British Journal of Pharmacology (1996) 118, 2152-2156

Facilitation of imipramine efflux from the brain by systemic specific antibodies

Corinne Ragusi, [1]Gabrielle Boschi, Richard Rips & Jean-Michel Scherrmann

INSERM U26, Hôpital Fernand Widal, 200 rue du Faubourg Saint Denis, F75475 Paris Cedex 10, France

1 This study investigated the capacity of circulating anti-tricyclic antidepressant (TCA) IgG to increase the efflux of imipramine (Imip) from the rat brain.

2 A tracer amount of [^{3}H]-Imip (40 pmol) was injected into the cerebral lateral ventricle and its efflux was determined in control rats and in rats given anti-TCA antibody. The monoclonal anti-TCA IgG_1 was injected i.v. 48 h before Imip at 4 IgG: Imip molar ratios (10, 100, 1000 and 10000). The [^{3}H]-Imip in arterial and venous plasma was measured for up to 60 min, and in the brain and peripheral organs (heart, liver, lung, kidney) 5 and 60 min after Imip injection.

3 The arterial plasma concentration of Imip in control rats was significantly higher (26.7 ± 2.1 pM) than the venous one (17.7 ± 2.0 pM) at 5 min, indicating that Imip released from brain becomes distributed in peripheral tissues. These concentrations were not significantly different at 60 min suggesting that Imip was, at this time, redistributing from extravascular tissues to the blood. In rats given anti-TCA IgG, any Imip leaving the brain was immediately bound by the circulating antibody at 5 min. This greatly reduced the Imip in the heart (63.9%) and lung (61.3%) at the highest IgG:Imip ratio. The brain Imip was markedly lower at 60 min (31.5% with an IgG:Imip ratio of 1000 and 57.5% at a ratio of 10000). The two lowest IgG:Imip ratios had less effect on the plasma Imip because of the relative low affinity of the anti-TCA IgG (3.8×10^7 M^{-1}).

4 These data indicate that the anti-TCA IgG facilitated the efflux of Imip from the brain, even though these antibodies cannot cross the blood-brain barrier. This may be an efficient system for increasing drug organ clearance, as more than half the Imip in the brain was actively removed by the antibody in 1 h.

Keywords: Imipramine; specific antibody; pharmacokinetics; cerebral efflux

Introduction

Drug-specific antibodies have been used successfully to reverse drug toxicity in peripheral organs since 1976 (Smith *et al.*, 1976). We have recently used them to reverse colchicine intoxication in man (Baud *et al.*, 1995). Detoxification by immunotoxicotherapy involves sequestration of the drug in the antibody distribution space, which results in extraction of the drug from deeper spaces, and elimination of the drug-antibody complex via the kidneys if antibody fragments used are small enough (e.g. fragment antigen binding: Fab, 50 kDa) (Scherrmann *et al.*, 1989). Thus, specific antibodies are able to redistribute drugs from deep reversible compartments to the vascular compartment and therefore prevent drug toxicity.

Tricyclic antidepressants (TCA), particularly imipramine (Imip), are psychotropic drugs that have been widely used since 1957. Although TCA overdose is a serious and common problem, there is no specific treatment for this intoxication, which mainly involves cardiac (Frommer *et al.*, 1987) and cerebral toxicity (Trimble, 1980; Frommer *et al.*, 1987). TCA immunotoxicotherapy has been proposed by several groups (Hursting *et al.*, 1989; Sabouraud *et al.*, 1990) with the main aim of reversing induced TCA cardiac toxicity. Previous studies in rats showed that pretreatment with specific-TCA immunoglobulin G (IgG) or Fab resulted in a marked reduction of the cardiac toxicity induced by desipramine, an active metabolite of imipramine (Pentel *et al.*, 1991). Although the removal of TCA from the brain was not extensively studied, these results suggest that specific antibodies could also be used to remove TCA from the brain, a target organ of TCA toxicity, even though most antibodies do not cross the blood-brain barrier (Juhler & Neuwelt, 1989).

The present study was carried out to demonstrate that the presence of specific anti-TCA IgG in the vascular compartment can facilitate the efflux of Imip from the brain. By this procedure, later, at the clinical stage, the use of a smaller antibody fragment such as Fab which is more rapidly effective should make possible detoxification in human subjects. In this study, [^{3}H]-Imip was injected into the cerebral lateral ventricle. The plasma concentration of Imip was set to zero at the time of the Imip administration. A monoclonal nortriptyline (NT)-specific IgG was injected previously intravenously (i.v.). One difference between NT and Imip is that the former has a secondary amino group at the end of the lateral chain, however, the monoclonal anti-NT IgG has good affinity for Imip. The measurement of [^{3}H]-Imip concentrations in the plasma, brain and peripheral organs of control and antibody treated rats should indicate any increase in the removal of Imip from the brain by NT-specific IgG.

