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Influence of various combinations of specific antibody dose and affinity on tissue imipramine redistribution

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1 This study was designed to evaluate the distribution kinetics of imipramine (Imip) in the brain and the main peripheral organs (heart, kidney, liver and lung) of rats, and to establish the relationship between the redistribution of Imip from these tissues and the immunoreactive capacity (dose and affinity) of anti-TCA IgG.

2 [^{3}H]-Imip (1 nmol kg^{-1} body weight) was injected intravenously 6 min before the i.v. injection of antibodies. At this time, the concentrations of Imip and its main metabolites in plasma were determined. The radioactivity measured corresponded to 91.7% Imip, indicating that the pharmacokinetics reflected essentially Imip. Plasma and tissue Imip contents were measured over the interval 1 to 90 min in control and in treated rats. The antibodies used were a murine monoclonal IgG_1 ($Ka = 3.8\ 10^7\ M^{-1}$) at an $IgG_1/_{Imip}$ molar ratio of 1000 (IgG_1 1000), and a sheep polyclonal IgG (TAb, $Ka = 1.3\ 10^{10}\ M^{-1}$) at IgG/Imip molar ratios of 1, 10 and 100 (TAb1, TAb10 and TAb100).

3 The anti-TCA IgG increased the plasma [^{3}H]-Imip concentrations: the $AUC_{1\rightarrow 60\ min}$ for [^{3}H]-Imip were 4 (IgG_1 1000), 9 (TAb1), 33.9 (TAb10) and 41.4 (TAb100) times higher in the treated groups than in the controls. The opposite effect occurred in the brain, heart and lungs, with large, rapid decreases in Imip. The increase in plasma Imip and the decrease in tissue Imip depended on the immunoreactive capacity (NKa) of the antibody, where N = molar concentration of IgG binding sites and Ka = IgG affinity constant. Maximal plasma and tissue redistribution occurred when $NKa = 33.8 \times 10^4$.

4 Imip redistribution can be controlled using various doses or affinities of specific antibodies, and the resulting rapid, extensive Imip redistribution from the main target organs could be very promising for TCA detoxification.

Keywords: Imipramine; antibody; tissue redistribution

Introduction

Tricyclic antidepressants (TCA), particularly imipramine (Imip), are psychotropic drugs that have been widely used since 1957. Although TCA overdose is a serious and common clinical problem, no antidotal treatment is currently available for the resulting cardiac (Frommer *et al*., 1987) and cerebral (Trimble, 1980; Frommer *et al*., 1987) toxicity. Immunotoxicotherapy is a potential alternative for TCA detoxification. The patient is given drug-specific antibodies which sequester the drug in the vascular compartment and thus remove drug molecules from the peripheral organs to the vascular compartment, leading to the reversal of drug toxicity (Scherrmann *et al*., 1989). Specific antibodies have been used in man to treat digitalis poisoning (Smith *et al*., 1976) and more recently, colchicine intoxication (Baud *et al*., 1995). Several groups (Hursting *et al*., 1989; Sabouraud *et al*., 1990; Pentel *et al*., 1991) have suggested using immunotoxicotherapy to treat TCA-induced cardiac toxicity, but it has not yet been tried in humans. There have been few attempts to remove TCA from the brain; except for a recent study in which we demonstrated that injection of TCA-specific immunoglobulins (IgG) facilitated the efflux of Imip from the brain (Ragusi *et al*., 1996), even though these antibodies do not cross the blood-brain barrier (Juhler & Neuwelt, 1989). Imip was injected directly into the brains of rats previously treated with a specific IgG. The efflux of Imip from the brain increased with the amount of antibody injected (31.5% was removed with an Imip : IgG ratio of 1 : 1000 and 57.5% with a ratio of 1 : 10 000), but this large excess of specific antibody could be difficult to use clinically. This problem could be overcome by using antibodies with a greater affinity. This affinity effect was first described by Cano *et al*. (1995) who showed that the capacity of anti-digoxin immunoglobulins to trap digoxin decreased with decreasing antibody affinity.

