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Pharmacokinetics and distribution of clioquinol in golden hamsters

Gianpietro Bondiolotti, Mariaelvina Sala, Claudia Pollera, Marco Gervasoni, Maria Puricelli, Wilma Ponti and Silvio R. Bareggi

Abstract

Clioquinol (5-chloro-7-iodo-8-quinolinol) is a zinc and copper chelator that can dissolve amyloid deposits and may be beneficial in Alzheimer's disease. Prion diseases are also degenerative CNS disorders characterised by amyloid deposits. The pharmacokinetics and tissue distribution of drugs active against prions may clarify their targets of action. We describe the pharmacokinetics of clioquinol in hamster plasma, spleen and brain after single and repeated oral or intraperitoneal administration (50 mg kg^{-1}), as well as after administration with the diet. A single intraperitoneal administration led to peak plasma clioquinol concentrations after 15 min (Tmax), followed by a decay with an apparent half-life of 2.20 ± 1.1 h. After oral administration, Tmax was reached after 30 min and was followed by a similar process of decay; the AUC_{0-last} was 16% that recorded after intraperitoneal administration. The Cmax and AUC values in spleen after a single administration were about 65% (i.p.) and 25% (p.o.) those observed in blood; those in liver were 35% (p.o.) those observed in blood and those in brain were 20% (i.p.) and 10% (p.o.) those observed in plasma. After repeated oral doses, the plasma, brain and spleen concentrations were similar to those observed at the same times after a single dose. One hour after intraperitoneal dosing, clioquinol was also found in the ventricular CSF. Clioquinol was also given with the diet; its morning and afternoon concentrations were similar, and matched those after oral administration. No toxicity was found after chronic administration. Our results indicate that clioquinol, after oral administration with the diet, reaches concentrations in brain and peripheral tissues (particularly spleen) that can be considered effective in preventing prion accumulation, but are at least ten times lower than those likely to cause toxicity.

Department of Pharmacology, Chemotherapy and Medical Toxicology, School of Medicine, University of Milan, Milan, Italy

Gianpietro Bondiolotti, Marco Gervasoni, Silvio R. Bareggi

Faculty of Sciences, University of Milan, Milan, Italy

Mariaelvina Sala

Department of Animal Pathology, Hygiene and Veterinary Public Health, Microbiological and Immunological Unit, Faculty of Veterinary Medicine, University of Milan, Milan, Italy

Claudia Pollera, Maria Puricelli, Wilma Ponti

Center of Excellence on Neurodegenerative Diseases, Milan, Italy

Gianpietro Bondiolotti, Mariaelvina Sala, Claudia Pollera, Marco Gervasoni, Maria Puricelli, Wilma Ponti, Silvio R. Bareggi

Correspondence: S. R. Bareggi, Dept. of Pharmacology, Via Vanvitelli 32, 20129 Milano, Italy. E-mail: silvio.bareggi@unimi.it

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Introduction

Transmissible spongiform encephalopathies (TSE) form a group of progressive, fatal neurodegenerative diseases affecting the central nervous system of man and animals (scrapie, bovine spongiform encephalopathy) (Will et al 1996; Hill et al 1997; Aguzzi et al 2001). They are characterised by a long incubation period and a very slow evolution that leads to typical histopathological changes in the CNS (Caughey & Chesbero 1997).

It is believed (Prusiner 1998) that the causative agents are proteinaceous infectious particles (prions). The pathological protein (PrP^{sc}) is the protease-resistant isoform of a glycosylphosphatidylinositol-anchored cell transmembrane molecule (PrP^{c}) that is mainly expressed in CNS neurons but also in many other cell types. As it is the main component of amyloid deposits and the cause of neurodegenerative CNS lesions, PrP^{sc} is the primary target for therapeutic strategies (Piccardo et al 1990; Ye et al 1998).