Methods

Animals and surgical procedures

All studies were carried out on male Sprague-Dawley rats (Iffa Credo, Lyon, France), 47-50 days old, weighing about 230-260 g. They were given food and water *ad libitum* and kept on a 12 h light-dark cycle at 22 ± 1°C. Rats were anaesthetized with chloral hydrate (400 mg kg^{-1}, i.p.) and the left jugular vein or caudal artery was cannulated with a teflon catheter for blood sampling. The rats were placed in a stereotaxic frame with atraumatic ear bars and the skull was exposed by a midline incision. A Hamilton syringe (10 μl) with a micrometer screw was placed on the stereotaxic micromanipulator. The needle was implanted stereotaxically into the right lateral ventricle (coordinates: 1.1 mm posterior to bregma, 1.5 mm lateral to sagittal suture, 5.6 mm below the skull surface) according to the atlas of König & Klippel (1967). Body temperature was controlled by a rectal thermoprobe and maintained at approximately 37°C with a heating pad.

[1] Author for correspondence.

Drug administration

Each rat ($n=60$) was given 40 pmol [^{3}H]-Imip in 2 μl (1 μl min^{-1}) into the right lateral ventricle via the Hamilton syringe. One group of rats were given [^{3}H]-Imip alone. Other groups of rats were given NT-specific IgG i.v. (10, 100, 1000 or 10000 fold the Imip dose in terms of binding sites (2 sites per IgG molecule), 0.2, 2, 20 or 200 nmol). The antibodies were dissolved in sterile 0.9% NaCl and injected in a volume of 750 μl. Control rats were given 750 μl normal saline containing 200 nmol of non-specific IgG. All antibody injections were given 48 h before the [^{3}H]-Imip injections. According to Redureau (1995), murine monoclonal IgG$_1$ given i.v. to rats has a distribution half-life of 8.4 h and an elimination half-life of 8.1 days.

Collection and treatment of samples

Blood was collected 5, 10, 20, 30 and 60 min after [^{3}H]-Imip injection via a caudal or a jugular catheter. Plasma samples (100 μl) were suspending in 9 ml of Picofluor 40 and radioactivity was measured by beta counting. Animals were killed by decapitation 5 or 60 min after the i.c.v. injection. Organs (brain, liver, kidney, heart, lung) were rapidly removed, rinsed with physiological saline, blotted dry, weighed and stored at -20°C until assayed. Tissues were homogenized in water with an Ultra-Turrax, and then with an ultrasound unit. Aliquots (up to 400 μl) of the mixture were placed in 1 ml of Soluene 350 for 1 h at 55°C, suspended in 9 ml of Picofluor 40 and the radioactivity measured in a Tri-Carb model 1900 Tr liquid scintillation spectrophotometer. The protein content was determined by Bio-Rad protein assay.

Preparation and characterization of a monoclonal anti-nortriptyline IgG$_1$

Monoclonal anti-NT IgG$_1$ was prepared as previously described (Marullo *et al.*, 1985). Briefly, nortriptyline (NT) was conjugated to serum albumin (BSA) by the method of Kamel *et al.* (1979). Succinylated BSA was coupled to NT via carbodiimide on the lateral chain secondary amino group. Spleen cells of Balb/c mice immunized with the BSA-nortriptyline conjugate were fused with NS-1 myeloma cells in the presence of polyethylene glycol. Hybridoma cell populations that were identified by ELISA as secreting NT-specific antibodies of IgG$_1$ isotype control were subcloned and injected i.p. in mice that had been primed with pristane. Ascitic fluid (2.4 l) containing 62 μM of the monoclonal IgG$_1$, was collected from mice, centrifuged to removed cells, and pooled. The anti-NT monoclonal antibody was purified by Pasteur-Mérieux Sérums et Vaccins (Marcy l'Etoile, France) by ammonium sulphate precipitation and S-Sepharose Fast Flow ion-exchange chromatography (Pharmacia, Orsay, France) with a 91% recovery. The IgG purity was found to be 96% by sodium lauryl sulphate polyacrylamide gel electrophoresis and high performance liquid chromatography. It was packed in 10 ml sterile bottles (0.33 mM) and stored at 4°C. Antibody titer was expressed as the dilution of antiserum that bound 50% of a standard amount of [^{3}H]-Imip (4.5 nM). The affinity constant was obtained by a competitive radioimmunoassay (RIA) and calculated according to Müller (1980). Specificity was studied by adding serial amounts of Imip and NT and the concentrations of Imip and NT that removed 50% of the tracer from the antibody binding were calculated. The ratio of the above concentrations to the reference NT values, expressed as a percentage, indicated the cross reactivity with Imip.