The present study was therefore carried out to investigate how the dose and affinity of an anti-TCA antibody, that is its immunoreactive capacity, influence its ability to reduce the TCA concentration in target organs of rats. These specific IgG were injected intravenously a few minutes after the systemic injection of Imip, and the kinetics of Imip redistribution in the brain and in the main peripheral organs (heart, kidney, liver and lung) were determined. We used our initial murine monoclonal IgG_1, which has a relatively low affinity ($Ka = 3.8 \times 10^7\ M^{-1}$), and a high affinity sheep polyclonal IgG ($Ka = 1.3 \times 10^{10}\ M^{-1}$) for which the cross-reactivities with imipramine and its main metabolites (desipramine, 2-hydroxy-imipramine, 2-hydroxy-desipramine) were estimated. Four doses of antibody and a single dose of Imip (a tracer dose) were used to establish a correlation between the redistribution of Imip and the immunoreactive capacities of the antibodies.

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Methods

Animals and surgical procedures

All studies were carried out on male Sprague-Dawley rats (Iffa Credo, Lyon, France) weighing 200–230 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 throughout the study (400 mg kg^{-1} i.p.) and the left femoral vein and artery were cannulated with teflon catheters, which were kept as short as possible to minimize the dead volume. The right femoral vein was exposed for direct intravenous injection.

Drugs

[benzene-ring 3H]-Imipramine (50.2 Ci $mmol^{-1}$) was obtained from New England Nuclear (Dupont de Nemours, Paris, France). The purity was checked at the start and in the course of the study. It was found to be 99%. Aqualyte scintillation liquid (Picofluor 40) and Soluene 350 were purchased from Packard (Rungis, France). 2-OH-Imipramine and 2-OH-desipramine were a generous gift from Novartis (Basel, Switzerland).

Nortriptyline-specific immunoglobulin (anti-NT IgG_1) The anti-NT IgG_1 was a murine monoclonal antibody (Ragusi *et al*., 1996). The affinity constants are (M^{-1}): 3.8 10^7 for Imip, 3.6 10^7 for desipramine (Desi), 0.17 10^7 for 2-OH-imipramine (2-OH-Imip) and 1.4 10^7 for 2-OH-desipramine (2-OH-Desi) when measured by the method of Müller (1980). Specificity was studied by adding serial amounts of Imip, Desi, 2-OH-Imip 2-OH-Desi and nortriptyline, and the concentrations that reduced the amount of the tracer bound by the antibody by 50% were calculated. The ratio of the above concentrations to the reference nortriptyline values, expressed as a percentage, indicated that the antibodies cross-reacted 152% with Imip, 64% with Desi, 2.3% with 2-OH-Imip and 15.4% with 2-OH-Desi.

Anti-desipramine/nortriptyline immunoglobulin (anti-DN IgG) This sheep polyclonal IgG was a generous gift from Therapeutic Antibodies (TAb London Ltd). The affinity constants are (M^{-1}): 6.0 10^{10} for Imip, 7.1 10^{10} for Desi, 2.6 10^{10} for 2-OH-Imip and 2.8 10^{10} M^{-1} for imipramine, desipramine, 2-OH-imipramine and 2-OH-desipramine, respectively, when measured by the method of Müller (1980). The antibodies cross reacted 112% with Imip, 115% with Desi, 75% with 2-OH-Imip and 67% with 2-OH-Desi.

Drug administration

Rats (160) were divided in five treatment groups (seven sample times for each group). Each rat was given [^{3}H]-Imip (50 μCi ml^{-1}, i.e. 1 nmol ml^{-1} 1 ml kg^{-1} body weight) in 0.9% NaCl *via* the catheterized femoral vein. The control group was given vehicle alone (750 μl 0.9% NaCl) 6 min after [^{3}H]-Imip injection. The other rats were given anti-NT IgG_1 or anti-DN IgG (collectively named anti-TCA IgG) *via* the right femoral vein in a total volume of 750 μl. Antibody doses were 1000 times the Imip dose in terms of binding sites (two sites per IgG molecule, i.e. 100 nmol) for anti-NT IgG_1 (IgG1000), and 1, 10 or 100 times the Imip dose (0.1, 1 or 10 nmol, respectively) for the anti-DN IgG (TAb1, TAb10 and TAb100 groups).