No therapy is yet available for the treatment of TSE, although a number of drugs have been tested (Kimberlin & Walker 1986b; Caughey & Race 1992; Caughey et al 1994; Ingrosso et al 1995; Caspi et al 1998; Demaimay et al 1998, 2000; Farquhar et al 1999; Beringue et al 2000; Milhavet et al 2000; Rudyk et al 2000; Poli et al 2003, 2004; Caramelli et al 2006).

Clioquinol (5-chloro-7-iodo-8-quinolinol) acts as a zinc and copper chelator, and it has been shown that metal chelation is a potential therapeutic strategy for Alzheimer's disease because the interaction of zinc and copper is involved in the deposition and

stabilisation of amyloid plaques and, by preventing metal–amyloid-beta interactions, chelating agents can dissolve the amyloid deposits (Cuajungco et al 2000; Cherny et al 2001; Bush 2003). As Alzheimer's disease and prion disease have a number of common features (they are CNS degenerative disorders characterised by amyloid deposits), it is conceivable that some drugs may be active in preventing both.

The hamster model is particularly suitable for TSE studies because the period required for development of experimental scrapie is shorter than in the mouse. When hamsters are intracerebrally infected by the 263K strain, the incubation period lasts two months and death occurs after about one month (Kimberlin & Walker 1977, 1986a). Preliminary results indicate that clioquinol may improve cognitive symptoms and prolong the survival of the infected animals (Ponti et al 2004).

Clioquinol is almost insoluble in water and many other vehicles, and is thus difficult to administer. When used as a drug in man (Enterovioform) it is combined with a surfactant (sapamine); in experimental animals it has been administered as a suspension in carboxymethylcellulose by means of oral gavage (Hayashi et al 1976a, b), but this is too distressing a method for long-term chronic treatment and a single daily administration cannot be used to measure active concentrations over 24 h. Its poor water solubility also makes it almost impossible to administer clioquinol intravenously without a specific pharmaceutical formulation, which is why most previous studies of acute toxicity in many species have been performed using intraperitoneal administration (Chen et al 1976; Kotaki et al 1983a).

Previous studies in rodents (mice and rats) have shown that clioquinol is extensively metabolised to glucuronated and sulfate metabolites (Chen et al 1976; Hayashi et al 1976a, b; Kotaki et al 1983b), but there are no data concerning hamsters.

To understand the absorption and distribution of clioquinol, and find the most appropriate way of chronically administering it to enable us to investigate its effects in the hamster model of TSE, we studied its pharmacokinetics after direct acute and chronic oral doses, as well as its concentrations after its addition to a standard diet. Furthermore, to obtain data concerning its relative bioavailability after parenteral administration, we also determined the concentrations of clioquinol at various times after intraperitoneal injection.

Materials and Methods

Chemicals and reagents

The 5-chloro-7-iodio-8-hydroxyquinoline, 5,7-dichloro-8-hydroxyquinoline, phenolphthalein glucuronic acid, carboxymethylcellulose (CMC-Na), polysorbate 80 (Tween 80) and β-glucuronidase (25 000 U/0.4 mL) were purchased from Sigma-Aldrich (St Louis, MO) and the analytical reagent from Merck (Darmstadt). The aqueous solutions were in reagent-grade water obtained using a Milli-Q system (Millipore, Bedford, MA). The stock solutions (0.5 mg mL^{-1} in methanol) were prepared every month and stored at 4°C.

Clioquinol administration

Female Syrian golden hamsters, 180–190 g (Charles River), were individually housed in cages with a controlled environment (lights on 0700–1900 h, temperature 22°C). Food and water were provided freely. After an overnight fast, clioquinol 50 mg kg^{-1} suspended in CMC-Na was given orally by gavage in single or repeated doses (twice a day for seven days); the same clioquinol dose suspended in Tween 80 was also given as a single intraperitoneal injection. Blood and tissues were obtained 0.5, 1.0, 2.0, 4.0 and 7.0 h after the single oral or intraperitoneal doses (at least five hamsters were used each time), and 2.0 and 4.0 h after the repeated doses (four hamsters).

