JPP Journal of Pharmacy and Pharmacology

JPP 2004, 56: 323–328

Received September 24, 2003
Accepted November 27, 2003
DOI 10.1211/0022357022854
ISSN 0022-3573

Pharmacokinetics and distribution of sodium 3,4-diaminonaphthalene-1-sulfonate, a Congo Red derivative active in inhibiting PrP^{res} replication

M. Gervasoni, R. Pirola, C. Pollera, S. Villa, G. Cignarella, P. Mantegazza, G. Poli and S. R. Bareggi

Abstract

Sodium 3,4-diaminonaphthalene-1-sulfonate (CRA) is a compound, synthesised by our group from Congo Red (CR), that is active in preventing the pathological conversion of normal prion protein (PrP). As the precise mechanisms controlling the ways in which prions are distributed and infect the brain and other organs are not fully understood, studying the pharmacokinetics of drugs that are active against prions may clarify their targets and their means of inhibiting prion infection. This paper describes the pharmacokinetics of CRA in plasma, spleen and brain after single or repeated intraperitoneal or subcutaneous administration, as determined by means of specific and sensitive fluorimetric HPLC. A single intraperitoneal administration led to peak plasma CRA concentrations after 15 min, followed by biphasic decay with an apparent half-life of 4.3 h. After subcutaneous administration, T_{max} was reached after 30 min, and was followed by a similar process of decay: C_{max} and the AUC_{0-last} were 25% those recorded after intraperitoneal administration. The mean peak concentrations and AUCs of CRA after a single intraperitoneal or subcutaneous administration in peripheral tissue (spleen) were similar to those observed in blood, whereas brain concentrations were about 2% those in plasma. After repeated intraperitoneal or subcutaneous doses, the C_{max} values in plasma, brain and spleen were similar to those observed at the same times after a single dose. After repeated intraperitoneal doses, CRA was also found in the ventricular cerebrospinal fluid at concentrations of $1.8 \pm 0.2\ \mu g\ mL^{-1}$, which is similar to, or slightly higher than, those found in brain. Brain concentrations may be sufficient to explain the activity of CRA on PrP reproduction in the CNS. However, peripheral involvement cannot be excluded because the effects of CRA are more pronounced after intraperitoneal than after intracerebral infection.

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

M. Gervasoni, R. Pirola, P. Mantegazza, S. R. Bareggi

Department of Animal Pathology, Hygiene and Public Health, Section of Microbiology and Immunology, School of Veterinary Medicine, University of Milan, Italy

C. Pollera, G. Poli

Pharmaceutical and Toxicological Chemistry Institute of the Faculty of Pharmacy Department of Chemistry, School of Pharmacy, University of Milan, Italy

S. Villa, G. Cignarella

Centre of Excellence on Neurodegenerative Disorders, Milan, Italy

M. Gervasoni, R. Pirola, C. Pollera, S. Villa, C. Cignarella, P. Mantegazza, G. Poli, S. R. Bareggi

Correspondence: S. R. Bareggi, Department of Pharmacology, Chemotherapy and Medical Toxicology, School of Medicine, University of Milan, Via Vanvitelli 32, 20129 Milano, Italy.
E-mail: silvio.bareggi@unimi.it

Funding: This study was supported by funds from the Italian Ministry of University and Research (COFIN).

Introduction

Prion diseases or transmissible spongiform encephalopathies (TSEs) are a group of neurodegenerative and invariably fatal diseases affecting the central nervous system (CNS) of man and animals, which are characterised by a long incubation time and a slow evolution. TSEs are mainly represented by Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker syndrome and fatal familial insomnia in man, and by scrapie and bovine spongiform encephalopathy (BSE) in animals (Will et al 1996; Hill et al 1997). The epizootic BSE that recently spread in the UK, and the first cases of a new human spongiform encephalopathy called new juvenile variant CJD (vCJD), alarmed the entire scientific community because of the risk of bovine prion transmission to man, a hypothesis that was supported by the discovery that vCJD is caused by the same prion as that found in BSE (Will et al 1996; Hill et al 1997; Aguzzi et al 2001). Individuals affected by TSE develop no inflammatory or immune response, and undergo typical histopathological changes in the CNS – vacuolisation, neuronal death, amyloid deposition, hypertrophy and the proliferation of astroglial cells (Caughey & Chesebro 1997).