Pharmacokinetic analysis

The maximal concentration (C_{max}) was obtained from experimental data. The area under the plasma concentration-time curve ($AUC_{0\rightarrow 60\ min}$) was calculated by the trapezoidal method (Gibaldi & Perrier, 1982) using InPlot 4 (GraphPAD, San Diego, U.S.A.).

Autoradiographic analysis

Control rats were decapited 5, 30, 60, 180, 270 or 360 min after Imip injection. The brains were removed and frozen at -20°C. Sections (20 μm) were cut on a cryostat, mounted on glass sides and exposed to Hyperfilm (Amersham, France) for 21 days. The autoradiograms were analysed with a computerized image-analysis system (Biocom, Les Ulis, France).

Drugs

[Benzene-ring ^{3}H]-Imipramine (53.2 Ci mmol^{-1}) was obtained from New England Nuclear (Dupont de Nemours, Paris, France). Aqualyte scintillation liquid (Picofluor 40) and Soluene 350 were purchased from Packard (Rungis, France). Imipramine and nortriptyline hydrochloride were gifts from Ciba Geigy (Rueil Malmaison, France), and the Bio-Rad Protein Assay Kit was from Bio-Rad (Ivry Sur Seine, France). Control antibody (a monoclonal mouse anti-human cells IgG) was a generous gift from Sanofi (Montpellier, France).

Statistical analysis

The effects of NT-specific IgG on the plasma and tissue concentrations of [^{3}H]-Imip were compared by multiple one-tailed t tests with the Bonferroni adjustment and a global critical level of 5%. When the homogeneous variance assumption was false, the degrees of freedom were adjusted according to Brownlee (1965). Differences between arterial and venous plasma concentrations in control rats at 5 and 60 min were compared by the Mann-Whitney U test. The changes in the Imip plasma $AUC_{0\rightarrow 60\ min}$ with increasing doses of specific IgG were analysed by one-way analysis of variance (Kruskall-Wallis) followed by the Mann-Whitney U test.

All calculations were made using the SYSTAT Software (SYSTAT, Evanston, Illinois, U.S.A.).

Results

Characterization of anti-NT IgG$_1$

NT-specific IgG was of the IgG$_1$ isotype and its affinity constants for NT and Imip were 5.9×10^7 M^{-1} and 3.8×10^7 M^{-1}, respectively. Binding experiments showed that the antibodies had 64% cross-reactivity with Imip. The monoclonal anti-human cells IgG used as the control antiserum did not bind Imip (data not shown).

Arterial and venous [^{3}H]-Imip plasma concentrations

Figures 1 and 2 show that the plasma [^{3}H]-Imip concentration in control rats rapidly increased at 5 min and then more slowly, towards a plateau at 60 min. The arterial plasma Imip concentration was 26.7 ± 2.1 pM, significantly higher ($P<0.02$) than the venous plasma concentration of 17.7 ± 2.0 pM at 5 min. The arterial (41.4 ± 3.6 pM) and venous (45.4 ± 2.3 pM) plasma concentrations were not significantly different at 60 min. The plasma [^{3}H]-Imip concentrations of the control group given Imip alone and rats given Imip plus non-specific IgG were essentially the same (data not shown). The rats given Imip alone were therefore used as controls in all further experiments.