Clioquinol was also chronically administered to normal hamsters by feeding the pellets of hamster standard diet (Piccioni Laboratories, Milan, Italy) containing clioquinol 0.5 mg g^{-1}, which is equivalent to a dose of about 50 mg kg^{-1} calculated on the basis of controlled food consumption of 10 g/day. Blood and tissues were obtained from four hamsters killed in the morning (0900 h) and afternoon (1600 h) after they had been fed the clioquinol-containing diet for one month.

All of the hamster care and treatment procedures respected the guidelines established by Italian Government Decree No. 94/2000-A and the EU International Guidelines, and every effort was made to minimise the number of hamsters used and their suffering.

Blood and tissue sampling

Blood was drawn by a syringe directly from the hearts of the hamsters under chloral hydrate anaesthesia using heparin as anticoagulant; after centrifugation (3500 *g*, 15 min, 4°C), the plasma was separated and stored at −80°C until analysis. The hamsters were then decapitated and their brains were rapidly removed, cut longitudinally into two parts and immediately frozen on dry ice; whole spleens and a piece of liver were also taken and stored at −80°C until analysis. Samples (0.05–0.2 mL) of the plasma or tissue supernatant were analysed within two weeks.

Clioquinol analysis

The plasma and tissue samples were prepared as described by Bondiolotti et al (2006); the CSF samples (50 μL) were injected directly into the chromatograph.

For plasma analysis, a volume of 0.02–0.2 mL was deproteinised with 0.2 mL of 0.6 N perchloric acid containing sodium disulfite 1 mg mL^{-1}, disodium ethylendiaminotetraacetate (EDTA) 0.5 mg mL^{-1}, and 40 ng of 5,7-dichloro-8-hydroxyquinoline as internal standard (IS), after which 0.2 mL of methanol was added and mixed by vortexing. After centrifuging at 13 000 *g* for 15 min at 4°C, 50 μL of clear supernatant was injected into the chromatograph.

For tissue analysis, the brain, spleen and liver were homogenised in 1 mL of 0.6 N perchloric acid containing 120 ng of IS, and then 0.5 mL of methanol was added and

the tissues homogenised again. After centrifugation, 50 μL of supernatant was injected into the chromatographic system.

Glucuronated clioquinol was examined in plasma after enzymatic deconjugation. A 500-μL sample was mixed with 400 μL of distilled water, 100 μL of 1 M acetate buffer (pH 5) and enough β-glucuronidase to reach a concentration of 200 U mL^{-1}; phenolphthalein glucuronide was used as the IS. The solutions were then incubated at 37°C for 2 h, and the free clioquinol and phenolphthalein were extracted and determined as described above. Clioquinol sulfate was determined after acid hydrolysis.

The clioquinol analysis was made using a new HPLC method with electrochemical detection described in detail elsewhere (Bondiolotti et al 2006). In brief, the chromatographic system consisted of a Shimadzu LC-6A pump (Kyoto, Japan), a refrigerated Dhimadzu SIL-9A autosampler and a Coulochem 5100A ESA dual potentiostat electrochemical detector (Bedford, MA). The data were collected using a Shimadzu CR-4A data processor. The samples were quantitated by interpolating the peak area ratios (analyte/internal standard) on a standard curve and the results expressed as ng mL^{-1} (plasma) or ng g^{-1} (tissue).