According to Prusiner's prion hypothesis (Prusiner 1998), the TSE agents consist of infectious abnormal prion protein (PrP^{res}), which is formed from a host-encoded

precursor protein (PrP^{sen}) by means of a post-translational process involving changes in its secondary structure (Collinge 2001). PrP^{res} is the main cause of the amyloid deposition and neurodegenerative lesions observed in the CNS of TSE-infected subjects, and is therefore the primary therapeutic target (Piccardo et al 1990; Ye et al 1998).

Although various drugs have been tested, there is still no treatment for TSE. Congo red, a sulfonated azo dye that is commonly used in histopathology to stain various amyloid deposits, including those encountered in TSE (Glenner 1980), can inhibit the replication of TSE agents and the accumulation of PrP^{res} in animals and scrapie-infected mouse neuroblastoma cells (Kimberlin & Walker 1986; 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), and this induced us to design molecules that are structurally related to Congo Red.

We have tested a number of synthesised Congo Red derivatives (Villa et al 2003) in cell-free systems consisting of partially purified PrP^{res} to evaluate their ability to over-stabilise PrP^{res} (as is done by Congo Red), as well as in the in-vivo scrapie-infected hamster model (Poli et al 2003, 2004).

Sodium 3,4-diaminonaphtalene-1-sulfonate (CRA) is the product of Congo Red hydrogenolysis, and it can be hypothesised that this reaction may also occur in-vivo as a result of hepatic azo reductase (Bos et al 1986). It is also the least toxic and most interesting compound that we have tested so far (Poli et al 2004). As its ability to cross the blood–brain barrier cannot be predicted by its physicochemical properties, and it is more active after intraperitoneal than after intracerebral infection (Poli et al 2004), its pharmacokinetics and distribution may help to clarify its mechanism of action.

This paper describes the pharmacokinetics of CRA in plasma and various tissues after single or repeated intraperitoneal or subcutaneous administration, as determined by means of a specific and sensitive HPLC method with fluorimetric detection. The possibility of the formation of CRA after Congo Red administration was also tested.

Materials and Methods

Chemicals and reagents

Congo Red was obtained from Sigma (Sigma-Aldrich, St Louis, MO), and CRA and sodium 4-aminonaphthalene-1-sulfonate (internal standard, IS), were synthesised by the Pharmaceutical and Toxicological Chemistry Institute of the Faculty of Pharmacy. The methanol was HPLC grade, and the tetra-n-butylammoniumiodide (TEBA), sodium disulphite ($Na_2S_2O_5$), sodium sulfate (Na_2SO_4), zinc sulfate ($ZnSO_4 \times 7H_2O$), barium hydroxide ($Ba(OH)_2 \times 8H_2O$) and cysteine hydrochloride were of analytical grade (Merck, Darmstadt, Germany). Isolute liquid–solid extraction columns, packed with 50 or 200 mg of ENV+ hydrophobic phase and with a 1- or 3-mL reservoir (International Sorbent Technology Ltd, Mid-Glamorgan, UK) were respectively used to process the plasma and brain tissue samples. Stock drug solutions ($1\,mg\,mL^{-1}$) in 1% sodium disulphite (antioxidant) in water were prepared weekly and stored protected from light at 4 °C. Standard working solutions were prepared daily immediately before use in 1% sodium disulphite in water.

CRA and CR administration

The plasma and tissues were obtained from 100–110-g female Syrian hamsters (6 for each time point) treated intraperitoneally or subcutaneously with a single dose of CRA $100\,mg\,kg^{-1}$ (acute administration) or eight doses on different days (chronic administration).

Before, and 15, 30, 45, 60, 90, 120, 180 and 240 min after, the single administrations, the hamsters were anaesthetised with diethyl ether. Blood was drawn by means of a heart puncture and centrifuged, after which the plasma was stored at −25 °C until analysis. After sacrifice, the hamsters' brains and spleens were removed, washed with physiological solution and 1% sodium disulphite, wiped with clean paper, weighed and frozen at −25 °C.

