Predictions of *in Vivo* Plasma Concentrations from *in Vitro* Release Kinetics: Application to Doxepin Parenteral (I.M.) Suspensions in Lipophilic Vehicles in Dogs

Christina Gido,[1] Peter Langguth,[2] and Ernst Mutschler[1,3]

Received March 31, 1993; accepted November 30, 1993

A flow through dissolution system was applied to obtain biorelevant dissolution rates from controlled release systems for parenteral administration using the antidepressant doxepin as a model compound. Plasma concentrations were simulated using the disposition function of doxepin obtained from administration of an aqueous doxepin solution (Aponal^R) to beagle dogs. Input functions were obtained from in vitro dissolution experiments with three parenteral controlled release suspensions of doxepin hydrochloride (DHCl), doxepin pamoate (DP-1), and microspheres of doxepin hydrochloride in poly D,L-lactid-co-glycolid (MC-1) in isopropyl myristate. The predicted plasma concentrations were compared with experimentally obtained concentrations in vivo. Good correlations ($r>0.88$) between observed and predicted data were obtained for all formulations investigated. Similarly, in vivo release kinetics calculated by the Loo-Riegelman method were compared with release kinetics measured in vitro and showed good correlations ($r>0.89$). It is anticipated that the in vitro dissolution system permits assessment of the clinical relevance of observed variations in dissolution rates e.g. between batches of one formulation.

KEY WORDS: biorelevant dissolution; simulation of plasma concentrations; in vivo release; in vitro/in vivo correlation.

Introduction

One of the major objectives of dissolution testing is quality control of solid and semi solid dosage forms. However, when one attempts to relate the in vitro dissolution rate of a drug from a particular dosage form to its in vivo dissolution behaviour, the applicability of currently used in vitro dissolution techniques to predict in vivo systemic drug concentrations, remains controversial (1). Experiments were primarily performed with oral controlled release products whereas with parenteral dosage forms few attempts to correlate in vitro and in vivo release rates have been reported (2, 3). Nevertheless, there are multiple benefits to be gained from such studies. A dissolution system providing biorelevant release rates could reduce the extent of animal studies and clinical testing in the development of sustained release formulations. Thus, we described the application of a flow through dissolution method for the testing of parenteral sustained release formulations in lipid vehicles, using doxepin as model compound (4).

Doxepin is a antidepressant with marked antipsychotic, anxiolytic and sedative properties (5). It is used in the long-term treatment of endogenous depression and is frequently administered orally. The therapeutically used drug represents a mixture of the cis- and trans-stereoisomers at a constant ratio of 15:85. The two isomers of the parent compound do not differ in their pharmacokinetic properties, i.e. a cis/trans-ratio of 15:85 is found in vivo as well (6), and it does not change with time. After a single oral dose, the disposition of doxepin was found to be biphasic and followed first order kinetics. Average plasma half-lives for doxepin and its pharmacologically active metabolite desmethyldoxepin in healthy subjects were 17 h and 50 h, respectively. Apparent volume of distribution was calculated to be 20 l/kg (7), absolute bioavailability 25% (8). There is no consensus yet on plasma doxepin concentrations necessary for clinical response. It is suggested that a minimum plasma concentration of 110 ng/ml doxepin must be reached to obtain an antidepressant response (9). A frequently used p.o. dosage schedule is 25–50 mg t.i.d. Yet, administration once daily is feasible as well, because of the comparatively long half-life of doxepin (10). Different administration routes for doxepin, e.g. parenteral, may be advantageous, since steady state plasma concentrations reached after p.o. dosage were found to be highly variable (7), which is in part due to the extensive first-pass effect of doxepin. Parenteral administration could also circumvent problems associated with the poor compliance of patients receiving antidepressant therapy (11). Since with a parenteral sustained release formulation more constant and reproducible plasma concentrations and hence a more predictable therapeutic effect at a lower dosage frequency could be expected, in the present work such formulations of doxepin were investigated.

The aims of the present studies were to derive the response function from a disposition function obtained from plasma concentration-time profiles following i.m. drug administration and an input function based on the in vitro drug dissolution, and to validate the dissolution method by comparison of simulated data and correlation with experimental data from studies in beagle dogs.

Materials and Methods

Reagents were of analytical grade, solvents were gradient grade and used as received from E. Merck (D-Darmstadt) unless otherwise indicated. Water was deionized and double distilled before use. Glass parts, silicon tubes, silicon plugs and polypropylene reducing fittings for the dissolution apparatus were obtained from W. Fischer (D-Frankfurt/M.).

Doxepin formulations

An aqueous isotonic solution of doxepin hydrochloride (Aponal^R, Boehringer Mannheim, D-Mannheim) was used for evaluation of the pharmacokinetic disposition character-

[1] Department of Pharmacology, Johann Wolfgang Goethe-University, Frankfurt/Main, Germany.

[2] Department of Pharmacy, Eidgenössische Technische Hochschule, Zürich, Switzerland.

[3] To whom correspondence should be addressed at Pharmakologisches Institut für Naturwissenschaftler, Biozentrum Niederursel, Marie-Curie-Str. 9, Gebäude N 250, D-60053 Frankfurt.

0724-8741/94/0600-0800$07.00/0

istics of the drug following i.m. administration to beagle dogs.

The preparation of oily suspensions as doxepin slow release formulations for i.m. administration was described in detail in a previous report (4). The formulations involved in the present study were

DHCl. doxepin hydrochloride suspended in isopropyl myristate containing 0.16% Epicuron^R 200. Doxepin content: 9.10%; particle size: 24 μm; density: 0.853 g/ml

DP-1. doxepin pamoate — precipitated from aqueous solution — suspended in isopropyl myristate containing 0.23% Epicuron^R 200. Doxepin content: 6.59%; particle size: 5.2 μm; density: 0.879 g/ml

MC-1. microspheres of doxepin hydrochloride in poly D,L-lactid-co-glycolid — prepared by spray drying — suspended in isopropyl myristate containing 0.16% Epicuron^R 200. Doxepin content: 8.35%; microsphere size: 2.5 μm; density: 0.954 g/ml