The analytes were separated on a reversed-phase Nucleosil C-18 column (7 μm, 300 × 3.9 mm), and detected using a dual electrode analytical cell (model 5011A) with the first electrode (E1) set at +300 mV and the second (E2) at +700 mV. The mobile phase consisted of a phosphate/citrate buffer 0.1 M (400 mL) with 600 mL of methanol–acetonitrile (1:1, v/v), and was delivered at a flow rate of 1.0 mL min^{-1}. All of the analyses were made at 22°C. The retention times were 8.1 min for IS and 11.6 min for clioquinol. The mean intra-day and inter-day assay accuracies of the method were more than 94% in plasma and tissues, with a mean precision (CV) of less than 8%. The results obtained using the quality control samples kept at −25°C for two weeks guaranteed sample stability under our conditions. The lowest clioquinol detection limit in plasma and tissues (a signal three times that of the blank) was 2 ng mL^{-1}; the lowest quantitation limit (CV ≤ 8%) was 5 ng mL^{-1} and the assay was linear from 5 to 2000 ng. The mean absolute recovery of clioquinol was more than 73% in plasma and 75% in tissues.

Pharmacokinetic and statistical analysis

After oral and intraperitoneal admininistration, the non-compartmental pharmacokinetic parameters, including elimination half-life (t½), the area under the concentration–time curve from the time of dosing to the last post-dose time (AUC_{0-last}; trapezoidal method) and the AUC extrapolated to infinity (AUC_{0-Last}/L_2, where L_2 is the elimination constant), were estimated using Kinetica software (Innaphase, USA); the experimental values were the peak concentrations (Cmax) and the time to reach them (Tmax).

The concentrations after single and repeated oral administration were compared by means of analysis of variance with Tukey's test for multiple comparisons; those obeserved in the morning and afternoon were compared by means of RM-analysis of variance with Tukey's test for multiple comparisons using the SigmaStat statistical package (Systat Software Inc., Point Richmond, CA)

Results

Plasma clioquinol concentrations

Single administration

The mean plasma concentrations after single oral and intraperitoneal administration (50 mg kg^{-1}) to hamsters are shown in Figure 1. After intraperitoneal administration (Table 1, Figure 1A), absorption was very rapid (Tmax 15 min) and the apparent half-life was 2.02 ± 0.5 h; after oral administration (Table 1, Figure 1B), the Tmax was reached after 30 min and the apparent half-life was 2.58 ± 0.9 h. Cmax and AUC_{0-last} after oral administration were about 16% of those after intraperitoneal administration (Table 1). After both oral and intraperitoneal administration, glucuronate metabolites were found as 27–35% of total clioquinol (Figure 1) but no sulfate metabolites were detected.

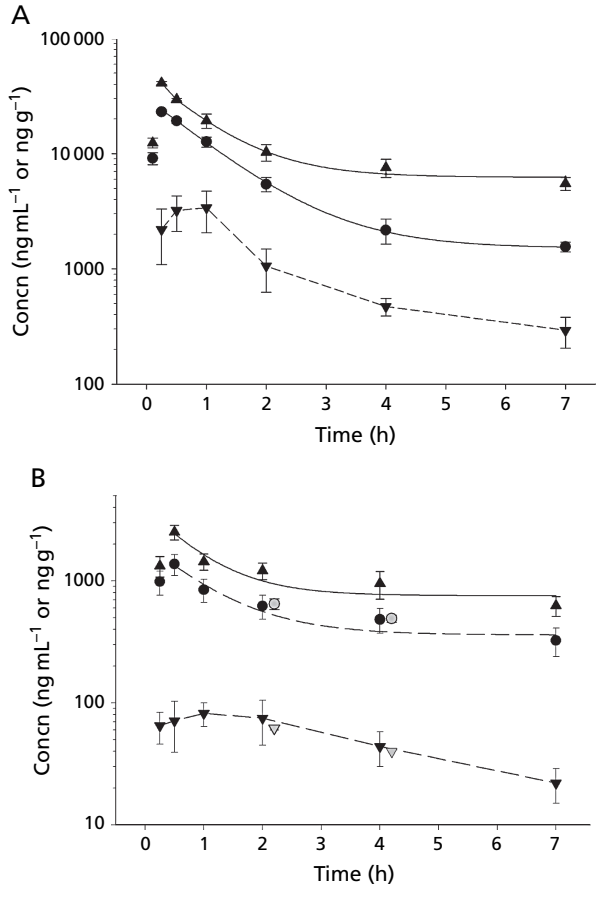

Figure 1 Time course of mean plasma free clioquinol (circles) and total (free + glucuronated) clioquinol (upward pointing triangles) concentrations, and brain concentrations (downward pointing triangles) in hamsters, after a single intraperitoneal dose of 50 mg kg 1 (A), after a single oral dose of 50 mg kg^{-1}, and after one week's administration (grey symbols) (B).