Sample collection and treatment

Arterial blood samples (100 μl) were collected *via* the teflon catheter into heparinized tubes from 5 s to 90 min after [^{3}H]-Imip injection. Plasma was separated by centrifugation and an aliquot (40 μl) was placed in 9 ml Picofluor 40 for counting. Rats were killed by decapitation 1, 5, 10, 20, 30, 60 or 90 min after [^{3}H]-Imip injection, and blood taken from the base of the neck. Aliquots of whole blood (10 μl) were placed in scintillation vials to measure the intravascular tracer content in the brain and other organs.

Organs (brain, liver, kidney, heart and lung) were quickly 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 500 μl) of the mixture were placed in scintillation vials containing 1 ml Soluene 350 (Packard), shaken for 1 h at 55°C, and suspended in 9 ml Picofluor 40. Radioactivity (plasma, blood and organs) was measured in a Tricarb model 1900 Tr liquid scintillation spectrophotometer (Packard, Rungis, France). The radioactivity in the brain was corrected for the brain blood content by determining the tissue blood content using [^{14}C]-inulin as a marker of cerebrovascular space. The radioactivity in organs was corrected for organ blood content using the blood radioactivity content measured just after decapitation and the vascular space volume of each organ determined according to Everett *et al*. (1956).

Determination of Imip metabolites in vivo

Blood was collected 6 min after Imip injection (50 μCi ml^{-1}, 1 ml kg^{-1} body weight) and centrifuged. An aliquot (20 μl) of a standard solution (250 μg ml^{-1}) containing Imip, Desi, 2-OH-Imip, 2-OH-Desi, was added to 200 μl of plasma. Imip and metabolites were then extracted from plasma according to the method of Foglia *et al*. (1991). Compounds were then separated on a C18 Lichrospher column; mobile phase: 0.01 M KH_2PO_4, 5 mM chloride tetramethylammonium, pH 2.4; acetonitrile (70 : 30 v/v) at 0.9 ml min^{-1}. Imip and its metabolites was detected at 215 nm with a Jasco UV-975 detector. Chromatograms was analysed with Borwin software. The standard compounds were used to estimate the extraction recovery (100% for Imip, Desi, 2-OH-Imip and 88% for 2-OH-Desi). Fractions of eluent were collected after the detector at times corresponding to the retention time for each compound. The radioactivity was measured, and the quantity of each compound determined.

Pharmacokinetic analysis

The maximum Imip concentration (Cmax), time to reach Cmax (Tmax), the minimal Imip concentration in the control group (Cmin) and the time to reach Cmin (Tmin) were determined from experimental data. The areas under the mean concentration-time curves (AUC) of Imip in plasma and tissues were calculated for 1–60 min by the trapezoidal method. Each AUC was plotted against the log NKa values for each tissue and plasma. NKa was defined as the immunoreactive capacity of the antibody, where N is the molar concentration of binding sites ($N = n \times C$ where n is the number of binding sites, i.e. 2 for IgG, and C the protein molar concentration) and Ka the affinity constant of the antibody (M^{-1}).

Statistical analysis

The effects of specific IgG on the plasma and tissue concentrations of [^{3}H]-Imip were analysed by one-way analysis of variance (Kruskall-Wallis) followed by the Mann-Whitney *U*-test. Significance was set at $P<0.05$.

Results

[^{3}H]-TCA plasma concentrations

The plasma of rats given [^{3}H]-Imip (1 nmol kg^{-1} i.v.) was analysed for Imip and its main metabolites just before injecting antibody (6 min after [^{3}H]-Imip injection). The radioactivity recovered was 100%, of which 91.7±1.1% was Imip, 1.9±0.3% Desi and 3.4±1.3% 2-OH-Imip. 2-OH-Desi was not detected. The radioactivity measured in blood was thus essentially due to the parent drug, imipramine.

[^{3}H]-Imip plasma concentrations

The plasma [^{3}H]-Imip concentration-time profiles in control and antibody-treated rats are shown in Figure 1. The plasma [^{3}H]-Imip concentration was maximal in the first sample (5 s) in the control group, and then decreased rapidly. The anti-TCA IgG increased the total plasma Imip concentration. The $AUC_{1\rightarrow 60\ min}$ for [^{3}H]-Imip were 4 fold higher than in the controls for the IgG_1 1000, 9 fold higher for TAb1, 33.9 fold for TAb10 and 41.4 fold for TAb100. This effect depended on the antibody dose and the antibody affinity indicating by the relationship between AUC and NKa (Figure 7).

