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

John W. Holladay,1 Michael J. Dewey,2 and Sun D. Yoo1,3

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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), triethyamine 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. (Muskogon, 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)F1 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 biopsies. 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.
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 isopropryl 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 −20oC 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 10−3 M EDTA and homogenized for 90 sec (12). Brain homogenates were then frozen at −20oC 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 −20oC 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 −20oC 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 C18 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).](image)

**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).](image)