Table 1 Main pharmacokinetic parameters of clioquinol in hamsters after a single intraperitoneal (i.p.) or oral (os) administration

	Tmax (min)		Cmax (ng mL^{-1} or g)		Half-life (t½β, h)		$AUC_{0-\infty}$, (ng·min mL^{-1} or ng·min g^{-1})		Tissue/plasma (AUC %)	
	i.p.	os	i.p.	os	i.p.	os	i.p.	os	i.p.	os
Plasma	15	30	23160 ± 860.1	1375.1 ± 296.0	2.20 ± 1.1	2.55 ± 0.7	41269.6 ± 2782.5	6880.9 ± 1580.4	—	16.3 ± 6.1[a]
Brain	60	60	3265.8 ± 1082.7	82.1 ± 29.3	2.25 ± 1.2	2.10 ± 0.6	8542.3 ± 1681.3	635.4 ± 148.2	20.5 ± 2.3	10.0 ± 1.2
Spleen	30	30	13358.4 ± 895.9	267.5 ± 139.2	2.11 ± 0.7	2.36 ± 1.0	25697.5 ± 3542.5	2061.3 ± 196.3	63.6 ± 3.9	29.5 ± 10.2
Liver	30	60	—	481.3 ± 165.2	—	5.26 ± 1.7	—	4325.6 ± 211.2	—	61.7 ± 11.7

Values are means ± s.d. of at least five determinations. [a]% vs i.p.

Repeated oral doses

The plasma concentrations after repeated oral doses to hamsters were not statistically different from those observed after a single dose at the same times ($P > 0.1$, analysis of variance, Tukey's test; Table 2, Figure 1).

Clioquinol administered with the diet

The plasma concentrations of clioquinol following administration in the hamster diet are shown in Table 3. The morning and afternoon values were not statistically different ($P > 0.1$, RM-analysis of variance, Tukey's test), and approximately those found 4–7 h after oral dosing. Conjugated clioquinol accounted for about 30% of the total.

Tissue clioquinol concentrations

Single administration

The mean peak clioquinol concentrations in brain, liver and spleen of hamsters after single intraperitoneal or oral administration are shown in Table 1, and the time-course in brain in Figures 1A and 1B. The time-course in peripheral tissues (not shown) paralleled that in plasma. The AUCs in the peripheral tissues (spleen, liver) were 25–35% of those in plasma after oral dosing, but larger after intraperitoneal dosing (65% in spleen). Brain concentrations were also higher after intraperitoneal dosing, being 21% of the plasma concentrations as against 11% after oral dosing.

One hour after intraperitoneal dosing, clioquinol was also found in ventricular CSF at concentrations of 228.2 ± 16.5 ng mL^{-1}, which were 7% of those found in brain at the same time.

Repeated administration

After repeated oral doses, the concentrations in hamster brain (Table 2 and Figure 1), spleen and liver (Table 2) were not statistically different from those observed after a single dose at the same times ($P > 0.1$, analysis of variance, Tukey's test).