[^{3}H]-Imip tissue redistribution

The distribution of [^{3}H]-Imip in the heart, kidneys, lungs, liver and brain of rats killed at different times after [^{3}H]-Imip (1 nmol kg^{-1}) injection was determined in control and antibody treated groups. The pharmacokinetic profiles in the lungs and heart of control rats were similar to those of the blood: Cmax was reached in the first sample (1 min after [^{3}H]-Imip injection), followed by a relatively rapid decline in 10 min, and a slow drop in radioactivity up to 90 min (Figures 2 and 3). The [^{3}H]-Imip Cmax was later in the brain, liver and kidneys (10 min after [^{3}H]-Imip injection) and then declined until 90 min (Figures 4, 5 and 6).

TAb1 did not significantly reduce the tissue concentrations of Imip in any of the organs examined. Rats treated with IgG_1 1000, TAb10 or TAb100 had significantly reduced Imip concentrations after 10 min (heart and kidneys) or after

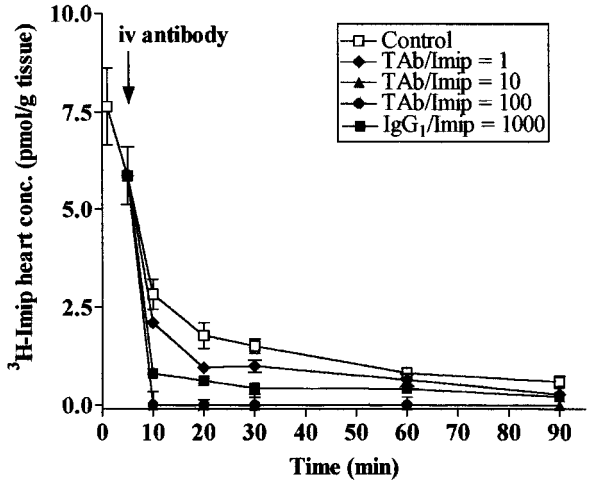

Figure 2 Effects of antibody treatment on Imip concentrations in the heart. [^{3}H]-Imip (1 nmol kg^{-1} of body weight) was injected i.v. at $t=0$. Controls were given 0.9% NaCl 6 min later. Treated rats were given high affinity (TAb) or low affinity (IgG_1) antibodies i.v. at different antibody/Imip molar ratios 6 min after Imip. Heart Imip concentrations were determined at different times after Imip injection. The values are means±s.e.m. S.e.m. not shown were less than the symbol size.

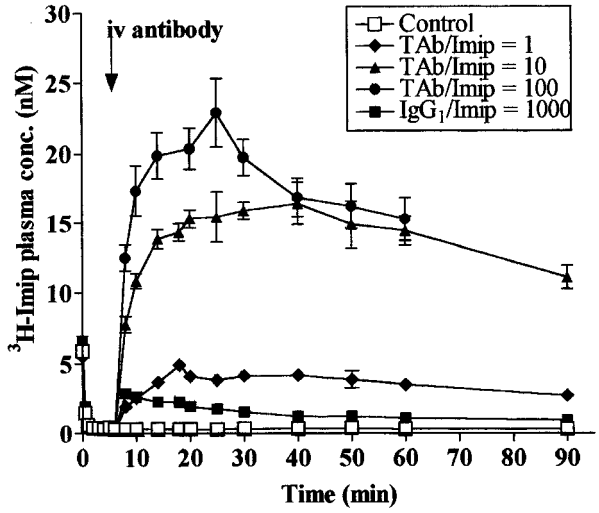

Figure 1 Changes in the plasma [^{3}H]-Imip concentrations in control and treated rats. [^{3}H]-Imip (1 nmol kg^{-1} of body weight) was injected i.v. at $t=0$. Treated rats were given high affinity (TAb) or low affinity (IgG_1) antibodies i.v. at different antibody/Imip molar ratios 6 min after Imip. Blood samples were taken from the femoral vein for up to 90 min. The values are means±s.e.m. S.e.m. not shown were less than the symbol size.

