Steady-State Kinetics of Imipramine in Transgenic Mice with Elevated Serum AAG Levels

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Purpose. The effect of elevated serum *alpha*-1-acid glycoprotein (AAG) concentrations on the steady-state serum and brain levels of imipramine and its metabolite desipramine was assessed. This was approached using a novel strain of transgenic mice whose basal endogenous serum AAG levels were 8.6-fold elevated over normal.

Methods. Imipramine was administered by s.c. infusion or i.p. injection into transgenic and control mice. After drug administration, serum and whole brain were harvested and analyzed for imipramine and desipramine concentrations. Equilibrium dialysis was performed to determine the extent of imipramine protein binding in transgenic and control sera. Serum and brain samples were analyzed for imipramine and desipramine content by an established HPLC method with UV detection.

Results. At steady-state, the mean serum imipramine concentration was significantly higher in transgenic mice than in control mice (859.0 *vs.* 319.9 ng/ml). In contrast, the mean steady-state brain imipramine concentration was significantly lower in transgenic mice (3,862.6 *vs.* 7,307.7 ng/g). Similarly, in transgenic mice, the mean steady-state serum desipramine concentration was significantly higher (176.7 *vs.* 39.0 ng/ml) while the mean brain desipramine concentration was lower (243.0 *vs.* 393.5 ng/g). The serum unbound fraction of imipramine was 3-fold lower in transgenic mice (0.03 *vs.* 0.09).

Conclusions. Elevated serum AAG impedes the transport of imipramine and desipramine into the brain. Further, in the presence of elevated serum AAG levels, imipramine and desipramine concentrations in the brain did not correlate with their respective concentrations in the serum.

KEY WORDS: imipramine; desipramine; transgenic mice; AAG.

INTRODUCTION

Imipramine, a tricyclic antidepressant routinely used in the treatment of depression, is known to be avidly bound to serum *alpha*-1-acid glycoprotein (AAG). Serum AAG levels are increased in several disease states including depression and such elevations have been shown to influence the disposition of imipramine (1–3). Despite its extensive binding to serum protein, imipramine is readily distributed into tissues, including the brain (4–6) and possesses a large volume of distribution (*e.g.*, 9.3–23 L/kg), consisting of a small central volume and a relatively large peripheral volume (7). Therefore, even a significant change in serum protein binding of imipramine is considered to have little effect on its concentrations in peripheral tissues (*e.g.*, brain) (8). Recently, Riant *et al.* (9) have reported that both free and bound imipramine are available for distribution into the brain and have further suggested that the total rather than the unbound serum concentration of imipramine may more accurately correlate with its antidepressant activity. Based on these observations, the protein bound species appears to traverse the blood-brain barrier and contribute to the pharmacological activity of imipramine.

We have previously reported the reduced pharmacological activity of imipramine in transgenic mice with elevated serum AAG levels (10). Furthermore, its antidepressant action correlated with the unbound serum drug concentration rather than the total drug concentration. Since the site of action of imipramine is brain tissue, elevated serum AAG levels could influence the antidepressant activity of imipramine *via* altered brain drug transport. To our knowledge, there is limited information on the extent of brain uptake of imipramine in relation to serum AAG concentrations (9).

The present study was conducted to examine the brain uptake of imipramine and its metabolite, desipramine as a function of altered serum AAG under steady-state and non-steady-state conditions utilizing the transgenic mice with elevated serum AAG levels. Our results indicated that, in the presence of significant alterations in serum binding, the total serum concentrations of imipramine and desipramine did not correlate with their brain concentrations.

MATERIALS AND METHODS

Materials

Imipramine, desipramine, clomipramine (all as hydrochloride salts), triethylamine and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma Chem. Co. (St. Louis, MO). Perchloric acid was obtained from J.T. Baker Chem. Co. (Phillipsburg, NJ). Ethyl ether and phosphoric acid 85% were purchased from Mallinckrodt Chem. Co. (Paris, KY). Acetonitrile, methanol and hexane (all HPLC grades) and 0.9% saline were purchased from Baxter Healthcare Co. (Muskegon, MI).