The arterial plasma concentrations of [^{3}H]-Imip in rats pretreated with the NT-specific IgG were not significantly different from those of the control group for the IgG:Imip molar ratios of 10 and 100, but were 33% higher, in term of $AUC_{0\rightarrow 60\ min}$, when the ratio was 1000 (Figure 1). Rats given with this last IgG:Imip ratio had venous plasma concentrations of [^{3}H]-Imip that were identical to the corresponding arterial concentrations (Figure 2). The highest dose of antibody (Figure 2) gave a C_{max} (160.53 ± 15.36 pM) 5 min after the i.c.v. injection of Imip that was 9 fold higher than the

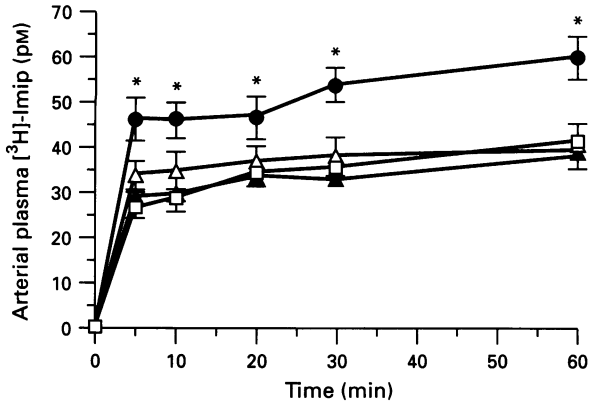

Figure 1 Time course of changes in the arterial plasma [^{3}H]-Imip concentration in control (□, $n=6$) and anti-NT IgG-treated rats (IgG:Imip molar ratios: ▲ 10, $n=4$; △ 100, $n=6$; ● 1000, $n=6$). [^{3}H]-Imip (40 pmol, i.c.v.) was injected at $t=0$; antibodies were given i.v. 48 h before [^{3}H]-Imip. Blood samples were collected for up to 60 min from the caudal artery. The values are means ± s.e.mean. Multiple one-tailed t tests with the Bonferroni adjustment and a global critical level of 5%. *$P<0.05$, significantly different from the control and treated (10 and 100) groups.

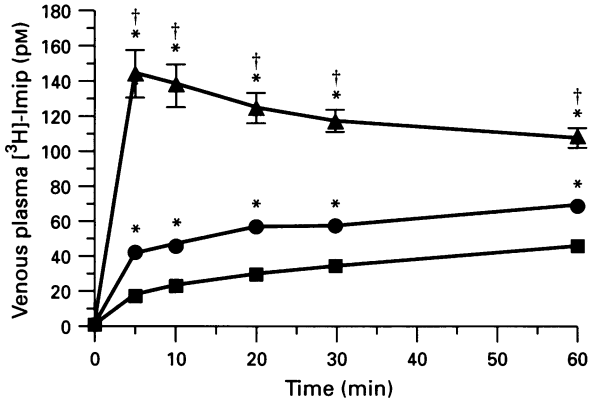

Figure 2 Time course of changes in the venous plasma [^{3}H]-Imip concentration in control (■ $n=6$) and anti-NT IgG-treated rats (IgG:Imip molar ratios: ● 1000, $n=8$; ▲ 10000, $n=6$). [^{3}H]-Imip (40 pmol, i.c.v.) was injected at $t=0$; antibodies were given i.v. 48 h before [^{3}H]-Imip. Blood samples were collected from the jugular vein for up to 60 min. The values are means ± s.e.mean. Multiple one-tailed t tests with the Bonferroni adjustment and a global critical level of 5%. *$P<0.05$, significantly different from the control group; †$P<0.05$, significantly different from the treated (1000) group.

corresponding concentration in the control group (17.70 ± 2.00 pM). Thereafter, the plasma concentration of Imip gradually decreased over 60 min, whereas it increased slightly in the rat having an IgG:Imip ratio of 1000. The relationship between the arterial and venous [^{3}H]-Imip plasma $AUC_{0\rightarrow 60\ min}$ and the four IgG:Imip molar ratios are presented in Table 1.

Tissue concentrations of [^{3}H]-Imip

The radioactivity was determined in the brain, heart, liver, kidney and lung of rats killed 5 or 60 min after i.c.v. injection of [^{3}H]-Imip (Table 2).

The radioactivity in the brains of control and treated rats was the same 5 min after Imip injection. In contrast, 60 min after Imip injection, the brain [^{3}H]-Imip concentrations in rats with IgG:Imip ratios 1000 and 10000 were significantly decreased (31.5%, $P<0.05$, for ratio 1000 and 57.5%, $P<0.05$, for the ratio 10000).

Rats given the highest dose of antibody had significantly reduced [^{3}H]-Imip concentration in the heart and lung 5 and 60 min after Imip injection, whereas it was increased in the liver. The kidney [^{3}H]-Imip concentration was reduced only at 60 min.