In vitro dissolution tests

In vitro dissolution experiments were performed in a flow-through apparatus described earlier (4). The system consisted of a U-shaped glass tube containing siliconized glass wool (Chrompack, Nl-EA Middelburg), 20 glass globules (2 mm diameter), and a glass half hollow sphere (7 mm diameter). The glass globules were coated with the formulation under investigation, placed on the glass wool and additionally 2 to 3 drops of the formulation were filled into the glass half hollow sphere and placed on top of the globules. All amounts of the formulation were weighed exactly on an analytical balance (Sartorius, D-Göttingen). The tube was filled with dissolution medium, air bubbles were removed, and the tube was closed with a silicon plug. The dissolution medium consisted of pooled human plasma and phosphate buffer 10 mmol$*$l^{-1} pH 7.4 (1/3, v/v) and was degassed ultrasonically at 37°C prior to use. Human plasma has been used in order to simulate physiological conditions for i.m. injectables as closely as possible. The dissolution medium passed through the tube at a rate of 7 ml per hour. Fractions were collected in 3 hourly intervals for 18 hours and in 9 hourly intervals thereafter up to 126 hours. The collection period for formulation DHCl was 36 hours because of the relatively fast drug release. Dissolution medium transport was performed by a peristaltic pump (Typ 131900, Desaga, D-Heidelberg). The system was thermostated at 37±0.5°C by a water bath. Each dissolution experiment was run in four replicates. Samples from the fractions collected were stored at −22°C until analysis.

In vivo studies

The investigation of single dose pharmacokinetics of doxepin formulations was performed in beagle dogs in a cross-over study design to minimize the influence of inter-individual variability. Each dog received a controlled release formulation and Aponal^R as a reference in order to estimate the relative bioavailability and the release prolonging effect of the novel formulation. Two female and two male healthy beagle dogs were used for the investigation of each formulation. They were given food and water ad libitum during the experiment. Injection was i.m. into the gastrocnemius muscle. A washout period of at least seven days was kept between the administrations to allow almost complete elimination of the drug from the body prior to the administration of the subsequent dose. For formulation DHCl pharmacokinetic data from only three dogs are available, because one female dog had to be removed from the study after drug administration. Blood samples of 4 ml were collected into heparinised tubes (Monovette^R, Sarstedt, D-Nümbrecht) before and from 0.5 to 168 h after administration. The exact sampling times are given in table I. The blood samples were centrifuged at 2000 rpm for 10 minutes. Subsequently the plasma was transferred to sample tubes and stored at −22°C until analysis. For details on pharmacokinetic studies performed see table I.

HPLC assay

Doxepin was assayed by a stereospecific HPLC method following an adequate sample pretreatment. The analytical method quantifies total (bound and unbound) doxepin in dissolution medium and plasma, respectively.

In vitro samples: A 200 μl aliquot of the dissolution sample was added to 100 μl of internal standard solution (20 μg desmethyloxaprotiline/ml water) placed in a centrifuge tube. Then 200 μl acetic acid 5% was added to improve

Table I — Pharmacokinetic studies of doxepin formulations in beagle-dogs

A: Aponal^R versus DHCl

dog number	sex	weight [kg] phase 1	weight [kg] phase 2	dose [mg/kg] Aponal^R	dose [mg/kg] DHCl
975	m	13.3	13.4	5	5
710	m	11.5	11.2	5	5
338	f	13.4	13.1	5	5

sample collection [h]: 2, 4, 7, 24, 48, 72, 144

B: Aponal^R versus DP-1

dog number	sex	weight [kg] phase 1	weight [kg] phase 2	dose [mg/kg] Aponal^R	dose [mg/kg] DE-1
253	f	8.5	8.6	5	5
256	f	8.9	8.7	5	5
716	m	10.3	10.4	5	5
701	m	11.5	12.1	5	5

sample collection [h]: 1, 3, 5, 7, 24, 28, 31, 48, 72, 96, 168

C: Aponal^R versus MC-1

dog number	sex	weight [kg] phase 1	weight [kg] phase 2	dose [mg/kg] Aponal^R	dose [mg/kg] MC-1
749	m	10.1	11.3	5	4.0
760	m	9.5	10.0	5	4.6
392	f	8.0	8.5	5	1.6
393	f	8.4	8.5	5	3.2

sample collection phase 1 [h]: 0.5, 1, 2, 3, 5, 7, 10, 24, 34

sample collection phase 2 [h]: 1, 2, 3, 5, 7, 10, 24, 34, 48, 72, 96, 100

phase separation after extraction. To make the sample alkaline 1.5 ml 0.2 N sodium hydroxide solution was added, and 6 ml of a mixture of n-hexane/ether 50/50 (v/v) were added for extraction. The tube was capped, shaken for 10 min and centrifuged for 10 min at 4000 rpm. Four ml of the organic phase were transferred to another tube, 500 µl of 1 mM hydrochloric acid were added. After shaking (10 min) and centrifugation (10 min) the organic phase was removed by suction (water jet vacuum pump) and subsequently by a gentle stream of nitrogen for 1 min. A 20 µl aliquot of the sample was assayed on an HPLC system, equipped with a Knauer HPLC pump 64 (Knauer, D-Berlin), an autosampler SP 8880 (Spectra-Physics, D-Darmstadt), a Hypersil silica column 5 µm, 250*4.6 mm (Gamma Analysen Technik, D-Bremerhaven), a UV spectrophotometric detector (SPD 6A, Shimadzu, J-Kyoto) at 225 nm, and a Chromjet integrator (Spectra-Physics). For the mobile phase as suggested by Dilger et al (12) 1.2 g of tetraethylammonium perchlorate (Fluka, CH-Buchs) were dissolved in 400 g of water and adjusted to pH 8.4 with sodium hydroxide solution. This solution was mixed with acetonitrile 15/65 (w/w). The flow rate was 1 ml/min. The areas under the peaks were integrated to quantify doxepin. The two isomers of the compound were separated, but since a constant ratio of about 5.5 between the peak areas of trans- and cis-doxepin was observed throughout all experiments, only the area under the trans-doxepin peak was used for quantification. Calculations were performed with the WINner/386 autolab software (Spectra Physics) on an IBM personal system/2 model 70. Calibration curves were run daily with concentrations of 0.6, 2, 10 and 40 µg doxepin / ml dissolution medium. Retention times for cis-doxepin, trans-doxepin, and desmethyloxaprotiline were 6.8, 7.2 and 10.1 minutes, respectively. Resolution factors were 0.84, and 3.39 for cis- / trans-doxepin, and trans-doxepin / internal standard, respectively.