Animals

A novel strain of transgenic mice with increased serum AAG levels and C57BL/6 mice (5–7 months old, male and female) were used in the study. Transgenic mice were produced utilizing an AAG gene construct derived from a 9.5-kb rat genomic clone containing the entire coding region along with 4.7-kb of 5′ flanking sequence (11). Briefly, the DNA construct was microinjected into the pronuclei of (C57BL/6 × DBA/2)F_2 embryos. The embryos were implanted into pseudopregnant foster females and were allowed to develop to term. Transgenic mice were identified among the offspring by Southern analysis of DNA from tail biopsy. These transgenic founders were subsequently mated to pure-strain C57BL/6 mice and positive offspring were crossed to each other to ultimately produce homozygous transgenic lines. Each line, subsequently maintained by sequential brother-sister mating of offspring from two homozygotes, reproducibly expressed the transgene at a characteristic level. The transgenic mice used in this study were hybrids of AGP 9.5-5 and C57BL/6, which had previously been shown to express AAG levels 8.6-fold over normal as

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determined by rocket immunoelectrophoresis (10,11). C57BL/6 mice (Jackson Laboratories, Bar Harbor, ME) were used as the nontransgenic control mice. All animals were maintained in a temperature controlled animal facility with 12/12 hr light/dark cycle and with free access to food and water.

Drug Administration

Imipramine was administered either by continuous s.c. infusion (1 μl/hr of 349 mg/ml imipramine in saline) using Alzet osmotic pumps (Model 1003D, Alza Corp, Palo Alto, CA) or by single i.p. injection (30 mg/kg). Prior to implantation of the osmotic pumps, the mice were weighed and anesthetized with diethyl ether. Incisions were made with dissecting scissors dorsally, left of the spine after cleansing the area with isopropyl alcohol. Each pump was inserted into the subcutaneous region and the incision closed with Michel wound clips (11 mm, Propper Manufacturing Co., Long Island, NY). At 4, 6, 8 and 16 hr after the initiation of infusion, whole blood was collected by cardiac puncture and allowed to stand for 1 hr (n = 4–5 for each sampling time). Serum samples were harvested by centrifugation and were kept in borosilicate tubes at −20°C until analysis. In addition, whole brains were removed from these mice promptly after cardiac puncture. Each brain was accurately weighed, placed in 10 ml of 0.4 *M* perchloric acid containing 10^{-5} *M* EDTA and homogenized for 90 sec (12). Brain homogenates were then frozen at −20°C until drug analysis. Single i.p. injections were performed to provide serum and brain concentrations during non-steady-state conditions. Control and transgenic animals (n = 5 for each group) were injected intraperitoneally (30 mg/kg) with imipramine (3 mg/ml in saline). At 30 min, whole blood and brain samples were taken. Serum samples and brain homogenates were prepared as described above and kept at −20°C until drug analysis.

Serum Protein Binding

Equilibrium dialysis was performed to determine the extent of serum protein binding of imipramine at concentrations of 500 and 1,000 ng/ml. Drug spiked transgenic and control serum was dialyzed against phosphate buffer (0.133 *M*, pH = 7.4) at 37°C. An optimum equilibrium time of 7 hr was determined in a preliminary study and used subsequently. At equilibrium, serum and buffer samples were collected into borosilicate tubes and stored at −20°C until drug analysis. Unbound fraction was calculated as the ratio of the buffer to serum drug concentrations.

Drug Analysis

Serum and brain samples were analyzed for imipramine and desipramine using a previously published protocol (13). Briefly, HPLC drug analysis was performed on a Shimadzu component system (Shimadzu, Columbia, MD) using a Microsorb® MV C_{18} column (Rainin, Woburn, MA). The mobile phase consisted of 60% acetonitrile and 40% 0.01 *M* triethylamine in distilled water, with the pH adjusted to 3.0 by dropwise addition of 85% phosphoric acid. The flow rate was set at 1.0 ml/min and the effluent monitored for UV absorption at 260 nm. The lower limit of quantitation was 10 ng/ml for both imipramine and desipramine, with intra- and inter-day coefficients of variation <10%.