Table 2 Concentrations of clioquinol in plasma and tissues of hamsters after repeated oral administration

	Free clioquinol concn (ng mL^{-1} or ng g^{-1})		Glucuronated clioquinol concn (ng mL^{-1}) (% total)		Tissue/plasma (%)	
	2 h	4 h	2 h	4 h	2 h	4 h
Plasma	648.7 ± 98.2[a]	590.5 ± 48.5[a]	189.1 ± 27.3 (30%)	177.3 ± 13.6 (27%)	—	—
Brain	62.3 ± 22.3[a]	28.0 ± 14.8[a]			11.7 ± 3.4	4.8 ± 2.1
Spleen	104.1 ± 57.6[a]	126.3 ± 66.4[a]			16.8 ± 9.1	21.3 ± 9.3
Liver	455.3 ± 92.5[a]	260.1 ± 102.3[a]			69.7 ± 14.4	44.5 ± 15.3

Values are means ± s.d., n = 4. [a]Not statistically different from concentrations after a single dose (analysis of variance, Tukey's test).

Table 3 Concentrations of clioquinol in plasma and tissues of hamsters after oral administration with diet

	Free clioquinol concn (ng mL^{-1} or ng g^{-1})		Glucuronated clioquinol concn (ng mL^{-1})		Tissue/plasma (Cmax %)	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
Plasma	381.5 ± 86.0	284.5 ± 37.1[a]	197.0 ± 39.6 (34%)	124.0 ± 21.1 (30%)[a]	—	—
Brain	62.2 ± 24.4	45.5 ± 13.5[a]	—	—	16.4 ± 6.2	15.5 ± 4.1
Spleen	114.7 ± 12.4	60.7 ± 12.8[b]			29.7 ± 5.2	21.9 ± 4.6
Liver	297.0 ± 89.2	246.0 ± 59.1[a]			76.2 ± 22.8	85.3 ± 20.1

Values are mean ± s.d., n = 5. [a]Not statistically different from morning concentrations; [b]$P < 0.01$ vs morning concentrations (RM-analysis of variance, Tukey's test).

Clioquinol administered with the diet

Table 3 shows the tissue clioquinol concentrations after administration with hamster diet. The morning and afternoon values were not statistically different ($P > 0.1$, RM-analysis of variance, Tukey's test), except in the case of the spleen concentrations, which were lower in the afternoon ($P < 0.01$, RM-analysis of variance, Tukey's test).

Discussion

We here describe the pharmacokinetics of clioquinol, a chelator of many metals, such as copper and zinc, that has amyloidolytic properties and improves the symptoms of Alzheimer's disease (Bush 2003); in hamsters experimentally infected with scrapie, it reduces amyloid formation in-vitro (Formentin et al 2005) and delays the appearance of clinical symptoms and increases survival time in-vivo (Ponti et al 2004).

Our analytical method is highly sensitive and specific (Bondiolotti et al 2006), and can therefore be used in pharmacokinetic studies in animals and man. As it does not require time-consuming sample preparation, it can also be used to monitor plasma clioquinol levels in patients.

The absorption and distribution of clioquinol was studied in golden hamsters after single and repeated oral doses, and compared with the results observed after it was administered intraperitoneally or added to standard hamster chow.

Clioquinol was rapidly absorbed after a single intraperitoneal injection as well as after oral dosing: peak plasma concentrations were reached after 15 (i.p.) or 30 min (p.o.), and then decayed with an apparent elimination half-life of about 2 h. There was no accumulation in peripheral tissue or brain as the concentrations after repeated doses were similar to those observed after a single dose.

To obtain constant concentrations through the day (an important factor for chronic studies, testing the effectivenes of a drug in preventing prion replication and diffusion), clioquinol was administered with the diet. This led to similar plasma and tissue levels in the morning and afternoon, and thus seems to be a valuable way of chronically administering the drug.

We also tested the alternative of administering clioquinol in drinking water, but its poor hydrosolubility made it impossible to dissolve clinically active doses without increasing or decreasing pH to values that are incompatible with animal administration.

Although to a lesser extent than suggested by its relatively high liposolubility, clioquinol crosses the blood–brain barrier: it was found in both brain tissue and CSF, the brain–plasma ratio being about 20% (7% of this was also found in ventricular CSF).