The accuracy of the HPLC method, defined as the percent deviation between the amount added to blank dissolution medium and the amount found, as well as the intra-day reproducibility, defined as the relative percent standard deviation of the amounts found, were evaluated by assaying 6 times each of the spiked samples with 40, 2 and 0.6 µg/ml. The accuracy was found to be 0.33, 2.5, and 8.3%, the intra-day reproducibility 0.72, 6.9, and 19.7%, respectively. The inter-day reproducibility, evaluated by using the obtained slope values of 9 calibration curves, each extracted and measured on different days, was found to be 7.07%.

Recovery of doxepin and desmethyloxaprotiline was studied in dissolution medium by assaying 6 times three types of samples. Type 1 consisted of 200 µl dissolution medium, 50 µl internal standard solution (40 µg/ml) and 50 µl aqueous doxepin standard solution (200 µg/ml and 3 µg/ml, respectively, each 3 times). Type 2 consisted of 200 µl dissolution medium, 50 µl internal standard solution, and 50 µl water. Type 3 consisted of 200 µl dissolution medium 50 µl water and 50 µl aqueous doxepin standard solution. After extraction and reextraction a 250 µl aliquot of the hydrochloric acid solution was added to 50 µl water (type 1), 50 µl aqueous doxepin standard solution (type 2), or 50 µl internal standard solution (type 3). A 20 µl aliquot of the resulting sample was assayed on the HPLC system. Recovery of doxepin was calculated from the means of peak areas obtained from samples type 1 and 2 and was found to be 77.9% and 79.1% for 200 µg/ml and 3 µg/ml, respectively. Recovery of desmethyloxaprotiline was calculated from the means of peak areas obtained from samples type 1 and 3 and was found to be 87.0%. Peak areas obtained from samples type 1 were multiplied by three in order to take account of the waste fractions appearing during sample pretreatment.

In vivo samples. A modified sample pretreatment and different calibration solutions were necessary because of the lower doxepin concentrations and the presence of the metabolite desmethyldoxepin in vivo. A 500 µl aliquot of the plasma sample was added to 100 µl of internal standard solution (200 ng/ml). Then 200 µl acetic acid 5%, 1.5 ml 0.2 N sodium hydroxide solution, and 6 ml of n-hexane/ether were added. After shaking (10 min) and centrifugation (10 min) 4 ml of the organic phase were transferred to another tube and 200 µl of 1 mM hydrochloric acid were added. After shaking (10 min) and centrifugation (10 min) the organic phase was removed by suction (water jet vacuum pump) and subsequently by a gentle stream of nitrogen for 1 min. A 100 µl aliquot of the sample was injected into the HPLC system. Calibration curves prepared from blank human plasma were run daily with concentrations of 2.74, 6.85, 13.7, 68.5, 274, and 685 ng/ml doxepin and 1.5, 3.75, 7.5, 37.5, 150, and 375 ng/ml desmethyldoxepin. Retention times were 8.75 min for cis-doxepin, 9.3 min for trans-doxepin, 12.1 min for desmethyloxaprotiline (internal standard), and 14.4 min for desmethyldoxepin. Resolution factors were 0.73, 3.7 and 2.3 for cis- / trans-doxepin, trans-doxepin / internal standard and internal standard / desmethyldoxepin, respectively. The accuracy for the quantification of 685, 68.5, and 2.74 ng/ml doxepin was 0.05, 2.19, and 1.31%, the intra-day reproducibility 0.62, 5.25, and 18.1%, respectively. The inter-day reproducibility was 7.88%, evaluated by using the obtained slope values of 6 calibration curves.

Recovery studies in plasma were performed according to the method described for dissolution medium with aqueous drug standard solutions containing 6 µg/ml or 20 ng/ml doxepin and 3 µg/ml or 10 ng/ml desmethyldoxepin and an internal standard solution containing 2 µg/ml desmethyloxaprotiline. Recovery of doxepin was found to be 74.3% and 72.5% for 6 µg/ml and 20 ng/ml, respectively. Recovery of desmethyldoxepin was found to be 79.7% and 82.5% for 3 µg/ml and 10 ng/ml, respectively. Recovery of desmethyloxaprotiline was found to be 85.6%.

Calculations

Dissolution rates from in vitro experiments, which were used for simulation of plasma concentrations, were obtained by fitting a biexponential model to the data according to

$$M_{(t)} = D_1 (1 - e^{-k_{01}t}) + D_2 (1 - e^{-k_{01}'t}) \quad (1)$$

$M_{(t)}$ = amount of drug released at time t, k_{01}, k_{01}' = rate constants of dissolution, D_1, D_2 = respective fractions of the dose.

Curve fits were calculated by the Fig.P software version 5.0 (Fig.P Software Corporation, Durham, North Carolina). For simulation of plasma concentrations mean parameters of four dissolution experiments were used. $t_{15\%}$ and $t_{30\%}$, the times when 15% and 30%, respectively, of the tested formu-

lation was released, were calculated from the resulting dissolution curves. Normal distribution was assumed when the data met the criteria: $0.9 < \text{median/mean} < 1.1$, and $3 * SD_{(n-1)} < \text{mean}$.

For comparison of mean values the Welch test was applied with $\alpha = 0.05$ in two tails and 95% confidence intervals (CIs) of differences of mean values were calculated.

Pharmacokinetic parameters of doxepin in beagle dogs following i.m. administration of Aponal^R were calculated after fitting a two compartment body model to the plasma-concentration-time data using Topfit^R (Thomae, D-Biberach). The parameters of interest were: V_C = apparent volume of distribution of the central compartment, k_{12}, k_{21} = microconstants of distribution for a two compartment model, k_{10} = microconstant of elimination from the central compartment,

$$\lambda_1 = \text{macroscopic rate constant} = \tfrac{1}{2}\,(k_{12}+k_{21}+k_{10}+([k_{12}+k_{21}+k_{10}]^{2}-4k_{21}k_{10})^{1/2}) \quad (2)$$

$$\lambda_2 = \text{macroscopic rate constant} = \tfrac{1}{2}\,(k_{12}+k_{21}+k_{10}-([k_{12}+k_{21}+k_{10}]^{2}-4k_{21}k_{10})^{1/2}) \quad (3)$$

For simulation of plasma concentrations mean pharmacokinetic parameters of the four dogs from study C were used.

