co(II) > Cu(II) > > Zn[5]. This is somewhat in contrast to Havlicek et al. who found Fe(III) > Co(II) = Zn[3], and Bozorgzadeh who found no differences at all between Co(II), Fe(III) and Ni(II) in vivo[6].

As the amount of absorbable transition metal ions from orally administered Cu-, Co-, and Ni-hexacyanoferrates(II) is not known, the use of these hexacyanoferrate derivatives in humans and domestic animals could raise some toxicological questions. This is not true for Fe(III)-hexacyanoferrates(II), where no toxic effects were found even at high dosage[7]. Some authors compared the efficacy of FeHCF ($Fe_4[Fe(CN)_6]_3$, 'insoluble Prussian blue'), KFeHCF ($KFe[Fe(CN)_6]$, 'soluble Prussian blue'), and NH_4HCF ($NH_4Fe[Fe(CN)_6]$) in vivo. Again, partly controversial results were obtained. On the one hand, evidence indicates that colloidal-soluble preparations are twice as effective in accelerating the elimination of ingested radiocesium in rats[8]. On the other hand, according to Bozargzadeh long-lasting administration of soluble Prussian blue (KFeHCF) was found to prolong the biological half-life of cesium, as a result of a complete blocking of the ^{134}Cs-excretion from liver, spleen, and skeleton[9]. However, the latter results were not confirmed by Müller et al. who found no exceptional behavior of soluble Prussian blue after prolonged administration of highly purified KFeHCF[10]. As pointed out by Müller, contradictory findings in some of these former studies may simply be caused by the use of poorly defined hexacyanoferrate compounds, which may contain unknown amounts of by-products.

In order to develop effective and practicable methods for the prevention of radiocesium uptake and/or elimination of incorporated cesium in domestic animals and humans, we compared the efficacy of different Fe(III)-hexacyanoferrates (II) on inhibiting radiocesium absorption in piglets.

Material and methods. $^{134}CsCl$ (88.4 MBq/mg Cs) was obtained from Amersham International, England. Chemicals of analytical grade were purchased from E. Merck, Darmstadt (FRG). Hexacyanoferrates(II) were synthesized as described elsewhere[11]. In brief: $KFe[Fe(CN)_6]$ (KFeHCF) was prepared by a 1:1 addition of $FeCl_3$ to $K_4[Fe(CN)_6]$. $NH_4Fe[Fe(CN)_6]$ (NH_4FeHCF) was prepared by the 1:1 addition of $FeCl_3$ to $(NH_4)_4[Fe(CN)_6]$. The latter compound was prepared from $K_4[Fe(CN)_6]$ by ion exchange reaction on AG 50 W X 8 (NH_4^+-form). The crude reaction products (KFeHCF and NH_4FeHCF) were dialyzed exhaustively against water. $Fe_4[Fe(CN)_6]_3$ (FeHCF) was obtained by the addition of excess $FeCl_3$ to KFeHCF. The precipitate formed was centrifuged and the pellet was washed with 0.1 M HCl and water. The compounds were freeze-dried and crushed in a mortar. For determination of K and Fe, the solid samples were treated with boiling H_2SO_4. Potassium was determined by flame photometry, iron was determined spectrophotometrically using bathophenanthrolin as color reagent. C, N, H, analyses were carried out by the Microanalytical Laboratory Beller, Göttingen. Piglets from different litters (Deutsche Landrasse, 25.8 ± 2.4 days of age, mean weight: 6.2 ± 1.4 kg, both sexes) were randomly pooled in the different experimental groups. Animals were nursed by sow and had access to creep feed from day 14 p.n., and water ad libitum.

