Efficacy of Different Hexacyanoferrates(II) in Inhibiting the Intestinal Absorption of Radiocaesium in Rats

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The inhibitory effect of various oral doses of different hexacyanoferrate(II) compounds (HCF) and the influence of the time interval of HCF-administration on intestinal ^{134}Cs-absorption was studied in rats. Optimum inhibition was obtained by administration of HCF together with or 2 min before oral ^{134}Cs loading. Using appropriate low amounts (0.1–0.5 mg) of the different HCF compounds, the inhibitory effect increased in the sequence KZnHCF < KCuHCF < FeHCF < KCoHCF = KNiHCF < NH_4FeHCF = KFeHCF. Oral administration of 5 mg (0.5 mg) of KFeHCF, together with ^{134}CsCl loading, reduces ^{134}Cs-absorption from 41% (control) to 0.8% (2.8%).

Zinc-, copper-, cobalt, and nickel hexacyanoferrates(II), despite showing a high caesium sorption capacity *in vitro*, were less effective in rats and are not suited for *in vivo* application, also because they may produce toxic side effects. As a consequence, the orally administered colloidal-soluble iron(III) hexacyanoferrates(II) ($NH_4Fe[Fe(CN)_6]$ and $KFe[Fe(CN)_6]$) have to be considered as the most valuable countermeasure against radiocaesium absorption for humans and domestic animals in the case of a severe nuclear accident in the future.

Manganese oxide, a non-hexacyanoferrate(II) compound with known *in vitro* caesium binding capacity, showed no inhibitory effect on radiocaesium absorption in rats.

Introduction

Under the impression of the overearth nuclear weapon tests (1954–1963), resulting in a hemisphere wide contamination of the food chain with the radionuclides ^{134}Cs and ^{137}Cs, the benefit of oral administered hexacyanoferrate(II) compounds (HCF) as antidote against radiocaesium incorporation in animals was demonstrated by several authors [1–6]. However, the efficacy of different HCF compounds and dosages in comparison remained obscure (Table I), and no admitted treatment procedure for the inhibition of radiocaesium absorption in humans and domestic animals was available as a result of these former studies. Some of the earlier contradictory results may have been caused by the fact that i) insufficiently purified and badly characterized compounds were used in most cases, and ii) the comparison of different compounds was influenced by overdosing the oral HCF (Table I).

The nuclear reactor accident in Chernobyl (1986) has stimulated the discussion on the risks of environmental radioactive contamination to man and animals and the use of HCF as potential countermeasure against radiocaesium absorption [7–10].

In a previous *in vitro* study, it was demonstrated that the sorption capacity for caesium of some transition metal hexacyanoferrates(II) (Cu, Ni, Co, Zn) was twice as high as that of the more common iron hexacyanoferrates ("soluble" (KFeHCF) or "insoluble" (FeHCF) Prussian blue) [9]. More recently, manganese oxide was described as a new compound for binding of cobalt and caesium isotopes *in vitro* [11].

The aim of the present study in rats was to find out the most effective compound for inhibiting intestinal radiocaesium absorption *in vivo* and to establish an appropriate treatment procedure against incorporation of $^{137/134}$Cs from contami-

Abbreviations: KHCF, potassium hexacyanoferrate(II) ($K_4[Fe(CN)_6]\cdot 3\,H_2O$); KFeHCF, potassium iron(III) hexacyanoferrate(II) ($KFe[Fe(CN)_6]\cdot 2\,H_2O$); FeHCF, iron(III) hexacyanoferrate(II) ($Fe_4[Fe(CN)_6]_3\cdot 15\,H_2O$); NH_4FeHCF, ammonium iron(III) hexacyanoferrate(II) ($NH_4Fe[Fe(CN)_6]\cdot 2\,H_2O$); KCuHCF, potassium copper hexacyanoferrate(II) ($K_2Cu[Fe(CN)_6]\cdot 2\,H_2O$); KCoHCF, potassium cobalt hexacyanoferrate(II) ($K_2Co[Fe(CN)_6]\cdot 2\,H_2O$); KNiHCF, potassium nickel hexacyanoferrate(II) ($K_2Ni[Fe(CN)_6]\cdot 3\,H_2O$); KZnHCF, potassium zinc hexacyanoferrate(II) ($K_2Zn[Fe(CN)_6]\cdot 2\,H_2O$).

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Table I. Inhibition of radiocaesium absorption by HCF in animals (summary from published data).