Simulated plasma-concentration-time data for the sustained-release formulations were calculated as product of an input function, obtained from the in vitro dissolution experiments, and a mean disposition function, obtained from the in vivo data of Aponal^R. The input function represents two parallel first order input steps and corresponds to equation 1:

$$M_{(t)} = D_1 - D_1 e^{-k_{01}t} + D_2 - D_2 e^{-k_{01'}t}. \quad (4)$$

Differentiation with respect to t yields

$$in = D_1 k_{01} e^{-k_{01}t} + D_2 k_{01'} e^{-k_{01'}t} \quad (5)$$

the Laplace transform of which is

$$in_s = D_1 k_{01}(s+k_{01})^{-1} + D_2 k_{01'}(s+k_{01'})^{-1} \quad (6)$$

$$= ((s+k_{01'})D_1 k_{01} + (s+k_{01})D_2 k_{01'})((s+k_{01})(s+k_{01'}))^{-1}, \quad (7)$$

where s is the Laplace operator from

$$e^{at} = (s-a)^{-1} \quad (8)$$

The disposition function for a two compartment body model

$$C = A_1 e^{-\lambda_1 t} + A_2 e^{-\lambda_2 t} \quad (9)$$

may be written in Laplace-transformed form

$$d_s = (s+k_{21})((s+\lambda_1)(s+\lambda_2))^{-1}. \quad (10)$$

The product of input and disposition function is

$$a_{s,c} = [D_1 k_{01}(s+k_{01'})(s+k_{21})][(s+k_{01})(s+k_{01'})(s+\lambda_1)(s+\lambda_2)]^{-1} + [D_2 k_{01'}(s+k_{01})(s+k_{21})][(s+k_{01})(s+k_{01'})(s+\lambda_1)(s+\lambda_2)]^{-1}. \quad (11)$$

Using the method of partial fractions one obtains

$$\begin{aligned} a_{s,c} = {} & [D_1 k_{01}(k_{21}-k_{01})][(\lambda_1-k_{01})(\lambda_2-k_{01})]^{-1}(s+k_{01})^{-1} \\ & + [D_1 k_{01}(k_{21}-\lambda_1)][(k_{01}-\lambda_1)(\lambda_2-\lambda_1)]^{-1}(s+\lambda_1)^{-1} \\ & + [D_1 k_{01}(k_{21}-\lambda_2)][(k_{01}-\lambda_2)(\lambda_1-\lambda_2)]^{-1}(s+\lambda_2)^{-1} \\ & + [D_2 k_{01'}(k_{21}-k_{01'})][(\lambda_1-k_{01'})(\lambda_2-k_{01'})]^{-1}(s+k_{01'})^{-1} \\ & + [D_2 k_{01'}(k_{21}-\lambda_1)][(k_{01'}-\lambda_1)(\lambda_2-\lambda_1)]^{-1}(s+\lambda_1)^{-1} \\ & + [D_2 k_{01'}(k_{21}-\lambda_2)][(k_{01'}-\lambda_2)(\lambda_1-\lambda_2)]^{-1}(s+\lambda_2)^{-1}. \end{aligned} \quad (12)$$

By taking the anti-Laplace of this equation, an expression for the amount of drug in the central compartment as a function of time can be obtained:

$$\begin{aligned} X_c = {} & [D_1 k_{01}(k_{21}-k_{01})][(\lambda_1-k_{01})(\lambda_2-k_{01})]^{-1} e^{-k_{01}t} \\ & + [D_2 k_{01'}(k_{21}-k_{01'})][(\lambda_1-k_{01'})(\lambda_2-k_{01'})]^{-1} e^{-k_{01'}t} \\ & + [(D_1 k_{01}(k_{21}-\lambda_1)][(k_{01}-\lambda_1)(\lambda_2-\lambda_1)]^{-1} \\ & + [D_2 k_{01'}(k_{21}-\lambda_1)][(k_{01'}-\lambda_1)(\lambda_2-\lambda_1)]^{-1} e^{-\lambda_1 t} \\ & + [(D_1 k_{01}(k_{21}-\lambda_2)][(k_{01}-\lambda_2)(\lambda_1-\lambda_2)]^{-1} \\ & + [D_2 k_{01'}(k_{21}-\lambda_2)][(k_{01'}-\lambda_2)(\lambda_1-\lambda_2)]^{-1} e^{-\lambda_2 t}. \end{aligned} \quad (13)$$

This equation may be expressed in terms of concentrations of drug in the central compartment employing the relationship

$$X_c = V_c * C. \quad (14)$$

Using equations 12 and 13 plasma-concentration-time data were simulated. The simulated plasma concentrations were compared to the actually measured plasma concentrations at the corresponding times after the start of the dissolution experiment and administration, respectively. Correlation coefficients for in vitro/in vivo data of all formulations investigated were calculated by linear least squares regression analysis.

Absorption kinetics from the in vivo data following administration of DHCl, DP-1, and MC-1 were calculated according to the Loo-Riegelman method (13). As it was reasonable to assume that doxepin release from the formulations was the rate limiting step in vivo, the obtained percent absorbed - time plots were compared to percent released - time plots resulting from in vitro studies. From the plot of in vitro release data versus in vivo absorption data at the respective times again correlation coefficients were calculated by linear regression analysis.

Table II — In vitro dissolution parameters for doxepin from formulations DHCl, DP-1, and MC-1: k_{01}, k_{01}, = rate constants of dissolution, D_1, D_2 = respective fractions of the dose, $t_{15\%}$, $t_{30\%}$ = times when 15% and 30%, respectively, of the tested formulation was released, means ± SD, n = 4.

Form.	D1[%]	$k_{01}[h^{-1}]$	D2[%]	$k_{01}'[h^{-1}]$	$t_{15\%}$[h]	$t_{30\%}$[h]
DHCl	69 ±12	1.68 ±0.72	18 ±6.3	0.11 ±0.016	0.12 ±0.024	0.26 ±0.054
DP-1	44 ±6.4	0.32 ±0.22	40 ±14	0.035 ±0.018	1.64 ±0.20	3.92 ±0.58
MC-1	56 ±15	0.53 ±0.18	41 ±19	0.048 ±0.017	0.44 ±0.11	1.03 ±0.26

Table III — CIs for the differences of $t_{15\%}$ and $t_{30\%}$ of different formulations

Formulations	Dif. of $t_{15\%}$	Dif. of $t_{30\%}$
MC-1 < DP-1	0.86 – 1.54	1.60 – 4.18
DHC1 < DP-1	1.12 – 1.93	2.48 – 4.84
DHC1 < MC-1	0.097 – 0.55	0.23 – 1.31

Results

In vitro dissolution parameters for doxepin release from formulations DHCl, DP-1, and MC-1 are listed in table II. Doxepin release from DHCl is shown to be faster than from DP-1 and MC-1 and faster from MC-1 than from DP-1. CIs for differences between the formulations are summarized in Table III. There was no difference in the dissolution parameters of the two doxepin stereoisomers, therefore all results are given in terms of total (cis + trans) doxepin.