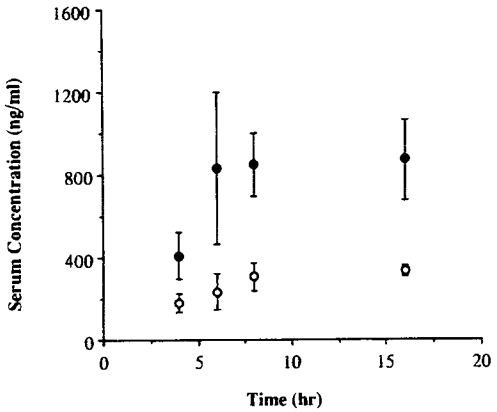

Fig. 1. Serum concentrations (mean ± s.d.) of imipramine in transgenic (closed circles) and control (open circles) mice during s.c. infusion (349 μg/hr) (n = 4–5 for each sampling time).

Data Analysis

Steady-state imipramine and desipramine concentrations were calculated as the mean of drug concentrations at 8 and 16 hr. The systemic clearance of imipramine was calculated as the infusion rate divided by the steady-state serum drug concentration (14). The brain to serum (B/S) drug concentration ratios were determined to be the quotient of the brain to serum drug concentrations at steady-state. Desipramine to imipramine serum concentration ratios were also determined for the 8 and 16 hr samples. Statistical analysis of brain and serum samples was performed by the computer program SAS using Multivariate Analysis of Variance (MANOVA) followed by Canonical Discriminant Analysis. Data were reported as the mean ± s.d. Statistical significance was set at $p < 0.05$.

RESULTS

The steady-state levels of imipramine and its metabolite, desipramine were achieved in the serum and the brain of both groups of mice within 8 hr of infusion (Figures 1–4). Throughout the infusion period, serum imipramine and desipramine concentrations were consistently higher in transgenic mice. At steady-state, the mean serum imipramine concentration was 2.7-fold higher in transgenic mice (859.0 ± 168.4 *vs.* 319.9 ±

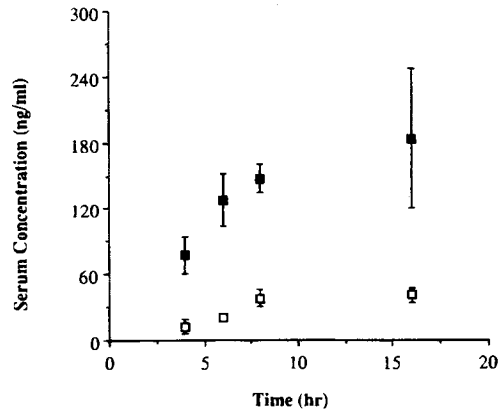

Fig. 2. Serum concentrations (mean ± s.d.) of desipramine in transgenic (closed squares) and control (open squares) mice during s.c. infusion of imipramine (349 μg/hr) (n = 4–5 for each sampling time).

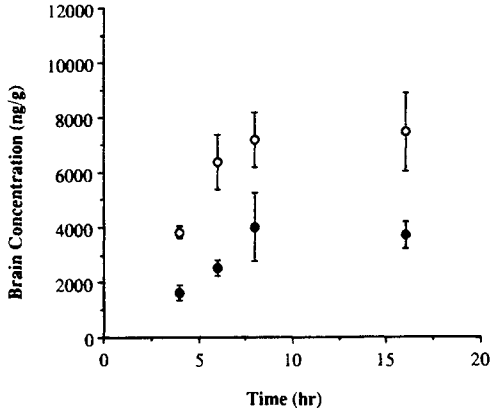

Fig. 3. Brain concentrations (mean ± s.d.) of imipramine in transgenic (closed circles) and control (open circles) mice during s.c. infusion (349 μg/hr) (n = 4–5 for each sampling time).