Author (*et al.*)	Ref.	Year	Animal	Dose [mg]	Time of HCF application	Efficacy of various HCF compounds
Nigrovic	(2)	1965	rat	50	"immediately after 137-Cs"	KFeHCF = NaFeHCF > KNiHCF > KCoHCF > KCuHCF > KZnHCF
Brenot	(3)	1967	rat	10	2 min after 134-Cs	KFeHCF = KCoHCF > KBiHCF
Giese	(4)	1971	rat	50	within 30 s after 137-Cs	NH_4FeHCF > FeHCF > NaFeHCF > KFeHCF > CsFeHCF
Iinuma	(5)	1971	rat	100	30 min after 137-Cs	FeHCF = KNiHCF
Seletskaya	(6)	1973	rat	50	together with 137-Cs	KNiHCF > KFeHCF > KCoHCF > FeHCF
				0.5		KNiHCF > KCoHCF > KFeHCF
				0.1		KNiHCF > KCoHCF > KFeHCF
Nielsen	(7)	1988	piglet	100	together with 134-Cs	KFeHCF = NH_4FeHCF = FeHCF
Dresow	*	1989	rat	5	2 min before 134-Cs	KFeHCF = NH_4FeHCF = KCoHCF = KNiHCF > FeHCF > KCuHCF ≫ KZnHCF
				0.5		KFeHCF = NH_4FeHCF > KCoHCF = KNiHCF
				0.1		KFeHCF = NH_4FeHCF

* Results of this study.

nated food in humans and domestic animals in the case of severe nuclear accident.

Materials and Methods

^{134}CsCl (12.49 μCi/μg Cs) was obtained from Amersham International, England. Chemicals of analytical grade were obtained from E. Merck, Darmstadt (F.R.G.). Colloidal "soluble" and "insoluble" hexacyanoferrates(II) of Fe^{3+}, Co^{2+}, Cu^{2+}, Zn^{2+} and Ni^{2+} were prepared as described previously [9]. The crude products were exhaustively dialyzed against distilled water, concentrated in vacuum and dried by lyophilization. Ammonium-ferric(III)-hexacyanoferrate(II) ("Giese-Salt", containing 30–35% ammonium chloride) was purchased from Riedel de Haen, Seelze (F.R.G.). Manganese oxide was prepared according to Mikhail and Misak [11]. *In vitro* ^{134}Cs-sorption capacity was measured as described [9].

Animals

Female Wistar rats (260–280 g) were obtained from Wiga (Hannover, F.R.G.) and fed a standard rat diet (Altromin 1324, Altromin, Lage F.R.G.). Rats were housed in polyethylene cages with stainless steel wire tops or in metabolic cages (polyethylene cages with stainless steel grid floors). Animals were fasted 24 h prior and 6 h after oral application in cages equipped with grid floors to minimize coprophagy.

Hexacyanoferrates(II) in 0.5 ml water and tracer doses ^{134}CsCl (0.3–0.8 μCi) in 0.5 ml water were administered separately through a gastric tube, control animals received ^{134}CsCl only. The ^{134}Cs whole body activity, measured immediately after radiocaesium application in the center of a 4 π-geometry whole body radioactivity detector with organic liquid scintillator for humans in the energy range of 500–1800 keV was taken as 100% reference value [12–13]. After oral administration of the test compounds, the rats were placed in metabolic cages for 7 days to collect urine and faeces separately. The ^{134}Cs whole body retention was measured again 7 days after ^{134}Cs application by whole body counting. ^{134}Cs-activity in urine and faeces of rats was measured in the whole body counter described above.

Results and Discussion

Effect of the time of HCF-administration on radiocaesium absorption

At various time intervals and with different sequence, 5 mg of KFeHCF and carrier free ^{134}CsCl were administered separately through a gastric

tube to 5 fasted rats and the ^{134}Cs absorption was calculated from the measured whole body retention after 7 days. Tracer amounts of orally administered radiocaesium were absorbed very quickly (Table II). HCF application 30 min after $^{134}CsCl$ ingestion exhibited only a small inhibitory effect on enteral caesium absorption compared to the control group receiving no HCF. Even an application 2 min after the radiocaesium administration was still leading to a distinct ^{134}Cs absorption in rats. For this small amount of HCF (5 mg), optimal inhibition results were obtained by application of the hexacyanoferrate together or a few minutes before loading with radiocaesium.

Table II. Influence of various time intervals and different sequence of administration of KFeHCF (5 mg) and carrier free $^{134}CsCl$ on the ^{134}Cs-absorption in fasted rats (n = 5; ^{134}Cs whole body retention ≡ ^{134}Cs-WBR in percentage of the dose 7 days after administration).