After the chronic intraperitoneal or subcutaneous administration of CRA, the blood and tissues were taken as described above 15, 30, 60 and 90 min after the last injection. Fifteen minutes after chronic intraperitoneal administration, ventricular cerebrospinal fluid (CSF) was also taken just before removing the brain.

Chronic Congo Red treatment (8 doses of $100\,mg\,kg^{-1}$ i.p. on different days) was also given to evaluate the possible formation of CRA, with blood samples being drawn as above 60, 90, 120 and 240 min after the last injection.

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

Samples (0.1–1 mL) of the plasma or tissue supernatant were analysed within two weeks according to a modified version of a previously described HPLC method (Pirola et al 2002) that uses fluorimetric, instead of UV, detection as described below.

CRA was extracted from the plasma or tissues in solid phase as previously described (Pirola et al 2002). The CSF samples (25–50 μL) were injected directly into the chromatograph.

CRA analysis

The HPLC analysis was made using a modified version of a previously described method (Pirola et al 2002) and an Agilent Technologies 1100 instrument (Wilmington, DE) equipped with an autosampler, quaternary pump and fluorimetric detector. The wavelengths were set at 350 nm for excitation and 480 nm for emission. The chromatograms were analysed and quantitated using Agilent Technologies ChemStation for LC 3D software. The CRA was separated on a 5 μm Symmetry C18 column (250×4.6 mm i.d.) (Waters, Milford, MA), with a 2-cm pre-column filled

with the same material and kept at 30 °C. The compounds were eluted at 1 mL min^{-1} using 50 mM Na_2SO_4 in 5 mM TEBA in water–methanol–acetonitrile (70:20:10 v/v) as mobile phase. Under these conditions, peak CRA and IS had retention times of 5.5 and 6.7 min, respectively, without any interfering peaks.

The mean intra-day and inter-day assay accuracies of the method were more than 95% in plasma and tissues, with a mean precision of less than 4%. The results obtained using the quality control samples kept at −25 °C for two weeks guarantee sample stability under our conditions. The lowest CRA detection limit in plasma and tissues (a signal three times that of the blank) was 1 ng mL^{-1}; the lowest quantitation limit (CV < 8%) was 2 ng mL^{-1}. The mean absolute recovery of CRA was more than 76% in plasma and 74% in tissue.

Pharmacokinetic analysis

After the subcutaneous and intraperitoneal administrations, non-compartmental pharmacokinetic parameters, including the elimination half-life ($t_{1/2}$) and the area under the concentration–time curve from the time of dosing to the last post-dose time point (AUC_{0-last}), were estimated by means of Kinetica software (Innaphase, USA) using the experimental peak concentration values (C_{max}) and the time to reach them (T_{max}).

Results

The HPLC method with fluorimetric detection for the determination of CRA is a modification of a previous UV method (Pirola et al 2002), and substantially improved both the sensitivity (more than 100 times) and specificity of the assay. It was linear over a wide range of concentrations (2–5000 ng mL^{-1} or ng g^{-1}), and therefore suitable for determining the concentrations of CRA in plasma and peripheral tissues, as well as in the brain and CSF samples, even at time-points long after the administration of a single dose. Furthermore, as this method is also more selective, the interfering peaks (which had to be separated when UV detection was used) are no longer detectable, thus allowing shorter analyses.

Plasma CRA concentrations

Single administration

The mean plasma CRA concentrations after single intraperitoneal or subcutaneous administration are shown in Figure 1. After intraperitoneal administration (Table 1, Figure 1A), absorption was very rapid (15 min), and the biphasic decay had apparent half-lives of 1.5 and 4.3 h (β phase). After subcutaneous administration (Table 1, Figure 1B), T_{max} was reached after 30 min and was followed by a similar process of biphasic delay. C_{max} and AUC_{0-last} were 25% of those recorded after intraperitoneal administration (Table 1).