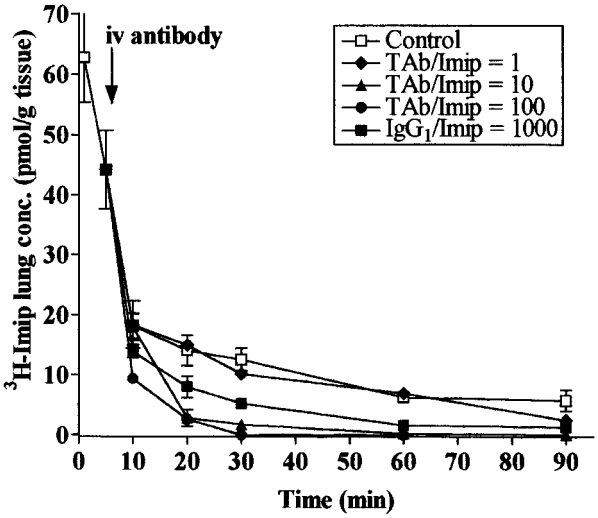

Figure 3 Effects of antibody treatment on lung Imip concentrations. [^{3}H]-Imip (1 nmol kg^{-1} of body weight) was injected i.v. at $t=0$. Controls were given 0.9% NaCl 6 min later. Treated rats were given high affinity (TAb) or low affinity (IgG_1) antibodies i.v. at different antibody/Imip molar ratios 6 min after Imip. Rats were decapited at various times post-injection and lung Imip concentrations were determined. The values are means±s.e.m. S.e.m. not shown were less than the symbol size.

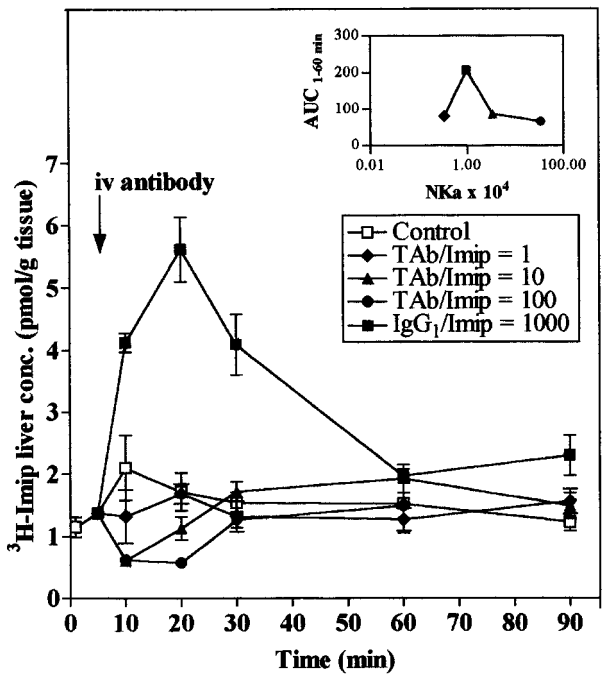

Figure 4 Effects of antibody treatment on liver Imip concentrations. [^{3}H]-Imip (1 nmol kg^{-1} of body weight) was injected i.v. at $t=0$. Controls were given 0.9% NaCl 6 min later. Treated rats were given high affinity (TAb) or low affinity (IgG_1) antibodies i.v. at different antibody/Imip molar ratios 6 min after Imip. Rats were decapited at various times post-injection and liver Imip concentrations were determined. The values are means$\pm$s.e.m. S.e.m. not shown were less than the symbol size. Inset: Relationship between the immunoreactive capacity (NKa) and the $AUC_{1\rightarrow 60\ min}$ for liver Imip concentrations.