In vivo studies

Preliminary studies have shown that there was no difference in the pharmacokinetics of cis- and trans-doxepin in dogs, thus all pharmacokinetic parameters are expressed in terms of total cis- and trans-doxepin. The area under the curves and terminal half-lives for all concentration-time data obtained from in vivo studies are listed in table IV. They reflect the relative bioavailability of the novel formulations and their release prolonging effect, respectively. AUC-values for formulations DHCl and DP-1 were 1275.5 $ng*h*ml^{-1}$ and 1462.0 $ng*h*ml^{-1}$, respectively (means, n = 3 and 4), and exceeded those for Aponal^R, which were 940.5 $ng*h*ml^{-1}$ and 905.5 $ng*h*ml^{-1}$, respectively (means, n = 3 and 4). AUC for formulation MC-1 was 938.6 $ng*h*ml^{-1}$ and was lower than the corresponding AUC-value for Aponal^R, which was 1064.6 $ng*h*ml^{-1}$ (means, n = 4). Terminal half-lives observed after administration of DP-1, 66.79 h, and MC-1, 33.98 h, were markedly prolonged compared to those after Aponal^R, 4.12 h and 5.36 h, respectively. The corresponding $t_{1/2}$-values for DHCl and Aponal^R were 7.21 h and 7.00 h, respectively (means, n = 4).

The pharmacokinetic parameters of doxepin after Aponal^R administration, which were used for simulation of plasma concentrations, were obtained from in vivo studies where the blood sampling schedule permitted an accurate description of the distribution and elimination phase of the drug (Table I, part C). Examples of fitted plasma-concentration-time curves are shown in Fig. 1. From the

Table IV—AUC-values and $t_{1/2}$ obtained from in vivo studies

A: Aponal^R versus DHCL					
dog	sex	AUC(0–24h)/(0–∞)[$ng*h*ml^{-1}$]		$t_{1/2}$[h]	
number		Aponal®	DHCl	Aponal^R	DHCl
975	m	703 / 858	630 / 696	11.5	7.0
710	m	841 / 881	1169 / 1210	4.8	4.8
338	f	1039 / 1082	1683 / 1920	4.7	9.9
mean		861 / 941	1161 / 1276	7.0	7.2
±SD		±169 / ±123	±526 / ±615	±3.9	±2.6
B: Aponal^R versus DP-1					
dog	sex	AUC(0–96h)/(0–∞)[$ng*h*ml^{-1}$]		$t_{1/2}$ [h]	
number		Aponal^R	DP-1	Aponal^R	DE-1
253	f	790 / 856	1052 / 1459	1.85	85.6
256	f	863 / 1001	1819 / 1932	2.58	71.5
716	m	540 / 626	1044 / 1062	2.82	53.3
701	m	1059 / 1139	1143 / 1395	9.24	56.8
mean		813 / 906	1264 / 1462*	4.12	66.8*
±SD		±215 / ±219	±373 / ±358	±3.44	±14.8
C: Aponal^R versus MC-1					
dog	sex	AUC(0–24h)/(0–∞)[$ng*h*ml^{-1}$]		$t_{1/2}$ [h]	
number		Aponal^R	MC-1	Aponal^R	MC-1
749	m	1699 / 1740	963 / 1126	4.89	72.5
760	m	901 / 905	863 / 888	3.41	26.8
392	f	689 / 719	742 / 1186	9.54	23.1
393	f	888 / 894	508 / 554	3.58	13.5
mean		1044 / 1065	769 / 939	5.36	34.0
±SD		±447 / ±459	±196 / ±287	±2.87	±26.3

* The difference between the means of the reference formulation versus the test formulation is significant ($p<0.05$)

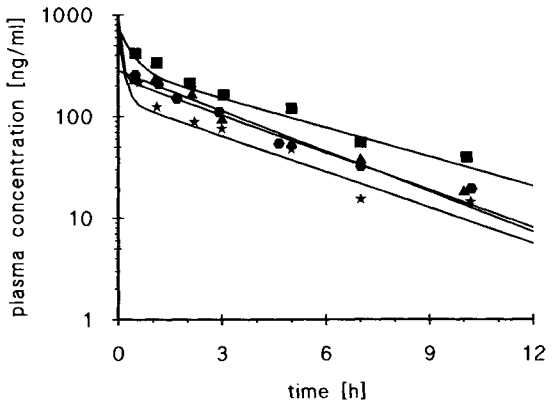

Fig. 1: Fitted plasma concentrations of total doxepin versus time after i.m. injection of Aponal^R to four beagle dogs

digital computer fits the relevant pharmacokinetic parameters were calculated: V_c = 84 l, k_{12} = 1.256 h^{-1}, k_{21} = 1.937 h^{-1}, k_{10} = 0.500 h^{-1}, λ_1 = 3.409 h^{-1}, λ_2 = 0.284 h^{-1} (medians, n = 4). Absorption of doxepin from the aqueous solution was very rapid and an absorption phase cannot be detected from the data.