Autoradiography

[^{3}H]-Imip was distributed in the vicinity of the right lateral ventricle 5 min after i.c.v. administration. There were narrow zones of dense radioactivity in the parenchyma around the injection site. The extent and the density of the radioactivity were reduced at 60 min and only traces were seen 180 min after Imip injection. There was little or no detectable radioactivity 360 min after injection.

Discussion

The study shows that anti-TCA IgG in the vascular compartment can facilitate the efflux of Imip from the brain even though these antibodies cannot cross the blood-brain barrier (Juhler & Neuwelt, 1989). This concept was studied with TCA drugs for two main reasons. The first is that TCA overdose or poisoning has adverse effects on the central nervous system

Table 1 Relationship between arterial and venous [^{3}H]-Imip plasma $AUC_{0\rightarrow 60\ min}$ and IgG:Imip molar ratios of 10, 100, 1000 and 10000

	$AUC_{0\rightarrow 60\ min}$ (min pM)	
	Arterial	*Venous*
IgG: Imip 10	1.91 ± 0.05	–
IgG: Imip 100	2.14 ± 0.20†	–
IgG: Imip 1000	3.00 ± 0.23**	3.33 ± 0.15**
IgG: Imip 10000	–	7.06 ± 0.42††

Values are means ± s.e. mean ($n=4-8$). One-way analysis of variance (Kruskall-Wallis), followed by the Mann-Whitney U test. †$P<0.05$, ††$P<0.01$, significantly different from the treated (1000) group; **$P<0.01$, significantly different from the treated (10) group.

Table 2 Imip tissue concentrations (fmol mg^{-1} prot) in control and anti-NT IgG-treated rats

	Time of death	
	5 min	*60 min*
Brain		
Control group	227.08 ± 16.49	161.24 ± 16.32
IgG: Imip 1000	223.98 ± 25.90	110.37 ± 19.73*
IgG: Imip 10000	264.90 ± 29.66	68.49 ± 8.00*†
Heart		
Control group	4.43 ± 1.01	2.46 ± .018
IgG: Imip 1000	2.25 ± 0.66	2.42 ± 0.61
IgG: Imip 10000	1.60 ± 0.18*	1.22 ± 0.23*
Kidney		
Control group	7.84 ± 0.96	11.06 ± 1.37
IgG: Imip 1000	6.87 ± 0.58	9.71 ± 1.52
IgG: Imip 10000	5.48 ± 0.25	6.96 ± 0.64*
Liver		
Control group	2.61 ± 0.37	2.58 ± 0.22
IgG: Imip 1000	3.91 ± 0.56	3.88 ± 0.37*
IgG: Imip 10000	6.11 ± 1.04*	4.56 ± 0.32*
Lung		
Control group	24.72 ± 3.01	15.04 ± 1.66
IgG: Imip 1000	13.63 ± 2.71*	14.87 ± 2.13
IgG: Imip 10000	9.57 ± 2.08*	6.57 ± 0.59*†

Values are means ± s.e. mean ($n=6-8$). Multiple one-tailed t tests with Bonferroni adjustment; *$P<0.05$, significantly different from the control group; †$P<0.05$ significantly different from the treated (1000) group.

(confusion, agitation, hallucinations, coma, myoclonus, seizures) (Frommer *et al.*, 1987), so that removal of TCA from the brain is of therapeutic significance. The second is that passage of TCA across the blood-brain barrier depends mainly on diffusion which can be considered as kinetically similar in both directions, from blood to the brain and from brain to the blood. As a consequence, the brain and plasma TCA concentrations are in equilibrium in steady-state conditions (DeVane *et al.*, 1984; Sugita *et al.*, 1987). The basic question is how to alter this equilibrium to improve the elimination of TCA from the brain. The efflux of TCA from the brain could be modified by trapping the TCA molecules leaving the brain in the vascular compartment. This would keep the concentration of free TCA in the blood low and set up a continuous gradient of TCA concentration between the brain and the blood. Antibodies to drugs are known to be powerful trapping systems because of their affinity and specificity. Digoxin and colchicine-specific Fab fragments effectively reverse drug toxicity in human subjects (Smith *et al.*, 1976; Baud *et al.*, 1995). Specific antibody IgG were useful to support the concept but cannot be used in therapy as they need too long for their distribution. We therefore prepared a TCA-specific antibody and investigated its ability to facilitate the efflux of Imip from the brain. TCA-specific antibodies have been prepared by several groups and used for the immunotoxicotherapy of several molecules of the TCA family. The monoclonal anti-NT IgG_1 produced had a significant cross reactivity with Imip (64%) and an affinity constant of 3.8×10^7 M^{-1} for Imip.