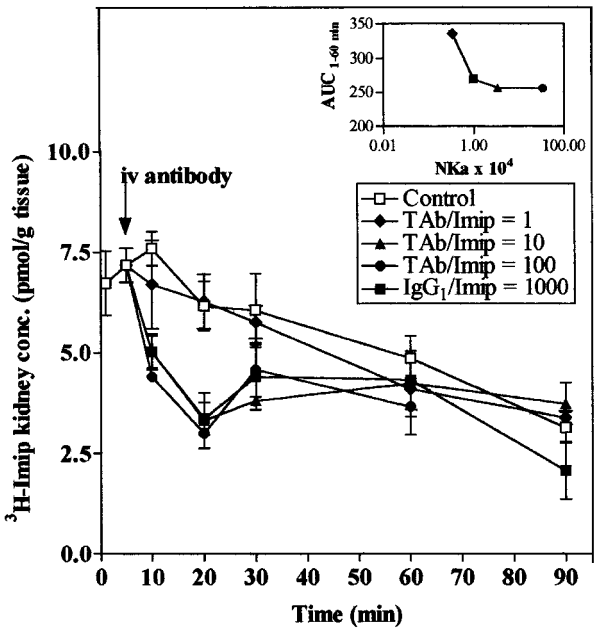

Figure 5 Effects of antibody treatment on kidney Imip concentrations. [^{3}H]-Imip (1 nmol kg^{-1} of body weight) was injected i.v. at $t=0$. Controls were given 0.9% NaCl 6 min later. Treated rats were given high affinity (TAb) or low affinity (IgG_1) antibodies i.v. at different antibody/Imip molar ratios 6 min after Imip. Rats were decapited at various times post-injection and kidney Imip concentrations were determined. The values are means$\pm$s.e.m. S.e.m. not shown were less than the symbol size. Inset: Relationship between the immunoreactive capacity (NKa) and the $AUC_{1\rightarrow 60\ min}$ for kidney Imip concentrations.

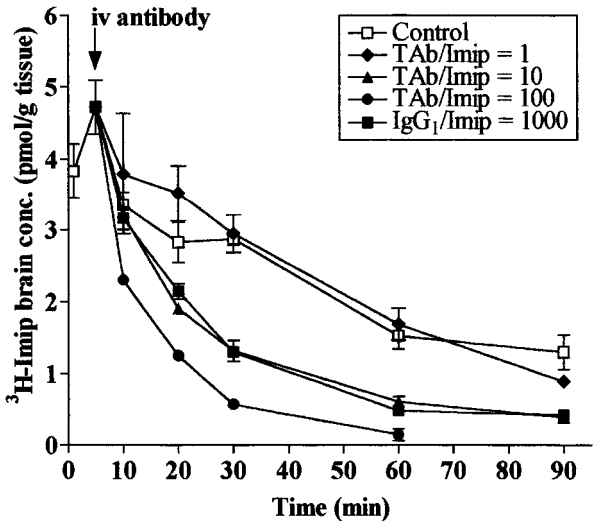

Figure 6 Effects of antibody treatment on brain Imip concentrations. [^{3}H]-Imip (1 nmol kg^{-1} of body weight) was injected i.v. at $t=0$. Controls were given 0.9% NaCl 6 min later. Treated rats were given high affinity (TAb) or low affinity (IgG_1) antibodies i.v. at different antibody/Imip molar ratios 6 min after Imip. Rats were decapited at various times post-injection and brain Imip concentrations were determined. The values are means$\pm$s.e.m. S.e.m. not shown were less than the symbol size.

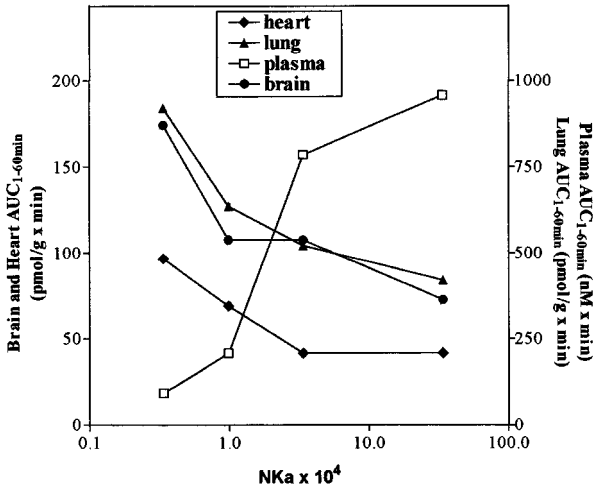

Figure 7 Relationship between the immunoreactive capacity (NKa) and the plasma, brain, heart and lung Imip $AUC_{1\rightarrow 60\ min}$.