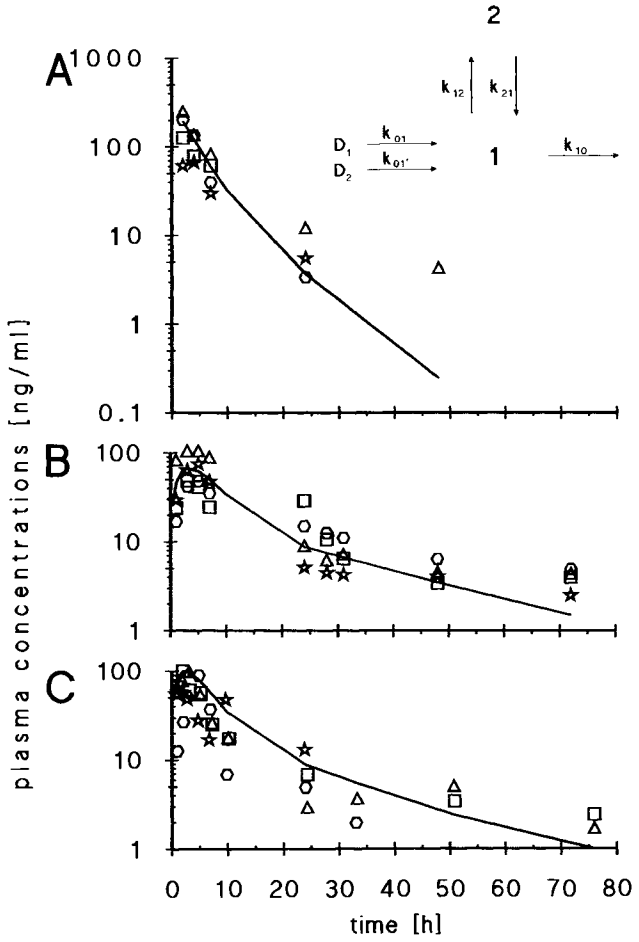

Fig. 2: Predicted total doxepin plasma concentrations calculated from in vitro dissolution data on the basis of the mean disposition parameters of a two compartment body model (lines) and measured concentrations in four dogs (symbols) for the formulations DHCl (A), DP-1 (B) and MC-1 (C)

In vitro/in vivo correlation

Fig. 2 shows in vivo doxepin plasma concentrations after administration of DHCl, DP-1, and MC-1 compared to predicted doxepin plasma concentrations calculated from in vitro dissolution data on the basis of a two compartment body model with two parallel first order input steps. Data are enumerated in table V.

Calculated plasma concentrations from in vitro release data plotted versus individual in vivo concentrations of four dogs are depicted in Fig. 3. Correlation coefficients ranged from 0.936 to 0.996, from 0.892 to 0.993, and from 0.876 to 0.968 for DHCl, DP-1, and MC-1, respectively.

Table V—Calculated total doxepin plasma concentrations from in vitro release data and concentrations measured in vivo at the respective times following administration

A: Formulation DHCl

time [h]	in vitro [ng/ml]	in vivo [ng/ml] dog 209	dog 338	dog 975	dog 710
2	196	127	237	61	200
4	122	78	129	66	135
7	61	61	79	30	40
24	3.7	0.0	12	5.5	3.4

B: Formulation DP-1

time [h]	in vitro [ng/ml]	in vivo [ng/ml] dog 253	dog 256	dog 716	dog 701
1	46	24	78	29	17
3	67	49	118	63	42
5	62	42	127	74	48
7	51	25	85	47	35
24	8.8	29	8.6	5.3	15
28	7.3	11	5.9	4.5	13
31	6.4	6.5	6.9	4.2	11
48	3.5	3.4	4.3	4.0	6.4
72	1.5	4.0	4.2	2.5	5.0
96	0.6	3.3	3.4	1.9	3.6

C: Formulation MC-1

time [h]	in vitro [ng/ml]	in vivo [ng/ml] dog 749	dog 760	dog 392	dog 393
1	80	66	62	54	#
2	100	107	74	57	51
3	99	62	94	48	#
5	79	57	51	28	90
7	57	25	25	17	37
10	35	17	17	#	#
24	8.8	6.8	2.8	13	4.9
33	5.5	*	3.5	0.0	2.0
51	2.4	3.4	4.8	0.0	0.0
76	1.0	2.4	1.6	0.0	0.0

missing data point; sample collection was impossible from this dog at this time

* missing data point; the blood sample has spilt in the centrifuge

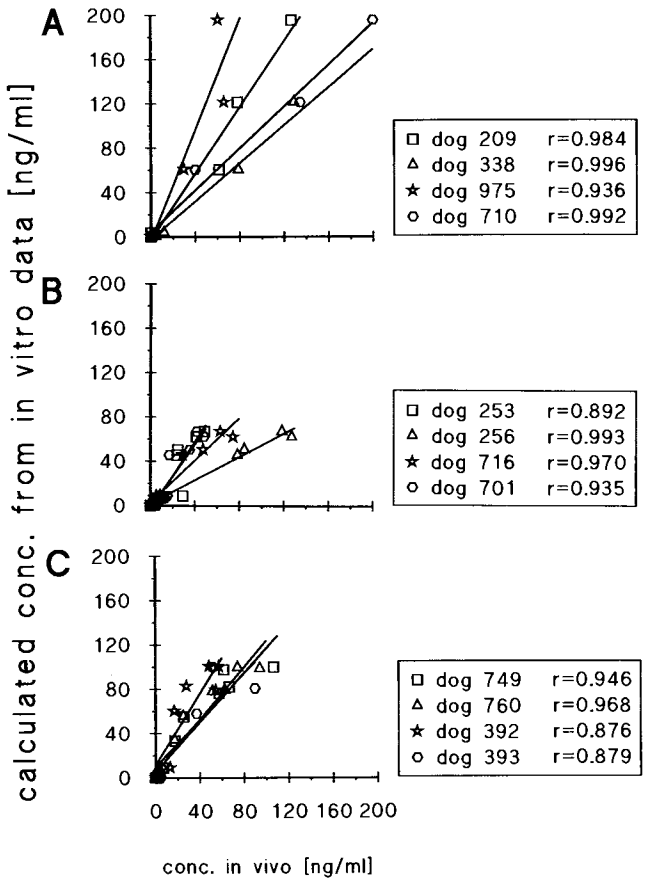

Fig. 3: Calculated total doxepin plasma concentrations from in vitro release data plotted versus concentrations measured in vivo at the respective times following administration for the formulations DHCl (A), DP-1 (B) and MC-1 (C)