52.1 ng/ml) and the serum desipramine concentration was 4.5-fold higher in transgenic mice (176.7 ± 55.3 *vs.* 39.0 ± 6.8 ng/ml) as compared to control. In both groups of mice, steady-state serum desipramine levels were consistently lower than their parent drug levels, with a mean metabolite to parent drug concentration ratio of 0.20 in transgenic and 0.12 in control mice (Figure 1–2). Imipramine and desipramine were extensively distributed into the brain in both groups of mice, with the B/S concentration ratios exceeding unity throughout the infusion period (Table I). Unlike the serum levels, the brain levels of imipramine and desipramine were consistently lower in transgenic mice than in control mice, with a mean of 3,862.6 ± 898.3 *vs.* 7,307.7 ± 1,148.9 ng/g for imipramine and a mean of 243.0 ± 42.6 *vs.* 393.5 ± 66.2 ng/g for desipramine.

Serum concentrations of imipramine and desipramine 30 min after i.p injection were significantly higher in transgenic mice than in control mice. Conversely, brain levels of imipramine and desipramine were lower in transgenic mice (Table I). The B/S ratios of imipramine and desipramine obtained after i.p. injection were higher than those found at steady-state in both groups of mice (Table I).

The systemic clearance of imipramine (204.4 ± 79.1 ml/min/kg *vs.* 461.8 ± 88.8 ml/min/kg) and its fraction unbound

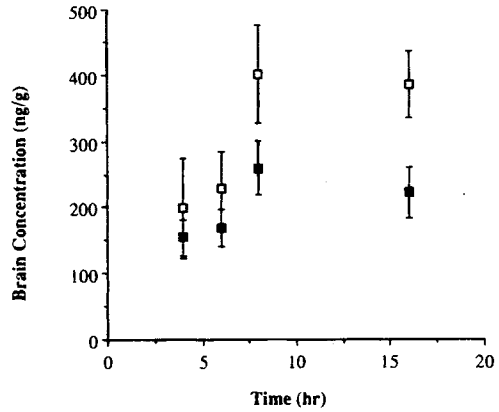

Fig. 4. Brain concentrations (mean ± s.d.) of desipramine in transgenic (closed squares) and control (open squares) mice during s.c. infusion of imipramine (349 μg/hr) (n = 4–5 for each sampling time).

to serum protein (0.03 *vs.* 0.09) were significantly reduced in transgenic mice as compared with control animals. Figure 5 shows the steady-state desipramine to imipramine serum concentration ratio as a function of the systemic clearance of imipramine in both groups of mice. Despite the reduced systemic clearance of imipramine, the desipramine to imipramine concentration ratios tended to be higher in transgenic mice than in control mice.

DISCUSSION

A significant finding of our present study is that the steady-state imipramine and desipramine total serum concentrations do not correspond to their brain concentrations in the presence of altered serum binding. We have previously examined the role of elevated AAG levels on the disposition and action of imipramine in these transgenic mice under non-steady-state conditions (10). The antidepressant activity of imipramine was significantly reduced in transgenic mice as determined 30 min after i.p. injection. Our current i.p. injection data were consistent with this finding in that significantly less amount of imipramine was accumulated in the brain tissue of transgenic mice. Hence, if serum protein binding is altered, monitoring the total serum concentrations may be misleading because serum drug concentrations may not reflect the drug concentrations in the brain.

Imipramine and desipramine are known to be rapidly and extensively distributed into the rat brain (4–5,15). The mean B/S ratio of imipramine determined 30 min after i.p. injection in control mice is similar to that found in the rat (43.8 *vs.* 33.7, respectively) (11). Likewise, the steady-state B/S ratio of imipramine found in control mice (mean 22.8) is similar to those values (25–35) found in the rat after chronic i.p. administration of ^{3}H-imipramine to steady-state (5). As in the rat, desipramine distributed into the mouse brain less extensively than the parent drug (11). Interestingly, the steady-state serum concentration of the active metabolite desipramine was lower than the parent drug concentration, indicating that the elimination of desipramine in mice was formation-rate limited as opposed to being elimination-rate limited, as found in humans (6,17). Therefore, the relatively low brain levels of desipramine (3.8–6.1%) may not significantly augment the antidepressant activity of imipramine under steady-state conditions in mice.