Time of administration of KFeHCF in relation to ^{134}Cs	$^{134}Cs-WBR$ [%] $X_a \pm SD$	% of control
control	40.7 ± 1.0	100
60 min before	5.2 ± 3.1	12.8
30 min before	1.8 ± 0.20	4.4
2 min before	0.77 ± 0.49	1.9
together with	0.70 ± 0.30	1.7
2 min after	6.4 ± 0.60	15.7
30 min after	36.9 ± 0.60	90.7

Efficacy of different HCF in rats

In a pilot experiment, 5 mg amounts of the different hexacyanoferrate(II) compounds were administered to 8–10 rats by gastric intubation 2 min before oral loading with carrier-free $^{134}CsCl$. As judged by the ^{134}Cs whole body retention after 7 days (Table III, Fig. 1), KHCF had no significant effect on the caesium absorption. The efficacy of KZnHCF and KCuHCF was only small in comparison, although these compounds showed a high caesium sorption capacity *in vitro* [9]. This difference might be caused by the much shorter contact time between these compounds and radiocaesium in the intestinal tract compared to the *in vitro* experiments.

A distinct reduction of enteral radiocaesium absorption was observed by FeHCF, but 5 mg of KFeHCF, KNiHCF, KCoHCF, and NH_4FeHCF resulted in an almost complete blockade of the radiocaesium absorption. Differences in efficacy between these four compounds could not be derived from this experiment because of the too high 5 mg HCF dose. This is in contrast to other authors (Table I), who could set up an order of efficacy even with much higher HCF doses.

The caesium sorption effect of hexacyanoferrates(II) in the gastroenteral tract becomes obvious by regarding the ^{134}Cs-excretion in urine and faeces during 7 days after oral loading with $^{134}CsCl$ (Table III). In the control group (receiving no HCF), only 10% of the administered ^{134}Cs-activity was found in the faeces, whereas ^{134}Cs-application combined with oral HCF treatment leads with the best compounds to an almost complete (>98%) faecal excretion of the label. Obviously, there was found a good negative correlation between ^{134}Cs whole body retention and the excretion of ^{134}Cs-

Table III. Accumulated faecal and urinary excretion, whole body retention of ^{134}Cs in rats (n = 9–10; in % of oral dose after 7 days; mean ± SD). 5 mg of HCF compounds were administered though a gastric tube 2 min before oral $^{134}CsCl$-loading.

HCF-compound	Faecal excretion	Absorption (100% − faecal excretion)	Urinary excretion	Whole body retention ($^{134}Cs-WBR$)	Recovery (faec. + urin. excret. + $^{134}Cs-WBR$)
No	10.9 ± 1.72	89.1	44.0 ± 3.50	40.7 ± 1.04	95.6
KHCF	36.6 ± 23.6	63.4	23.4 ± 8.63	38.6 ± 1.51	98.6
KZnHCF	48.2 ± 13.0	51.8	19.6 ± 5.26	25.5 ± 6.31	93.3
KCuHCF	87.9 ± 5.73	12.1	7.93 ± 2.92	6.90 ± 6.87	102.7
FeHCF	93.9 ± 2.29	6.1	3.04 ± 0.85	3.32 ± 1.57	100.3
KFeHCF	97.2 ± 2.30	2.8	1.86 ± 1.72	0.77 ± 0.49	99.8
NH_4FeHCF	98.2 ± 1.60	1.8	0.77 ± 0.37	0.66 ± 0.53	100.7
KCoFeHCF	97.8 ± 2.19	2.2	1.24 ± 0.50	0.53 ± 0.32	99.6
KNiHCF	99.3 ± 1.07	0.7	1.00 ± 0.57	0.22 ± 0.21	100.5

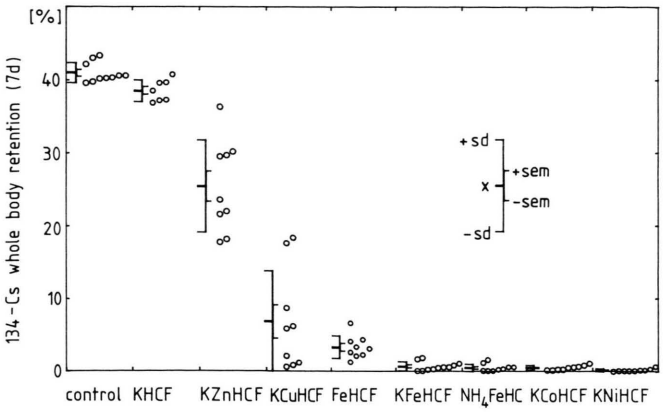

Fig. 1. Prevention of intestinal radiocaesium absorption in rats. *5 mg* of HCF compounds were administered through a gastric tube 2 min before oral ^{134}Cs-loading (carrier free ^{134}CsCl, 0.3–0.8 μCi). ^{134}Cs whole body retention was measured 7 days after oral application.

activity in faeces within 7 days after administration (Table III).

For the comparison of the most effective compounds (KFeHCF, NH_4FeHCF, KNiHCF, and KCoHCF) the HCF dose had to be reduced considerably since a tracer dose of ^{134}Cs was used. The administration of only 0.5 mg HCF 2 min before oral loading with carrier free ^{134}CsCl still showed an inhibiting effect (5.2–2.8% average ^{134}Cs-WBR) on the intestinal radiocaesium absorption. KFeHCF and NH_4FeHCF were effective to the same extent, whereas the inhibition effect of KNiHCF and KCoHCF was somewhat smaller (Fig. 2).