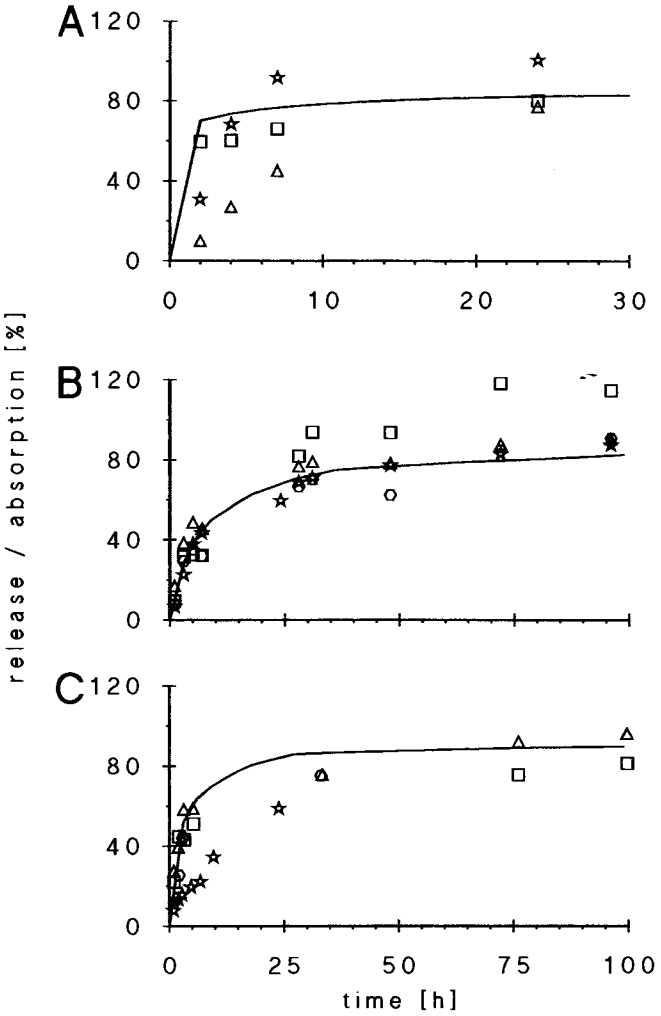

Fig. 4: Measured total doxepin release rates in vitro (lines) and calculated doxepin absorption from beagle dogs (symbols) for the formulations DHCl (A), DP-1 (B) and MC-1 (C)

Fig. 4 shows in vivo doxepin absorption kinetics after administration of DHCl, DP-1, and MC-1 compared to in-vitro release kinetics. Data are enumerated in table VI.

In vitro release data plotted versus individual in vivo absorption data from four dogs are depicted in Fig. 5. Correlation coefficients ranged from 0.918 to 0.999, from 0.967 to 0.994, and from 0.893 to 0.999 for DHCl, DP-1, and MC-1, respectively.

Discussion

Although there are great interindividual differences in doxepin pharmacokinetics in volunteers and patients (5), formulation DHCl did not lead to a prolongation of doxepin terminal half-life (Table IV). This result is not surprising since the formulation DHCl also showed the fastest release of doxepin in in vitro dissolution experiments (Table II). Formulations DP-1 and MC-1, however, markedly extended doxepin terminal half-life. In vitro the poorly water soluble doxepin pamoate led to clearly sustained release profiles compared with the doxepin hydrochloride. Embedding doxepin hydrochloride into polymer microspheres also led to sustained release rates compared to the free drug. Several mechanisms of release from poly D,L-lactid-co-glycolid microcapsules may occur (14): Initial release from the capsule surface, release through pores, diffusion through the intact polymer barrier, diffusion through the water swollen polymer barrier, and polymer erosion. The relatively rapid release of doxepin from formulation MC-1 might be due to a fast degradation of the polymer caused by the basic drug as reported by Maulding et al for thioridazine (15). They found no acceleration of polymer hydrolysis when the amino group of thioridazine was protonated in the form of the pamoate salt. Incorporation of doxepin pamoate into polymer microspheres appeared not to provide a clinically useful formulation, because the resulting low drug content would require a larger volume of suspension to be administered than would be possible for the intramuscular route.

Good in vitro/in vivo correlation coefficients by correlating simulated and observed plasma concentrations were found for all formulations investigated. In vivo dissolution rates may be obtained from plasma-concentration-time data by various methods, such as the Wagner-Nelson or Loo-Riegelman procedure based on a compartmental pharmacokinetic approach, the deconvolution procedure or the calculation of statistical moments. The underlying assumption for obtaining the correct in vivo dissolution rate is that absorption is dissolution rate-limited. This is the case for the slow

Table VI—Measured release data from in vitro studies and calculated absorption data from in vivo studies at the respective times following administration

A: Formulation DHCl

time [h]	in vitro [%]	in vivo [%] dog 209	dog 338	dog 975	dog 710
2	70	/	60	9	31
4	74	/	60	26	68
7	77	/	66	44	92
24	82	/	80	76	100

/ dog 209 had to be removed from the study after administration of Aponal^R

B: Formulation DP-1

time [h]	in vitro [%]	in vivo [%] dog 253	dog 256	dog 716	dog 701
1	11	9	16	7	8
3	27	32	37	22	29
5	38	33	48	38	35
7	45	32	45	43	32
28	69	82	76	69	67
31	71	94	78	71	70
48	77	94	77	77	62
72	81	118	87	83	85
96	83	115	89	87	91

C: Formulation MC-1

time [h]	in vitro [%]	in vivo [%] dog 749	dog 760	dog 392	dog 393
1	28	22	26	8	#
2	43	45	38	13	25
3	52	43	57	16	#
5	61	51	58	19	44
7	66	56	57	22	50
10	71	59	61	#	#
24	84	57	71	59	70
33	87	*	75		75
51	88	73	83		
76	89	76	91		

missing data point; sample collection was impossible from this dog at this time

* missing data point; the blood sample has spilt in the centrifuge

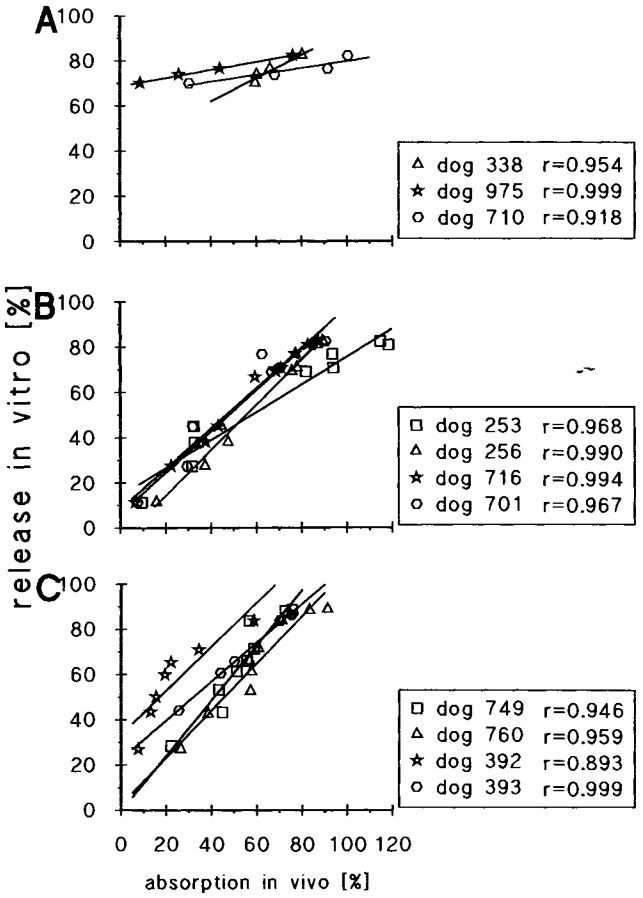

Fig. 5: Measured release data from in vitro studies plotted versus calculated absorption data from in vivo studies at the respective times following administration for the formulations DHCl (A), DP-1 (B) and MC-1 (C)