The amount of clioquinol metabolised to conjugated compounds is much lower in hamsters (30% glucuronate, and hardly any sulfate metabolites) than in rats which, in comparison with the free compound, have four-fold higher concentrations of glucuronate metabolites and ten-fold higher concentrations of sulfate metabolites (Hayashi et al 1976a, b; Kotaki et al 1983b).

Plasma clioquinol concentrations were lower after oral than after intraperitoneal administration, with a relative bioavailability (never previously determined) of about 16%. This low level of absorption obtained using an oral suspension in carboxymethylcellulose justifies the use of a surfactant (Hayashi et al 1976a, b) as in the case of the pharmaceutical formulation of Enterovioform.

Relatively high concentrations of clioquinol were found in peripheral tissues although they were lower than those found for the previously tested Congo Red derivative (Gervasoni et al 2004). The dietary administration of clioquinol led to spleen concentrations that were as much as 30% those observed in plasma, and it is known that the spleen is important in peripheral PrP invasion (McBride et al 1992; Lazmenas et al 1996; Klein et al 1997; Glatzel & Aguzzi 2000; Race et al 2000). The plasma and tissue concentrations obtained after chronic administration are in the range of those capable of reducing amyloid levels: in-vitro studies have shown that 120 ng mL^{-1} can dissolve amyloid by 200% (Cherny et al 2001) and 50 ng mL^{-1} can reduce PrP expression in cell cultures (Formentin et al 2005).

In the early 1970s, the toxic syndrome known as subacute myelo-optic neuropathy (SMON) was associated with the use of Enterovioform, particularly in the Japanese population, and for this reason it was withdrawn from the market. Many toxicity studies were performed in various animal species to define the toxic doses (Tateishi & Otsuki 1975) and concentrations of the drug (Chen et al 1976; Kotaki et al 1983a). The plasma and tissue clioquinol concentrations in our hamsters were always lower than the toxic levels indicated for rats, which showed some signs of toxicity after the chronic administration of 200 mg kg^{-1} daily and had free clioquinol concentrations of more than 15 $\mu g mL^{-1}$ (Kotaki et al 1983a), and dogs, which showed signs of toxicity after 100 mg kg^{-1} daily with concentrations of about 14 $\mu g mL^{-1}$ (Chen et al 1976; Hayakawa et al 1977). Tateishi & Otsuki (1975) report a study by Egashira & Kitamura who found toxicity of clioquinol administered with pesticides at 500 mg kg^{-1}. Moreover, our hamsters that were chronically treated with the diet were used as controls in an experiment to test the effects of clioquinol on the behavioural modifications induced by prion infection (Bareggi et al 2003). They received 50 mg kg^{-1} daily for up to four months and never showed any signs of toxicity in terms of behavioural changes (motility and cognitive functions) or histologically at the end of the experiment.

Clioquinol has been used to treat Alzheimer's disease at doses of up to 750 mg per day. The dose–concentration ratios of free clioquinol found in hamster were similar to those found in other rodents (0.03 in hamsters, 0.07 in mice, 0.09 in rats, 0.01 in rabbits), but much lower than those found in man, in whom the dose–concentration ratio is 0.64–1.4 at doses of 250 or 500 mg (Chen et al 1976; Jack & Riess 1973) (i.e. a mean of 33 times the free clioquinol concentrations found in hamsters). It is known that clioquinol is much less metabolised to conjugates in man than in rodents (Chen et al 1976; Hayashi et al 1976b), and it can therefore be estimated that the 50 mg kg^{-1} daily dose used here is seven times lower than the average dose used in man to treat Alzheimer's disease (7.1 mg kg^{-1} with a 500-mg daily dose for a 70-kg man) and very far from the estimated toxic dose (SMON) of 20–30 mg kg^{-1} per day.

Conclusions

Our results in hamsters indicate that clioquinol after oral administration with the diet reaches concentrations in brain

and peripheral tissues (particularly spleen) that can be considered effective in preventing prion accumulation but are at least ten times lower than those estimated to produce toxicity.

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