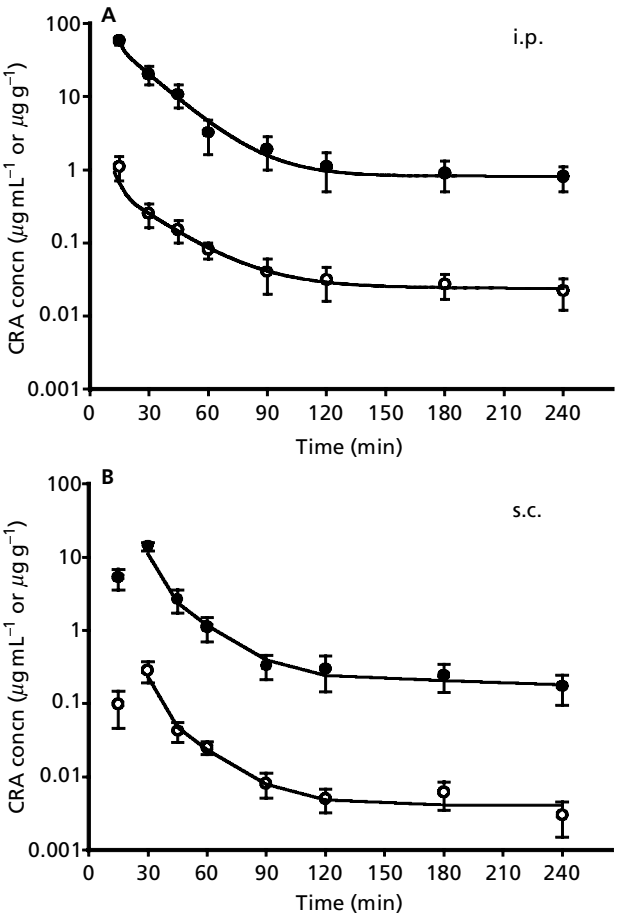

Figure 1 CRA concentrations in plasma (•) or in brain (○) in hamsters after intraperitoneal (A) or subcutaneous (B) administration of 100 mg kg^{-1}. Data are presented as means ± s.d., n = 6.

Repeated doses

After repeated intraperitoneal or subcutaneous doses the C_{max} in plasma (Table 2) was similar to that observed at the same times after single doses (Table 1).

CRA concentration after Congo Red administration

After repeated high doses of Congo Red, only very small amounts of CRA (mean: 0.07 ± 0.01 μg mL^{-1}) were found at all times, without any trend to increase or decrease over time.

Tissue CRA concentrations

Single administration

The mean peak CRA concentrations in brain and spleen after a single intraperitoneal or subcutaneous administration are shown in Table 1, and the time-course in brain is shown in Figures 1A and 1B. The peak concentrations and AUCs in peripheral tissue (spleen) were similar to those observed in blood, whereas those in brain were about 2% of those in plasma (Table 1).

Table 1 Pharmacokinetic parameters of CRA in plasma and tissues after a single intraperitoneal or subcutaneous administration.

	T_{max} (min)		C_{max} ($\mu g\,mL^{-1}$ or $\mu g\,g^{-1}$)		Half-life ($t_{1/2}\,\beta$, h)		AUC_{0-last}, ($\mu g \cdot min\,mL^{-1}$ or $\mu g \cdot min\,g^{-1}$)		Tissue/plasma (AUC %)	
	Intra-peritoneal	Subcuta-neous	Intra-peritoneal	Subcuta-neous	Intra-peritoneal	Subcuta-neous	Intra-peritoneal	Subcuta-neous	Intra-peritoneal	Subcuta-neous
Plasma	15	30	57.9 ± 6.6	14.1 ± 4.2	4.3 ± 0.8	5.2 ± 1.2	2936.2 ± 654.7	752.7 ± 165.5	—	—
Brain	15	30	1.1 ± 0.7	0.28 ± 0.05	3.3 ± 0.7	3.8 ± 1.1	49.7 ± 10.3	12.2 ± 2.9	1.9 ± 0.2	1.7 ± 0.5
Spleen	15	30	60.2 ± 8.4	14.4 ± 3.3	3.9 ± 1.0	4.9 ± 1.3	2415.3 ± 455.6	705.9 ± 145.0	92.4 ± 2.8	94.4 ± 1.9

Data are means ± s.d., n = 6.