20 min (brain and lungs) and the AUC for the tissue concentration-time curves decreased as the NKa value increased (Figure 7 and inset Figure 5). The time to reach the Imip Cmin, which was 90 min in the control group, was shorter in heart, brain and lungs of rats treated with specific antibodies; it varied inversely with the NKa value (Table 1).

The kidney Imip concentration was decreased by IgG_1 1000, TAb10 or TAb100 for only the 10–30 min period post-injection and was then the same as the Imip concentrations in control rats (Figure 5). The [^{3}H]-Imip concentrations in the liver of rats given IgG_1 1000 were significantly higher than those in the control group over the whole experiment. Conversely, the [^{3}H]-Imip liver concentrations at the TAb10 group were significantly reduced at 10 min and then tended to be similar to those of the control group. There was no

Table 1 Relationship between the immunoreactive capacity (NKa) and the time (Tmin) taken to reach the minimal value of the control in various tissues after specific-antibody treatment.

	NKα (10^4)	*Tmin* (min)		
		Brain	*Heart*	*Lung*
Control	–	90	90	90
TAb1	0.338	70	64	68
IgG_1 1000	0.998	30	21	28
TAb10	3.38	31	9.5	18
TAb100	33.8	19.5	9.5	15

significant difference between the Imip levels in TAb1, TAb 100 and control groups throughout the experiment (Figure 4). The $AUC_{1\rightarrow 60\ min}$ of the Imip liver concentration-time curve did not depend on the NKa value (inset Figure 4).

Discussion

The present study was carried out to demonstrate the influence of the immunoreactive capacity of anti-TCA IgG (dose and affinity) on the removal of imipramine from brain and peripheral organs. Antibodies were given at the single time of 6 min after imipramine because the concentration of the drug was maximal 5 min after its injection. The kinetics of imipramine redistribution from organs into plasma can be evaluated at this time. As imipramine is efficiently extracted by the liver, it is essential to estimate whether the observed effects were partially attributable to imipramine metabolites. The identification and measurement of radioactive molecules in plasma showed that there was considerable (91.7%) unmetabolized drug present 6 min after imipramine injection. This indicates that the pharmacokinetics obtained accurately reflect the parent drug distribution and not that of its metabolites. This is of great importance because the two antibodies used recognize both imipramine and its two main metabolites, desipramine and 2-hydroxy imipramine. However, desipramine is an active metabolite of imipramine, so that it is important that the antibodies react equally with both, with similar affinities. Because of the small amount of desipramine (1.9%) detected in plasma, the results described represent only imipramine.

Anti-TCA IgG dramatically increased the Imip concentration in the vascular compartment of the rats. The increase depended on two factors; the ratio of Imip to IgG and the affinity constant of the antibody, which defines the stability of the Imip-antibody complex. The effect of these factors can be seen by comparing the Imip plasma AUC resulting from the redistribution of Imip in the plasma to the immunoreactive capacity of the antibody, defined by the concentration of binding sites and their affinity, NKa. The relationship between AUC and NKa appears to be described by a sigmoidal curve, indicating a maximal effect that cannot be exceeded, despite an increase in antibody dose or affinity constant. TAb10 ($NKa = 3.38 \times 10^4$) and TAb100 ($NKa = 33.8 \times 10^4$) produced maximal plasma Imip concentrations. This effect of anti-TCA IgG on the plasma Imip concentration raises the question of the effect on the Imip concentrations in tissues, particularly in Imip target organs such as the brain and the heart.

The Imip pharmacokinetics in control rats indicate that two groups of tissue can be distinguished kinetically: those with profiles similar to the plasma (Cmax reached immediately), such as the heart and lung, and those in which Cmax is delayed, such as the brain (Tmax at 5 min), liver and kidney (Tmax at 10 min).