Consistent with our previous i.v. injection study, the systemic clearance of imipramine was reduced in transgenic mice as compared to control (10). Whether the reduced systemic clearance in transgenic mice is due to alterations in intrinsic clearance or due to the reduced rate of drug uptake by the liver deserves further examination (16). There was no significant correlation found between the steady-state desipramine to imipramine serum concentration ratios and the systemic clearance of imipramine within each group (Figure 5). Assuming that the systemic clearance of desipramine is consistent across individual animals, the non-significant correlation within group indicates that the formation of a metabolite other than desipramine may be a contributing factor in the variations seen in the systemic clearance of imipramine. This is in agreement with an observation made in humans in which the steady-state serum concentrations of desipramine and imipramine are independent of the demethylation clearance and inversely proportional to the hydroxylation clearance of imipramine (18). Nevertheless, the formation clearance of desipramine appears significantly increased in transgenic mice as evidenced by the steep slope of the plot (Fig. 5).

In summary, the reduced brain uptake of imipramine and desipramine in transgenic mice was consistent with the elevated

Table I. Serum and Brain Levels of Imipramine and Its Metabolite Desipramine Following S.C. Infusion or I.P. Injection of Imipramine in Transgenic and Control Mice (n = 5)

Parameter	Imipramine		Desipramine	
	Transgenic	Control	Transgenic	Control
infusion				
Css,serum (ng/ml)	859.0 ± 168.4[a]	319.9 ± 52.1	176.7 ± 55.3[a]	39.0 ± 6.8
Css, brain (ng/g)	3862.6 ± 898.3[a]	7307.7 ± 1148.9	243.0 ± 42.6[a]	393.5 ± 66.2
B/S Ratio	4.5 ± 0.9[a]	22.8 ± 4.4	1.4 ± 0.6[a]	10.1 ± 2.8
Cls (ml/min/kg)	204.4 ± 79.1[a]	461.8 ± 88.8		
i.p. injection				
C30 min, serum (ng/ml)	983.7 ± 239.7[a]	357.4 ± 41.5	232.1 ± 16.2[a]	41.1 ± 13.0
C30 min, brain (ng/g)	10056.4 ± 1085.6[a]	15639.2 ± 2554.1	654.5 ± 83.9	762.4 ± 112.4
B/S Ratio	10.2 ± 2.7[a]	43.8 ± 4.6	2.8 ± 0.33[a]	18.5 ± 7.5

[a] Significantly different from control values ($p < 0.05$).

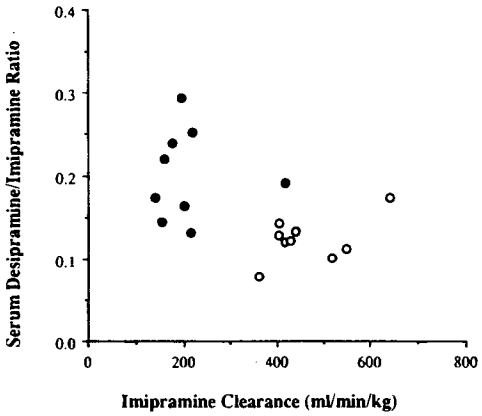

Fig. 5. Relationship between the steady-state serum desipramine to imipramine concentration ratios and the systemic clearance of imipramine obtained from s.c. infusion of imipramine (349 μg/hr) in transgenic (closed circles) and control (open circles) mice.

serum AAG levels at steady-state. Further, drug concentrations in the brain did not correlate with the total serum drug concentrations in the presence of altered serum AAG levels in mice. Therefore, in disease states in which serum AAG levels are elevated, monitoring the total imipramine serum concentration may be misleading.