Table 2 Concentrations of CRA in plasma and tissues after repeated intraperitoneal or subcutaneous administration.

	T_{max} (min)		C_{max} ($\mu g\,mL^{-1}$ or $\mu g\,g^{-1}$)		Tissue/plasma (C_{max} %)	
	Intraperitoneal	Subcutaneous	Intraperitoneal	Subcutaneous	Intraperitoneal	Subcutaneous
Plasma	15	30	68.9 ± 15.6	16.4 ± 2.5	—	—
Brain	15	30	1.6 ± 1.2	0.4 ± 0.3	2.4 ± 1.3	1.9 ± 1.4
Spleen	15	30	65.2 ± 6.1	15.9 ± 5.6	96.8 ± 5.3	97.2 ± 6.5

Data are means ± s.d., n = 6.

Repeated administration

After repeated intraperitoneal or subcutaneous doses, the C_{max} in brain and spleen (Table 2) was similar to that recorded at the same times after a single dose (Table 1). Fifteen minutes after repeated intraperitoneal doses, CRA was also found in ventricular CSF at a concentration of $1.8 \pm 0.2\,\mu g\,mL^{-1}$, which was slightly higher than that found in brain at the same time.

Discussion

This paper describes the pharmacokinetics of CRA, the hydrogenolytic product of Congo Red that has been found to possess anti-PrP activity in-vitro, and which delays the appearance of clinical symptoms and increases the survival of hamsters experimentally infected with scrapie (Poli et al 2003, 2004).

CRA was very rapidly absorbed after intraperitoneal injection; its plasma concentrations decayed biphasically, with the second phase showing very low concentrations and a $t_{1/2}$ of 4.3 h. The drug did not accumulate in peripheral tissue or brain as its C_{max} after repeated doses was similar to that observed after a single dose. However, it does cross the blood–brain barrier because, although lower than those in plasma, the concentrations found in brain tissue were not due to contamination from capillary blood, as is confirmed by the relatively high concentrations found in CSF, which can be regarded as a physiological brain perfusate.

In comparison with the intraperitoneal route, the subcutaneous route of administration, which is typically used for the chronic treatment of prion-infected hamsters (Poli et al 2004), led to lower but still significant plasma and tissue concentrations, which peaked after 30 min and then decayed in a similar way to that observed after intraperitoneal injection. As can be expected from the $t_{1/2}$ values, repeated intraperitoneal or subcutaneous administration did not cause any accumulation in plasma or tissue and the concentrations were similar to those observed after a single dose.

Although it is difficult to extrapolate in-vivo concentrations from in-vitro data because the models are different, the in-vitro concentrations of CRA inhibiting PrP expression were of the same order of magnitude as the concentrations of CRA found in brain (Poli et al 2003) and may be sufficient to explain the activity of CRA on PrP reproduction in the CNS. However, it is not possible to exclude a peripheral effect because the effects of CRA are more pronounced after intraperitoneal than after intracerebral infection (35% vs 10% increase in survival time, Poli et al 2004). In other words, the drug is effective when PrP^{res} must move from peripheral sites toward the CNS, thus mimicking the conditions of the natural route of BSE infection in which the cells of the immune system are probably involved in the initial replication and progression of PrP^{res} (McBride et al 1992; Lazmesas et al 1996), and can interact in peripheral organs with the PrP^{res} slowly progressing towards the CNS (Klein et al 1997; Glatzel & Aguzzi 2000; Race et al 2000). This hypothesis is consistent with the high concentrations of CRA found in the spleen,

and the same mechanism has been described in the case of other anti-prion agents (Ehlers & Diringer 1984).

CRA concentrations after treatment with a very high dose of Congo Red (three times that normally used to treat infected animals) were low and showed no clear time course. These findings refute the hypothesis that Congo Red can be transformed into CRA in-vivo (Bos et al 1986), and that the latter is the active agent. Congo Red, therefore, seems to act by itself, although the formation of other metabolites cannot be excluded.