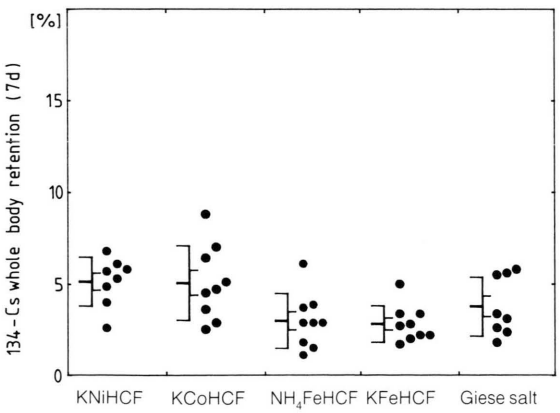

Fig. 2. Prevention of intestinal radiocaesium absorption in rats. *0.5 mg* of HCF compounds (Giese-Salt, 0.75 mg) were administered through a gastric tube 2 min before oral ^{134}Cs-loading (carrier free ^{134}CsCl, 0.3–0.8 μCi). ^{134}Cs whole body retention was measured 7 days after oral application.

As a result of this study at low HCF doses of 0.1 and 0.5 mg, the colloidal KFeHCF and NH_4FeHCF have to be considered as the most efficient antidotes for inhibiting the absorption of tracer doses of radiocaesium in rats. Higher doses of 5 mg HCF are required for obtaining the same inhibitory effects with FeHCF, KCoHCF and KNiHCF. This is in contrast to former studies of Seletskaya [6] or Giese [4] but can be compared to some extent with the results of Nigrovic [2], despite this author used 50 mg doses of HCF, which, as our data clearly show, was overdosed.

After the Chernobyl accident, Giese, based on his study in rats 1971 [4], suggested NH_4FeHCF (Giese-Salt, Riedel de Haen, F.R.G.) to be the most effective compound as antidote against radiocaesium absorption and for promoting the decorporation of already absorbed radiocaesium in domestic animals [8]. As shown in Fig. 2, the commercial preparation (administered dose: 0.75 mg NH_4FeHCF, because of its known content of 30–35% ammonium chloride) was somewhat less effective as compared to the purified (non-commercial) NH_4FeHCF and KFeHCF-preparations, used in this study. An equal potency of purified KFeHCF and NH_4FeHCF for *in vivo* radiocaesium elimination is demonstrated in Table IV.

As shown previously, only small amounts of ferric and ferrous iron, as well as of cyanide are released and absorbed from oral doses of ^{59}Fe and ^{14}CN labelled KFe[Fe(CN)$_6$] in piglets, rats and man [10, 14, 15]. This indicates that soluble Prussian blue can be used without reservation and therefore must be considered as the most valuable

Table IV. Efficacy of different oral doses of KFeHCF, NH_4FeHCF and MnO_2 on the radiocaesium absorption in fasted rats (n = 8–10) (^{134}Cs–WBR ≡ ^{134}Cs whole body retention in percentage of the dose 7 days after administration; mean ± SD).

Compound	Dose	^{134}Cs–WBR [%]	% of control
Control	–	40.7 ± 1.0	100
KFeHCF	0.1 mg	21.4 ± 4.8	52.6
	0.5 mg	2.8 ± 1.0	6.9
	5.0 mg	0.77 ± 0.49	1.9
NH_4FeHCF	0.1 mg	24.0 ± 3.9	59.0
	0.5 mg	3.0 ± 1.5	7.4
	5.0 mg	0.66 ± 0.53	1.6
MnO_2	5.0 mg	40.3 ± 2.4	99.0
	10.0 mg	39.4 ± 2.3	96.8

antidote for the inhibition of Cs-absorption and decorporation of already absorbed radiocaesium. For medical use in man a separation of low molecular by-products in crude commercial preparations is recommendable.

More recently, the capability of manganese oxide for the binding of radiocaesium isotopes *in vitro* was demonstrated by Mikhail and Mizak [11]. However, in comparison with HCF compounds, we found a much lower caesium sorption capacity for MnO_2 (water, 0.07 mmol Cs^+/g MnO_2; artificial duodenal juice pH 6.8, 0.03 mmol/g; artificial gastric juice pH 1.2, 0.005 mmol/g) as compared to KFeHCF (about 1.0 mmol Cs^+/g KFeHCF). In addition, oral administered MnO_2 (5 or 10 mg/rat), showed no significant inhibiting effect on the absorption of carrier free ^{134}Cs (Table IV). Therefore, this compound is not suitable as antidote against intestinal radiocaesium absorption.

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