Murine monoclonal IgG_1 given i.v. to rats was mainly distributed in plasma and not in brain parenchyma. Arizono *et al.* (1994) recently confirmed, in the rat that, 48 h after injection of a radiolabelled monoclonal IgG, the brain contained less than 1.3% of the plasma radioactivity, and that most of this brain radioactivity was attributable to blood which remained in the tissues. Hence, the circulating antibody cannot interact directly with the brain Imip molecules. Imip was given as a tracer dose after antibody injection because this study was designed to ascertain whether the efflux of Imip was facilitated, and not if toxicity was reversed. The latter would require higher doses of Imip and antibody. The molar ratios of antibody and Imip used were based on the value of the antibody affinity for Imip. A stoichiometric amount of antibody for the drug dose is usually administered in immunotoxicotherapy. But, this can only be done if the affinity constant of the antibody is sufficiently high. Cano *et al.* (1995) used three anti-digoxin monoclonal antibodies having a log range difference in their affinity constants ($10^7 - 10^9$ M^{-1}), to show that the efficacy of the antibody in sequestering digoxin decreased as the antibody affinity became lower. They also demonstrated that this lack of efficacy of the low affinity antibody can be offset by increasing the antibody concentration. We therefore performed a dose-range study with antibody-Imip molar ratios from 10 to 10000, knowing that our anti-NT IgG has a relative low affinity for Imip (3.8×10^7 M^{-1}). We first evaluated the efflux of Imip from the brain by measuring the Imip concentration in arterial plasma for 60 min. The anti-NT IgG has no significant effect on the Imip cerebral efflux kinetics at the two lowest doses of antibody. However, an antibody-Imip molar ratio of 1000 resulted in all measured plasma Imip concentrations being significantly higher than in the previous experiments. These initial data suggested that, as seen by Cano *et al.* (1995), the effect of the antibody was dose-dependent, and this strategy would be appropriate in the present study. The apparent lack of effect with the two lowest doses of antibody could be because blood taken from the caudal artery may allow the dissociation of Imip from the antibody. We therefore took blood samples from the jugular vein, closer to the brain, with the two highest doses of antibodies, and measured the Imip concentrations in the brain and peripheral organs.

The venous plasma concentration of Imip in control rats, 5 min after injection, was significantly lower than the arterial plasma concentration, indicating that the Imip released from the brain was distributed in the other body tissues. In contrast, the venous concentration of Imip at 60 min was not significantly different from the arterial concentration, suggesting that Imip was, at this time, redistributing from extravascular tissues to the blood. The significance of the difference between the arterial and venous plasma concentrations of TCAs has been discussed by Baud *et al.* (1985). These kinetic properties are also consistent with the Imip distribution half-life of 0.21 ± 0.05 h in the rat (Okiyama *et al.*, 1986). As expected, most of the Imip was found in the brain 5 and 60 min after its intracerebroventricular injection. The autoradiography confirmed the diffusion of some Imip from the cerebrospinal fluid into the cerebral parenchyma 5 min after i.c.v. administration and its lowering at 60 min. There was more Imip in the lung, which contains high affinity binding sites for Imip (Morin *et al.*, 1984), than in the kidney, heart or liver. However, even at 60 min, brain concentration of Imip was 10 fold higher than that of the lung. The concentrations of Imip were lower at 60 min that at 5 min, except in the kidney, where it was increased and reflected the elimination of Imip and metabolites by this organ.