Conclusions

This study describes the pharmacokinetics of CRA in plasma, spleen and brain after single or repeated intraperitoneal or subcutaneous administration. After subcutaneous administration, the concentrations of CRA in plasma and tissues were 25% of those recorded after intraperitoneal administration. The mean peak CRA concentrations and AUCs after a single intraperitoneal or subcutaneous administration in peripheral tissue (spleen) were similar to those observed in blood, whereas the concentrations in brain were about 2% those in plasma. The ability of CRA to cross the blood–brain barrier is proved by the fact that its CSF concentrations are similar to those found in brain tissue. After repeated intraperitoneal or subcutaneous doses, the C_{max} in plasma, brain and spleen was similar to that observed at the same times after a single dose. Brain concentrations may be sufficient to explain the activity of CRA on PrP reproduction in the CNS, although peripheral involvement cannot be excluded because the effects of CRA are more pronounced after intraperitoneal than after intracerebral infection.

References

Aguzzi, A., Montrasio, F., Kaeser, P. S. (2001) Prions: health scare and biological challenge. *Nat. Rev. Mol. Cell Biol.* **2**: 118–126

Beringue, V., Adjou, K. T., Lamoury, F., Maignien, T., Deslys, J. P., Race, R., Dormont, D. (2000) Opposite effects of Dextran Sulfate 500, the polyene antibiotic MS-8209, and Congo Red on accumulation of the protease-resistant isoform of PrP in the spleens of mice inoculated intraperitoneally with the scrapie agent. *J. Virol.* **74**: 5432–5440

Bos, R. P., van der Krieken, W., Smeijsters, L., Koopman, J. P., de Jonge, H. R., Theuws, J. L., Henderson, P. T. (1986) Internal exposure of rats to benzidine derived from orally administered benzidine-based dyes after intestinal azo reduction. *Toxicology* **40**: 207–213

Caughey, B., Chesebro, B. (1997) Prion protein and the transmissible spongiform encephalopathies. *Trends Cell Biol.* **7**: 56–62

Caughey, B., Race, R. E. (1992) Potent inhibition of scrapie-associated PrP accumulation by Congo Red. *J. Neurochem.* **59**: 768–771

Caughey, B., Brown, K., Raymond, G. J., Katzenstein, G. E., Thresher, W. (1994) Binding of protease-sensitive form of prion protein PrP to sulfated glycosaminoglycan and Congo Red. *J. Virol.* **68**: 2135–2141

Caspi, S., Halimi, M., Yanai, A., Sasson, S. B., Taraboulos, A., Gabizon, R. (1998) Antiprion activity of Congo Red. Putative mechanism. *J. Biol. Chem.* **273**: 3484–3489

Collinge, J. (2001) Prion diseases of humans and animals: their causes and molecular basis. *Annu. Rev. Neurosci.* **24**: 519–550

Demaimay, R., Harper, J., Gordon, H., Weaver, D., Chesebro, B., Caughey, B. (1998) Structural aspects of Congo Red as an inhibitor of protease-resistant prion protein formation. *J. Neurochem.* **71**: 2534–2541

Demaimay, R., Chesebro, B., Caughey, B. (2000) Inhibition of formation of protease-resistant prion protein by Trypan blue, Sirius Red and other Congo Red analogues. *Arch. Virol.* **16** (Suppl.): 277–283

Ehlers, B., Diringer, H. (1984) Dextran sulfate 500 delays and prevents mouse scrapie by impairment of agent replication in spleen. *J. Gen. Virol.* **65**: 1325–1330

Farquhar, C., Dickinson, A., Bruce, M. (1999) Prophylactic potential of pentosan polysulphate in transmissible spongiform encephalopathies. *Lancet* **353**: 117

Glatzel, M., Aguzzi, A. (2000) Peripheral pathogenesis of prion diseases. *Microbes Infect.* **2**: 613–619

Glenner, G. G. (1980) Amyloid deposits and amyloidosis. The beta-fibrilloses. *N. Engl. J. Med.* **302**: 1333–1343

Hill, A. F., Desbrulais, M., Joiner, S., Sidle, K. C. L., Gowland, I., Collinge, J. (1997) The same prion strain causes vCJD and BSE. *Nature* **389**: 448–450

Ingrosso, L., Ladogana, A., Pocchiari, M. (1995) Congo red prolongs the incubation period in scrapie-infected hamsters. *J. Virol.* **69**: 506–508