releasing formulations DP-1 and MC-1, where a flip-flop-case could be observed by comparison of the terminal half-lives of doxepin with the half-life following administration of the aqueous solution formulation Aponal^R, although, for MC-1, the difference was only close to reaching statistical significance ($p = 0.073$). The rapid release of doxepin from DHCl formulations, however, may indicate that the contribution of the absorption to the overall input rate is more pronounced. For calculation of the in vivo dissolution rates the Loo-Riegelman method was applied. Correlation between in vitro release and in vivo release calculated by this method was also good.

It has been found in preliminary experiments that the dissolution of the active species was more rapid – significant differences of $t_{15\%}$ and $t_{30\%}$ were observed — and correlated better with in vivo dissolution when diluted plasma was used as dissolution medium as compared to phosphate buffer pH 7.4. Using buffer in vitro/in vivo correlation coefficients ranged from 0.285 to 0.992. The improved correlation by using plasma may be due to the binding of doxepin to plasma proteins. The protein binding of doxepin in vivo was measured to be about 80% (16). This value was explained by binding to albumin and to α_1-acid glycoprotein. Faster dissolution rates may also be traceable to a reduced surface tension of the proteinaceous solution.

For doxepin as a model compound one may conclude that from the applied dissolution system biorelevant dissolution rates can be obtained on accurate control of experimental parameters and that in vivo plasma levels can be predicted. For the future the general applicability of this method to other drugs and parenteral sustained-release formulations remains to be investigated.

Acknowledgments

This study was supported by a grant from Boehringer Mannheim GmbH.

References

1. P.G. Welling. In Vitro Methods to Determine Bioavailability:

In-Vitro/In-Vivo Correlations. In P.G. Welling, F.L.S. Tse, and S.V. Dighe (eds.), *Pharmaceutical Bioequivalence, Drugs and the Pharmaceutical Sciences,* Marcel Dekker, New York, 1991, pp. 223–232

2. S. Benita, D. Friedman, and M. Weinstock. Pharmacological Evaluation of an Injectable Prolonged Release Emulsion of Physostigmin in Rabbits. *J. Pharm. Pharmacol.* **38**; 653–658 (1986)
3. K. Hirano and H. Yamada. Studies on the Absorption of practically Water-insoluble Drugs following Injection. IV. An Approach for Predicting Relative Intramuscular Absorption Rates of a Drug in Oily Solution, Aqueous Suspension and Aqueous Surfactant Solution in Rats. *Chem. Pharm. Bull.* **29**; 1410–1415 (1981)
4. C. Gido, P. Langguth, J. Kreuter, G. Winter, H. Woog, and E. Mutschler. Conventional versus Novel Dissolution Conditions for In Vitro Testing of Parenteral Slow Release Formulations: Application to Doxepin Parenteral Dosage Forms. *Die Pharmazie;* **48**; 764–769 (1993)
5. R.M. Pinder, R.M. Brogden, T.M. Speight, and G.S. Avery. Doxepin up-to-date: A Review of Its Pharmacological Properties and Therapeutic Efficacy with Special Reference to Depression. *Drugs* **13**; 161–218 (1977)
6. K.K. Midha, J.W. Hubbard, G. McKay, E.M. Hawes, E.D. Korchinski, T. Gurnsey, J.K. Cooper, and R. Schwede. Stereoselective Pharmacokinetics of Doxepin Isomers. *Eur J Clin Pharmacol* **42**; 439–544 (1992)
7. V.E. Ziegler, J.T. Biggs, L.T. Wylie, S.H. Rosen, D.J. Hawf, and W.H. Coryell. Doxepin kinetics. *Clin. Pharmacol. Ther.* **23**; 573–579 (1978)
8. G. Neugebauer, unpublished data
9. R.O. Friedel and M.A. Raskind. Relationship of blood levels of Sinequan to clinical effects in the treatment of depression in aged patients. In J. Mendels (Ed.), *Sinequan, a Monograph of Recent Clinical Studies,* Excerpta Medica, 1975, pp. 31–53
10. J.T. Biggs, S.H. Preskorn, V.E. Ziegler, S.H. Rosen, and D.A. Meyer. Dosage Schedule and Plasma Levels of Doxepin and Desmethyldoxepin. *J. Clin. Psychiatry* **39**; 740–742 (1978)
11. P.R. Joyce, and J.R. Sharman. Doxepin Plasma Concentrations in Clinical Practice. *Clin. Pharmacokinet.* **10**; 365–370 (1985)
12. C. Dilger, Z. Salama, and H. Jaeger. Highperformance Liquid Chromatographic Determination of trans-Doxepin and Desmethyldoxepin. *Drug Res* **38(II)**; 1525–1530 (1988)
13. M. Gibaldi and D. Perrier. *Pharmacokinetics,* Marcel Dekker, New York, 1982
14. R. Jalil and J.R. Nixon. Biodegradable Poly(Lactic Acid) and Poly(Lactide-co-Glycolide) Microcapsules: Problems Associated with Preparative Techniques and Release Properties. *J. Microencapsulation* 7; 297–325 (1990)
15. H.V. Maulding, T.R. Tice, D.R. Cowsar, J.W. Fong, J.E. Pearson, and J.P. Nazareno. Biodegradable Microcapsules: Acceleration of Polymeric Excipient Hydrolytic Rate by Incorporation of a Basic Medicament. *J Controlled Rel* 3; 103–117 (1986)
16. R. Virtanen, E. Iisalo, and K. Irjala. Protein binding of doxepin and desmethyldoxepin. *Acta pharmacol. et toxicol.* **51**; 159–164 (1982)