The Imip arterial plasma $AUC_{0 \rightarrow 60\ min}$ in anti-NT IgG-treated rats was not significantly different from the venous $AUC_{0 \rightarrow 60\ min}$ at an antibody-Imip molar ratio of 1000. This does not support our hypothesis that a difference in arterial or venous blood sampling site would reflect the stability of the Imip-IgG complex. The antibody-Imip molar ratio of 10000 had a more pronounced effect on the elevation of Imip plasma level than that of 10 – 1000 range. This confirmed that the antibody dose-effect on the plasma redistribution of Imip probably follows a E_{max} sigmoidal function or Hill equation, as we found previously for digoxin (Cano *et al.*, 1995). Further experiments using TCA-specific antibody with affinity in the range $10^9 - 10^{10}$ M^{-1} would help to describe the complete relationship. This antibody dose-dependent Imip plasma redistribution shows that Imip molecules leaving the brain are neutralized in the vascular compartment, allowing the creation of a concentration gradient of Imip between the brain and the blood. The brain concentrations of Imip were determined to verify the efficacy of this mechanism. There was no decrease in Imip concentration at 5 min with the IgG:Imip molar ratios 1000 and 10000, but at 60 min, the brain Imip concentrations had dropped 31.5% (ratio 1000) and 57.5% (ratio 10000). Thus, specific antibodies can facilitate the efflux of Imip from the brain. The lack of antibody effects on brain Imip at 5 min could be due to the injection procedure. Some of the Imip injected i.c.v. into the cerebrospinal fluid quickly passes directly into the blood, without entering the brain parenchyma. This Imip was immediately bound by the plasma circulating antibodies. This could explain the abrupt rise in the plasma concentration of Imip at 5 min. This sequestration of Imip by the plasma also partly prevents the Imip distribution into the peripheral organs. The Imip concentrations were markedly reduced in the heart (63.9% and 50.4%) and lung (61.3% and 56.3%) at 5 min and 60 min, respectively, with the highest dose of antibody. The efficacy of the low dose of the antibody on the Imip concentration in lung at 5 min may reflect the more rapid redistribution of a part of Imip in this tissue than in others. However, the lack of effect of the low dose in lung at 60 min suggest that in this organ, which contains high affinity binding sites for Imip (Morin *et al.*, 1984), Imip was sufficiently strongly bound at its receptors to require a higher dose of anti-TCA antibody to produce its redistribution. The 130% increase in the Imip concentration in the liver at 5 min was probably due to the metabolism of IgG in this organ and also to the high degree of first-pass elimination of Imip through the liver (Gram & Christiansen, 1975). The lack of significant effect of anti-TCA IgG on the kidney Imip concentrations at 5 min suggests that the efficacy of these antibodies on Imip redistribution is not uniform (Pentel *et al.*, 1991) and may be slower in this organ. A reduction (37.1%) in the kidney Imip concentrations was observed at 60 min.

In conclusion, the data demonstrate that a TCA-specific antibody that does not cross the blood-brain barrier binds Imip released from the brain. This may be an efficient system for increasing drug organ clearance, as more than half the Imip in the brain was actively removed by the antibody in 1 h. The kinetics for the reversal of the central nervous clinical signs of TCA toxicity remains to be evaluated. For this, a TCA-specific antibody with high affinity and rapid onset of action should be used in future so as to reduce the stoichiometry between the amounts of drug and antibody and to evaluate the efficacy of the antibody administered after the drug intoxication. It may be possible to extend this concept to other drugs or toxins that are toxic in the brain.

The authors thank Marcel Debray for help in the statistical analysis, Jean Carcagne (Pasteur-Mérieux Sérums et Vaccins, Paris) for assistance in antibody preparation, Bernard Pau (Sanofi, Montpellier) for the gift of the control antibody and Owen Parkes for editing the english text.

References

ARIZONO, H., ISHII, S., NAGAO, T., KUDO, S., SASAKI, S., KONDO, S. & KIYOKI, M. (1994). Pharmacokinetics of a new human monoclonal antibody against cytomegalovirus. *Arzneim.-Forsch./Drug Res.*, **44,** 890–898.

BAUD, F.J., BUISINE, A., BISMUTH, C., GALLIOT, M., VICAUT, E., BOURDON, R. & FOURNIER, P.E. (1985). Arterio-venous plasma concentration differences in amitriptyline overdose. *Clin. Toxicol.*, **23,** 391–406.

BAUD, F.J., SABOURAUD, A., VICAUD, E., TABOULET, P., LANG, J., BISMUTH, C., ROUZIOUX, J.M. & SCHERRMANN, J.M. (1995). Brief report: treatment of severe colchicine overdose with colchicine-specific Fab fragments. *N. Engl. J. Med.*, **332,** 642–645.

BROWNLEE, K.A. (1965). *Statistical Theory and Methodology in Science and Engineering*. New York: John Wiley and Sons.

CANO, N., SABOURAUD, A., BENMOUSSA, K., ROQUET, F., NAVARRO-TEULON, I., MANI, J.C. & SCHERRMANN, J.M. (1995). Monoclonal digoxin-specific antibodies induce dose- and-affinity-dependent plasma digoxin redistribution in rats. *Pharm. Res.*, **12,** 709–714.