The first group of organs (heart and lung) belongs to the same compartment as the plasma and the redistribution effect of anti-TCA IgG is similar in these organs. The time to reach the control Cmin in the heart was greatly decreased when the NKa value increased. Rats in the Tab10 group had reached the control heart Cmin by 10 min after Imip injection. This ability to reduce Tmin is of clinical importance as most deaths from cardiac toxicity occur within a few hours after TCA ingestion (Pentel & Benowitz, 1986). Immediate redistribution also occurs in the lungs, which kinetically belong to the blood compartment and contain specific Imip binding sites (Morin *et al*., 1984) similar to those in the brain (Raisman *et al*., 1979). The lung contained the highest concentrations of Imip of all the tissues examined, in agreement with previous reports (Dingell *et al*., 1964; Bickel *et al*., 1983). This accumulation in the lung is typical of basic lipophilic drugs. It has been attributed to the lower pH of the extravascular space in this tissue, and to a special affinity of Imip for the neutral and acidic phopholipids which are major components of surfactant (Kodavanti & Mehendale, 1990). Nevertheless, the Imip redistribution caused by the antibodies was not limited by this tissue binding and was of considerable degree.

In contrast, the anti-TCA antibody had different effects on the Imip profiles of the second group of tissues with slower Imip uptakes. All the antibody treatments except TAb1 had the same effect on kidney Imip with the Imip concentration being reduced during the first few minutes after their administration. This is probably because the marked early increase in the plasma Imip concentration due to the binding of Imip by IgG decreases the renal elimination of Imip, especially as the Imip-antibody complex cannot be excreted by the kidney because of its size (154 kDa). This is supported by the identical times for reaching Tmax in the plasma and kidney Tmin (20 min). The organ most involved in Imip metabolism is the liver. The monoclonal IgG_1 antibody greatly increased the Imip concentration in liver, as in our previous study (Ragusi *et al*., 1996). Pentel *et al*. (1987) used an anti-desipramine antiserum and also observed an increase in liver desmethylimipramine (DMI). They argued that the binding of DMI to IgG increased the delivery of DMI to the liver, thereby increasing its rate of metabolism. The increase in Imip concentration caused by IgG_1 1000 treatment agrees with this. But there was a decrease after the TAb treatments, perhaps due to the difference in the IgG species origin: IgG_1 1000 is a murine monoclonal immunoglobulin, whereas TAb is a sheep polyclonal immunoglobulin. The hepatic uptake of a circulating immune complex results from IgG interacting with Fc-receptors on the surface of the liver cells (Gudmundsen *et al*., 1986), but Fc piece may vary from one IgG to another. Rat liver may interact better with the murine IgG_1 1000 than with the sheep TAb.

The brain had distinct changes in its Imip distribution kinetics in response to the various antibody treatments. The effects of increasing doses of TAb were positively correlated with the NKa values, in the same way as in the heart and lung. TAb1 did not reduce the brain Imip concentrations as it did for peripheral organs, while TAb10 and TAb100 produced sustained declines in Imip, so that the concentrations at 20 min were less than a half (TAb10) or less than a third (TAb100) of those at Tmax (5 min). The ability of this high-affinity antibody to remove quickly significant amounts of the drug is a promising sign that it may diminish the risk of CNS toxicity. We have also demonstrated previously (Ragusi *et al*., 1996) that the presence of specific anti-TCA IgG in the blood

compartment increases the efflux of Imip from the brain even though the antibodies do not cross the blood-brain barrier. The anti-TCA antibodies were injected after Imip in the present study so as to mimic the clinical situation, and the results show that the blood-brain barrier is not an obstacle to the efflux of Imip from the brain induced by anti-TCA antibodies in the blood. This is not surprising, as the influx and efflux of Imip across the blood-brain barrier depend only on diffusion and not on active transport. These movements are not membrane-limited because Imip is hydrophobic. All the organs studied here have blood perfusion rates that facilitate the removal of Imip from the organs into blood by the anti-TCA antibodies in the blood. For example, 71% of Imip was removed from the lungs at 10 min (perfusion rate 28.7 ml min^{-1} g^{-1}), while only 29% was removed from the brain, which is less well perfused (0.74 ml min^{-1} g^{-1}) during this time.

Thus the present study provides a detailed kinetic profile of the Imip redistribution produced by anti-TCA antibodies under controlled conditions of binding site concentration and affinity. It confirms that using a higher affinity IgG reduces the amount of antibody needed and that anti-TCA IgG does remove Imip from target organs with an efficiency that depends on its immunoreactive capacity, defined by NKa. These kinetic data should be extremely helpful in investigations into the benefit of anti-TCA antibodies for treating Imip intoxication.

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