Piglets were fasted for 4 h prior to oral administration of the test compounds. 1 ml of the aqueous $^{134}CsCl$ solution (37 kBq, 1 μCi) was mixed with 10 ml of a solution of the respective hexacyanoferrate(II) within 30 s before administration through a gastric tube. The intubation tools were rinsed with deionized water to a final applicated volume of 50 ml. The retention of ^{134}Cs was measured 7 and 14 days after application, by whole body counting in a 4π whole body liquid scintillation counter for humans in the energy range of 500–1800 keV[12]. Piglets were measured in a small plastic cage to keep the efficiency deviations due to animal movements within counting statistics[13]. The activity registered immediately after administration of ^{134}Cs, was taken as 100% reference. Due to weight gain in the 14-day period (from $6.2 \text{ kg} \pm 1.4$ to $10.0 \text{ kg} \pm 2.1$) the counting efficiency decreased about 8%. As all groups are affected to the same extent, retention values were not corrected for the ^{134}Cs-uptake measurements. The decreasing efficiency was taken into consideration for the calculation of the biological half-life of ^{134}Cs. The whole body counter used cannot discriminate ^{134}Cs from ^{40}K. However, the apparent increase in the 14-day ^{134}Cs-retention due to increasing natural ^{40}K-radioactivity of growing piglets was found to be negligible, and was not corrected for either.

Results and discussion. To remove most of the low molecular weight by-products, the hexacyanoferrates(II) were purified by dialysis against water[10]. The analytical data given in table 1 are in good agreement with the theoretical values. The biological half-life of the radiocesium in piglets was determined by repeated whole-body-counting from 7 to 28 days after oral ingestion of ^{134}Cs (without hexacyanoferrate (II)). The retention values were corrected for decreasing counting efficiency due to weight gain in the measurement period. From the semilogarithmic analysis of the ^{134}Cs-retention curve a biological half-life of 21.6 days ± 3.3 (mean $\pm$ SD, $n = 9$) was calculated. As the retention measurements started

Table 1. Analytical data for the hexacyanoferrate(II) compounds under study

	$K\ Fe[Fe(CN)_6]\ 2\ H_2O$ (MW = 342.9)		$NH_4Fe[Fe(CN)_6]\ 2\ H_2O$ (MW = 321.9)		$Fe_4[Fe(CN)_6]_3\ 15\ H_2O$ (MW = 1129.3)	
	calc. (%)	found (%)	calc. (%)	found (%)	calc. (%)	found (%)
Fe	32.57	32.43	34.70	33.93	34.62	34.60
C	21.02	21.83	22.39	23.46	19.14	19.36
H	1.12	1.19	2.49	2.15	2.66	2.64
N	24.51	25.03	30.46	31.26	22.33	22.11
K	11.40	11.2	0	ND	0	0.26

ND not detectable by flame photometry.

Table 2. ^{134}Cs-whole body retention in percent of the administered dose in piglets 14 days after oral uptake of 37 kBq (1 μCi) ^{134}Cs together with different hexacyanoferrates(II)

Control No.		$K\ Fe[Fe(CN)_6]$ No.	$2\ H_2O$ 10 mg (0.03 mmol)	$K\ Fe[Fe(CN)_6]$ No.	$2\ H_2O$ 103 mg (0.3 mmol)	$NH_4Fe[Fe(CN)_6]$ No.	$2\ H_2O$ 97 mg (0.3 mmol)	$Fe_4[Fe(CN)_6]_3$ No.	$15\ H_2O$ 113 mg (0.1 mmol)
W211	63.98	W505	9.30	W206	1.79	W602	1.09	W208	2.20
W502	64.50	W506	11.94	W207	2.67	W603	1.12	W209	2.21
W503	66.92	W507	6.23	W509	1.76	W604	0.82	W608	1.08
W504	70.33	W508	5.20	W510	1.89	W605	1.33	W609	1.15
				W601	2.28	W606	1.56		
MIL04	52.35			LOT02	0.99	PEL07	0.61	ABO09	1.99
MIL08	52.78			LOT05	1.08	PEL08	0.63	PEL10	1.08
PEL01	50.79			PEL04	0.55	PEL09	0.71	PEL11	1.27
PEL02	53.17			PEL05	0.74			PEL12	1.06
PEL03	55.24			PEL06	0.74				
n	9		4		10		8		8
Xa	58.90		8.17		1.45		0.98		1.51
SEM	2.48		1.53		0.23		0.12		0.19
SD	7.45		3.06		0.73		0.35		0.53
RR[1]	100		13.9		2.46		1.66		2.56
U-test					p = 0.2 (not significant)				
							p = 0.1 (not significant)		
						p = 0.5 (not significant)			

[1] RR = relative retention ((^{134}Cs retention/ ^{134}Cs retention of controls)* 100).