Kimberlin, R. H., Walker, C. A. (1986) Suppression of scrapie infection in mice by heteropolyanion 23, dextran sulfate, and some other polyanions. *Antimicrob. Agents Chemother.* **30**: 409–413

Klein, M. A., Frigg, R., Raeber, A. J., Flechsig, E., Kalinke, U., Bluethmann, H., Bootz, F., Suter, M., Zinkernagel, R. M., Aguzzi, A. (1997) A crucial role for B cells in neuroinvasive scrapie. *Nature* **390:** 687–690

Lasmezas, C. I., Cesbron, J. Y., Deslys, J. O., Demamay, R., Adjou, K. T., Rioux, R., Lemaire, C., Locht, C., Dormont, D. (1996) Immune system-dependent and in-dependent replication of the scrapie agent. *J. Virol.* **70**: 1292–1295

McBride, P. A., Eikelenboom, P., Kraal, G., Fraser, H., Bruce, M. E. (1992) PrP protein is associated with FDC cells of spleens and lymph nodes in uninfected and scrapie-infected mice. *J. Pathol.* **168**: 413–418

Milhavet, O., Mangé, A., Casanova, D., Lehmann, S. (2000) Effect of Congo Red on wild-type and mutated Prion Proteins in cultured cells. *J. Neurochem.* **74**: 222–230

Piccardo, P., Safar, J., Ceroni, M., Gajdusek, D. C., Gibbs, C. J. (1990) Immunohistochemical localization of prion protein in spongiform encephalopathies and normal brain tissues. *Neurology* **40**: 518–522

Pirola, R., Gervasoni, M., Pollera, C., Villa, S., Mantegazza, P., Bareggi, S. R. (2002) Determination of sodium 3,4-diaminonaphthalene-1-sulfonate, a Congo Red derivative, in plasma and brain of hamsters by high-performance liquid chromatography after solid-phase extraction and ultraviolet absorbance. *J. Chromatogr. B* **769**: 27–33

Poli, G., Ponti, W., Carcassola, G., Ceciliani, F., Colombo, L., Dall'Ara, P., Gervasoni, M., Giannino, M. L., Martino, P. A., Pollera, C., Villa, S., Salmona, M. (2003) In vitro evaluation of the anti-prionic activity of newly synthesized Congo Red derivatives. *Arzneimittelforschung. Drug Res.* **53**: 875–880

Poli, G., Martino, P., Ceccarelli, A., Cignarella, G., Carcassola, G., Dall'Ara, P., Giannino, M. L., Pollera, C., Ponti, W., Bareggi, S. R., Mantegazza, P. (2004) Evaluation of anti-prion activity of Congo Red and its derivatives in experimentally infected hamsters. *Arzneimittelforschung. Drug. Res.* In press

Prusiner, S. B. (1998) Prions. *Proc. Natl Acad. Sci. USA* **95**: 13363–13383

Race, R., Oldstone, M., Chesebro, B. (2000) Entry versus blockade of brain infection following oral or intraperitoneal scrapie administration: role of prion protein expression in peripheral nerves and spleen. *J. Virol.* **74**: 828–833

Rudyk, H., Vasiljevic, S., Hennion, R. M., Birkett, C. R., Hope, J., Gilbert, I. H. (2000) Screening Congo Red and its analogues for their ability to prevent the formation of PrPres in scrapie-infected cells. *J. Gen. Virol.* **81**: 1155–1164

Villa, S., Cignarella, G., Barlocco, D., Gervasoni, M., Carcassola, G., Giannino, L., Mantegazza, P. (2003) Congo red analogues as potential anti-prion agents. *Il Farmaco* **58**: 929–937

Will, R. G., Ironside, J. W., Zeidler, M., Cousens, S. N., Estibeiro, K., Alperovitch, A., Poser, S., Pocchiari, M., Hofman, A., Smith, P. G. (1996) A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet* **347**: 921–925

Ye, X., Scallet, A. C., Kascsak, R. J., Carp, R. I. (1998) Astrocytosis and amyloid deposition in scrapie infected hamsters. *Brain Res.* **809**: 277–287