DEVANE, C.L., SIMPKINS, J.W. & STOUT, S.A. (1984). Cerebral and blood pharmacokinetics of imipramine and its active metabolites in the pregnant rat. *Psychopharmacology*, **84,** 225–230.

FROMMER, D.A., KULIG, K.A., MARX, J.A. & RUMACK, B. (1987). Tricyclic antidepressant overdose. *J. Am. Med. Ass.*, **257,** 521–526.

GIBALDI, M. & PERRIER, D. (1982). *Pharmacokinetics*. New York: Marcel Decker.

GRAM, L.F. & CHRISTIANSEN, J. (1975). First pass metabolism of imipramine in man. *Clin. Pharmacol. Ther.*, **17,** 555–563.

HURSTING, M.J., OPHEIM, K.E., RAISYS, V.A., KEBBY, M.A. & METZGER, G. (1989). Tricyclic antidepressant-specific Fab fragments alter the distribution and elimination of desipramine in the rabbit: a model for overdose treatment. *J. Toxicol. Clin. Toxicol.*, **27,** 53–66.

JUHLER, M. & NEUWELT, E.A. (1989). The blood-brain barrier and the immune system. In *Implications of the Blood-Brain Barrier and its Manipulation*. ed. Neuwelt, E.A., vol. 1, pp. 261–292. New York: Plenum Medical Book Company.

KAMEL, R.S., LANDON, J. & SMITH, D.S. (1979). Novel ^{125}I-Labeled nortriptyline derivates and their use in liquid-phase or magnetizable solid-phase second-antibody radioimmunoassays. *Clin. Chim.*, **25,** 1997–2002.

KÖNIG, J.F.R. & KLIPPEL, R.A. (1967). *The Rat Brain: a Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem*. Huntington: Krieger.

MARULLO, S., HOEBEKE, J., GUILLET, J.G. & STROSBERG, A.D. (1985). Structural analysis of the epitope recognized by a monoclonal antibody directed against tricyclic antidepressants. *J. Immunol.*, **135,** 471–477.

MORIN, D., ZINI, R., LANGE, J. & TILLEMENT, J.P. (1984). Evidence for high affinity [^{3}H] imipramine binding sites in human lung. *Biochem. Biophys. Res. Commun.*, **120,** 926–932.

MÜLLER, R. (1980). Calculation of average antibody affinity in anti-hapten sera from data obtained by competitive radioimmunoassay. *J. Immunol. Methods*, **34,** 345–352.

OKIYAMA, M., UENO, K., OHKAWARA, S., OHMORI, S., IGARASHI, T. & KITAGAWA, H. (1986). Effects of combined administration of diazepam and imipramine hydrochloride in rats. *J. Pharm. Sci.*, **75,** 1071–1075.

PENTEL, P., BRUNN, G.J., POND, S.M. & KEYLER, D.E. (1991). Pretreatment with drug specific antibody reduces desipramine cardiotoxicity in rats. *Life Sci.*, **48,** 675–683.

REDUREAU, M. (1995). Pharmacocinétique et catabolisme hépatique des anticorps et fragments. *PhD Thesis, Paris V University*.

SABOURAUD, A., DENIS, H., URTIZBEREA, M., CHAPPEY, O. & SCHERRMANN, J.M. (1990). The effect of nortriptyline-specific active immunization on amitriptyline toxicity and disposition in the rabbit. *Toxicology*, **62,** 349–360.

SCHERRMANN, J.M., TERRIEN, N., URTIZBEREA, M., PIERSON, P., DENIS, H. & BOURRE, J.M. (1989). Immunotoxicotherapy: present status and future trends. *J. Toxicol. Clin. Toxicol.*, **27,** 1–35.

SMITH, T.W., HABER, E., YEATMAN, L. & BUTLER, V.P. (1976) Reversal of advanced digoxin intoxication with Fab fragments of digoxin-specific antibodies. *N. Engl. J. Med.*, **294,** 797–800.

SUGITA, S., KOBAYASHI, A., SUZUKI, S., YOSHIDA, T. & NAKAZAWA, K. (1987). High-performance liquid chromatographic determination of imipramine and its metabolites in rat brain. *J. Chromatogr.*, **421,** 412–417.

TRIMBLE, M.R. (1980). New antidepressant drugs and the seizure threshold. *Neuropharmacology*, **19,** 1227–1228.

(Received February 19, 1996
Revised May 8, 1996
Accepted May 21, 1996)