7 days after application, this value does not include a fraction of absorbed cesium which is more rapidly excreted by the kidneys within the first days after administration. The biological half-life determined in this study by whole-body-counting is in good agreement with earlier measurements in organs of growing pigs [14].

In order to find the compound with the greatest efficacy in inhibiting enteral ^{134}Cs-absorption, carrier-free ^{134}Cs (controls) or ^{134}Cs together with the hexacyanoferrate(II) to be tested were administered by gastric tube to piglets pooled in groups of 8–10 animals. The first experiments were designed to obtain the optimal dose level for hexacyanoferrate in the 6.4 kg body weight piglets. As shown in table 2, a dosage of 10 mg KFeHCF reduced the absorption of ^{134}Cs only to a minor extent (from 59% to 8.2%). For the comparison of different hexacyanoferrates(II) 0.3 mmol (103 mg or 97 mg, respectively) of KFeHCF and NH_4HCF and 0.1 mmol of FeHCF (FeHCF contains three hexacyanoferrate(II)-complexes per mol) were administered. The whole-body-retention of ^{134}Cs was reduced from 58.9% ± 7.5 of the controls to 1.5% ± 0.7 by KFeHCF, 1.0% ± 0.4 by NH_4HCF, and 1.5% ± 0.5 by FeHCF (table 2, mean ± SD). As compared to the controls, reduction of the ^{134}Cs-body-burden is better than 97% for all compounds. Differences in efficacy between these three hexacyanoferrates(II) were not significant. Thus, 'soluble' and 'insoluble' Prussian blue are both effective in preventing radiocesium absorption to the same extent in piglets. This is in some contrast to earlier results in rats, where FeHCF was found to be less effective in elimination of radiocesium as compared to KFeHCF [8, 10].

After the Chernobyl accident in April 1986, NH_4HCF was used in pilot studies for the prevention of radiocesium uptake in cows and sheep [15]. This hexacyanoferrate(II) compound was suggested by Giese, based on his earlier study in rats [16]. However, the experimental evidence demonstrating the advantage of NH_4FeHCF over other hexacyanoferrate(II) compounds was rather limited. From our results, the efficacy in preventing the uptake of ^{134}Cs is comparable in all compounds under study. Differences between these compounds were also found to be slight in in-vitro studies regarding the ^{137}Cs-absorption capacities of different hexacyanoferrates(II) [11]. More detailed studies in domestic animals are needed to find out the most favorable compound. Until this has been done only the difference in price has to be considered. As FeHCF and KFeHCF are produced in large quantities for the making of iron blue pigments, these compounds are cheap as compared to NH_4FeHCF. Another problem is the purity of the hexacyanoferrate(II) preparations used [10]. Commercially available compounds can contain some low molecular weight by-products (i.e. free $[Fe(CN)_6]^{4-}$, KCl, NH_4Cl) which may influence the efficacy of suppressing radiocesium absorption or enhancement of radiocesium elimination. For further studies, only chemically pure hexacyanoferrate(II) preparations should be used.

It should be mentioned that a pharmaceutical FeHCF-preparation (Radiogardase-Cs) is available in West Germany for treatment of radiocesium intoxication in humans.

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The nature of posterior hypothalamic projections to cardiorespiratory centers in the brainstem

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Summary. Focal electrical stimulation of the midlateral posterior hypothalamus in the rat produces rapid shallow respiration accompanied by a rise in arterial blood pressure. Stimulation of presumably intrinsic neurons only by glutamate induces slower deeper respiration associated with a fall in blood pressure.
Key words. Posterior hypothalamus; blood pressure; respiration.