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REFERENCES

1. M. H. Bickel. Binding of chlorpromazine and imipramine to red cells, albumin, lipoproteins and other blood components. *J. Pharm. Pharmacol.* **27**:733–738 (1975).
2. C. B. Nemeroff, K. R. Krishinan, D. G. Blazer, D. L. Knight, D. Benjamin and L. R. Meyerson. Elevated plasma concentrations of *alpha*-1-acid glycoprotein, a putative endogenous inhibitor of the tritiated imipramine binding site in depressed patients. *Arch. Gen. Psychiatry* **47** :337–340 (1990).
3. W. A. Kehoe, J. A. Kwentus, W. B. Sheffel and A. F. Harralson. Increased alpha-1-acid glycoprotein in depression lowers free fraction of imipramine. *Biol. Psychiatry* **29**:489–493 (1991).
4. C. L. DeVane, J. W. Simpkins and S. A. Stout. Cerebral and blood pharmacokinetics of imipramine and its active metabolite in the pregnant rat. *Psychopharmacology* **84**:225–230 (1984).
5. M. van Wijk and J. Korf. A quantitative study in the rat on the relationship between imipramine levels in brain and serum. *Psychopharmacology.* **76**:48–51 (1982).
6. Y. Sato, S. Shibanoki, M. Sugahara and K. Ishikawa. Measurement and pharmacokinetic analysis of imipramine and its metabolite by brain microdialysis. *Br. J. Pharmacol.* **112**:625–629 (1994).
7. C. L. DeVane and C. R. Jarecke. Cyclic antidepressants. In W. E. Evans, J. J. Schentag and W. J. Jusko (eds.), Applied Pharmacokinetics: Principles of Therapeutic Drug Monitoring, Applied Therapeutics, Vancouver, 1992, pp. 33-1–33-47.
8. D. J. Greenblatt, E. M. Sellers and J. Koch-Weser. Importance of protein binding for the interpretation of serum or plasma drug concentrations. *J. Clin. Pharmacol.* **22**:259–263 (1982).
9. P. Riant, S. Urien, E. Albengres A. Renouard and J. P. Tillement. Effects of the binding of imipramine to erythrocytes and plasma proteins on its transport through the rat blood-brain-barrier. *J. Newrochem.* **51**:421–425 (1988).
10. S. D. Yoo, J. W. Holladay, T. K. Fincher, H. Baumann and M. J. Dewey. Altered disposition and antidepressant activity of imipramine in transgenic mice with elevated alpha-1-acid glycoprotein. *J. Pharmacol. Exp. Ther.* **276**:918–922 (1996).
11. M. J. Dewey, C. Rheaume, F. G. Berger and H. Baumann. Inducible and tissue-specific expression of rat alpha-1-acid glycoprotein in transgenic mice. *J. Immunol.* **144**:4392–4398 (1990).
12. S. A. Stout and C. L. DeVane. Quantification of imipramine and its major metabolites in whole blood, brain and other tissues of the rat by liquid chromatography. *Psychopharmacology* **84**:39–41 (1984).
13. S. D. Yoo, J. W. Holladay, T. K. Fincher and M. J. Dewey. Rapid microsample analysis of imipramine and desipramine by reversed-phase high performance liquid chromatography with ultraviolet detection. *J. Chromatogr.* **668**:1338–342 (1995).
14. W. D. Wessinger. Chronic administration of pencyclidine: pharmacokinetic comparison of intravenous and subcutaneous infusions in Sprague-Dawley rats. *Drug Metab. Dispos.* **19**:719–721 (1991).
15. W. Daniel, L. Danek, L. Janczar, H. Nocon and M. Melzacka. Regional distribution of imipramine, desipramine and specific [^{3}H]desipramine binding sites in the rat brain after acute and chronic treatment with imipramine. *J. Pharm. Pharmacol.* **43**:31–35 (1991).
16. M. Qin, M. Nilsson and S Øie. Decreased elimination of drug in the presence of alpha-1-acid glycoprotein is related to a reduced hepatocyte uptake. *J. Pharmacol. Exp. Ther.* **269**:1176–1181 (1994).
17. K. Brøsen and L. F. Gram. First-pass metabolism of imipramine and desipramine: impact of the sparteine oxidation phenotype. *Clin. Pharmacol. Ther.* **43**:400–406 (1988).
18. S. Sindrup, K. Brøsen and L. F. Gram. Nonlinear kinetics of imipramine in low and medium plasma level ranges. *Ther. Drug Monit.* **12**:445–449 (1990).