Stimulation of points throughout the length of the hypothalamus in the rat evokes a variety of behavioral functions such as attack, flight, defence, copulation and feeding[1]. There is some separation of response depending upon whether sites medial or lateral to the fornix are stimulated. Nevertheless, anatomically many nuclear groups such as the posterior hypothalamic nucleus, dorsal premammillary nucleus, submammillary nucleus and nucleus geminus as well as the subthalamic nucleus and zona inserts have been stimulated when producing these behaviors.

Focal electrical stimulation of a small locus of the midlateral posterior hypothalamus (MLPH)[2] in chloral hydrate anesthesized rats produces a rise in arterial blood pressure accompanied by rapid shallow respiration[3]. MLPH is, however, a conduit for caudally directed axons originating from centers which, when stimulated also result in alterations in cardio-respiratory function. These areas include the hippocampus[4], septum[4,5], habenula[6], medial hypothalamus[7], lateral hypothalamus[8–10], anterior hypothalamus[7,11], thalamus[12], cingulate cortex[13,14], and prefrontal cortex[13,15]. In this study gluatmate was used by both pressure and iontophoretic injection to stimulate neurons intrinsic to MLPH avoiding stimulation of axons of passage[16].

Materials and methods. Twenty 300-g Wistar rats were used in this study. The common carotid artery on the side opposite to that used for brain stimulation was cannulated, flushed with heparinized saline, and arterial blood pressure was monitored with a tham P23AA pressure transducer. Respiration was monitored with an impedance converter from electrodes inserted in intercostal muscles in the midaxillary line. Body temperature was maintained at 37 °C by a heating pad activated by a rectal thermistor probe. The site stimulated is immediately lateral to the mamillothalamic tract and above the fornix lateral to the posterior hypothalamic nucleus (AP – 3.8 mm, lat. 1.1 mm, vertical 8.2 mm in the atlas of Paxinos and Watson 17).

In 10 rats, MLPH was stimulated by a pressure injection of 50 or 100 nl of M sodium glutamate pH 7.5 –8 over 30–60 s through a glass electrode (tip 40 μm) using a nanoliter pump (WPI) or 0.5 μl over 10 s using a Hamilton syringe. The site of injection was verified histologically after substituting the electrode with one containing Methyl Blue in potassium acetate and making a 100-nl injection.

In a further series of 10 rats, one side of a double glass electrode was filled with 2 M NaCl (used to verify electrode location by electrical stimulation) and the other with 3 M sodium glutamate which was injected by a constant current of 12 μA of 15–60 s duration. Controls for this procedure comprised observing respiration or arterial blood pressure while passing the same current through the saline electrode or the same current of opposite polarity through the glutamate electrode. The site of injection was verified histologically after substituting the electrode with one containing Methyl Blue in potassium acetate and marking the site by passing negative current through the electrode[18,19].

In some cases the carotid bodies were denervated by the method of Favier and Lacaisse[20].

Results. Electrical stimulation of MLPH produced rapid shallow respiration for the duration of the stimulus in which both inspiration and expiration were equally shortened (fig., A). Arterial blood pressure showed a rapid rise and began to fall before cessation of the stimulus. A similar response could be elicited from points following the medial forebrain bundle forward to the anterior hypothalamic and preoptic areas. Following Carotid Body denervation, stimulation of MLPH produced a rise in blood pressure followed by a prolonged period of post stimulus recovery. Respiration also showed some post stimulus augmentation (fig., B).

Slow pressure injection of M glutamate produced a different response (fig., C). After a delay, blood pressure fell and respiration slowed and became shallow. This was followed by rebound of deeper and more rapid respiration during which blood pressure returned toward preinjection levels. Blood pressure was allowed to recover completely before subsequent stimulations and no more than two injections were made at one site.