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Kinetic Interaction between Fluoxetine and Imipramine as a Function of Elevated Serum Alpha-1-acid Glycoprotein Levels

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Abstract

The effect of elevated serum alpha-1-acid glycoprotein (AAG) levels on the pharmacokinetic interaction between imipramine and fluoxetine has been examined by utilizing a novel strain of transgenic mice which express serum AAG levels several times greater than normal.

Before fluoxetine treatment, serum imipramine levels were approximately three times greater in transgenic mice than in control mice. Despite higher serum imipramine levels in transgenic mice, brain drug levels were lower than those found in control mice. Fluoxetine pre-treatment (20 mg kg^{-1} for 5 days) resulted in an increase in serum imipramine levels in both groups of mice and the extent of the increase was greater in transgenic mice than in control mice (4·5-fold increase compared with 3·1-fold). Similarly, fluoxetine pre-treatment resulted in an increase in brain levels of imipramine in both groups of mice and the extent of the increase was greater in transgenic mice than in control mice (3·0-fold increase compared with 2·0-fold). Similar trends were observed for levels of desipramine in the serum and brain. Serum imipramine and desipramine levels did not correlate with their respective brain levels in the presence of elevated serum AAG levels before and after pre-treatment.

These findings indicate that the extent of increases in imipramine and desipramine serum and brain levels are greater during elevated serum AAG states than during normal AAG states when imipramine is co-administered with fluoxetine.

Imipramine, a tricyclic antidepressant routinely used for treatment of depression, is strongly (>90%) bound to serum alpha-1-acid glycoprotein (AAG) (Bickel 1975). After 40 years of use, imipramine is still considered to be a cost-effective, first-line therapy for depressive illnesses (Simon et al 1996). Fluoxetine, a selective 5-hydroxytryptamine (5-HT) re-uptake inhibitor, is often given in combination with tricyclics, e.g. imipramine, to reduce the lag-time for clinical response and to augment its antidepressant action (Weilburg et al 1989). Highly extracted by the liver, imipramine is primarily metabolized by hydroxylation (by CYP2D6, 1A2, 2C19 and 3A4) and by demethylation (by CYP1A2 and 2C19) (Koyama et al 1997). Concomitantly administered fluoxetine is known to inhibit several of the cytochrome P450 isoforms responsible for the hydroxylation of imipramine (Brøsen & Skjelbo 1991; Crewe et al 1992).

Vaughan (1988) was among the first to report an anomaly in tricyclic antidepressant serum levels when members of this class were co-administered with fluoxetine. Since then it has been well documented that fluoxetine induces a 3- to 5-fold elevation in tricyclic serum levels (Bergstrom et al 1992; Vandel et al 1992; El-Yazigi et al 1995). Similar results have been found with other selective 5-HT re-uptake inhibitors such as fluvoxamine (Spina et al 1993; Maskall & Lam 1993; Vandel et al 1995). The mechanism of this interaction is thought to be a consequence of inhibition of CYP2D6, an enzyme responsible for the hydroxylation of imipramine. Fluoxetine is considered to have little effect on the *N*-demethylation of imipramine, which is mediated by CYP1A2 and CYP3A4 (Lemoine et al 1993).

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AAG is an acute-phase protein the serum levels of which are not constant and are elevated in a variety of disease states, including depression (Kremer et al 1988). Previous data from this laboratory have indicated that the systemic clearance of imipramine is reduced in the presence of elevated serum AAG (Holladay et al 1996). This result is contrary to accepted pharmacokinetic theory for high-extraction-ratio drugs in that the clearance of imipramine is proportional to the unbound fraction of imipramine. Similar results have been found for the systemic clearance of disopyramide, which is also a highly extracted drug (Huang & Øie 1985). Because cytochrome P450 metabolism of imipramine is dependent on the concentration of unbound drug at the enzyme site, increased serum binding as a result of elevated serum AAG might influence the rate and extent of drug metabolism of imipramine. Further, the reduction in the systemic clearance of imipramine during elevated AAG states might be amplified by the concomitant administration of fluoxetine.

Because no studies have yet evaluated the effects of elevated serum AAG levels on the interaction between fluoxetine and imipramine, this study was undertaken to examine the serum and brain concentrations of imipramine and its metabolite desipramine during concomitant administration of imipramine and fluoxetine. This was approached by utilizing a novel strain of transgenic mice which expressed serum AAG levels 8·6 times greater than normal.

Materials and Methods

Chemicals

Fluoxetine and norfluoxetine (as hydrochlorides) were kindly supplied by Eli Lilly (Indianapolis, IN). Imipramine, desipramine and clomipramine (all as hydrochlorides), ethylenediaminetetraacetic acid (EDTA), bovine serum albumin, BioRad protein dye reagent, NADPH, potassium phosphate (monobasic and dibasic, anhydrous forms), magnesium chloride, triethylamine and sodium hydroxide were purchased from Sigma (St Louis, MO). Acetonitrile, methanol and hexane (all HPLC-grade) and sodium chloride were obtained from V.W.R. Scientific (Suwanee, GA). Diethyl ether and 85% phosphoric acid were purchased from Mallinckrodt (Paris, KY). Perchloric acid was obtained from J. T. Baker (Phillipsburg, NJ).

Animals

Studies were performed on adult control (C57BL/6) mice, 4–5 months old, male and female, 20–30 g (Jackson Laboratories, Bar Harbor, ME) and on transgenic mice expressing serum AAG levels severalfold higher than normal (Dewey et al 1990) produced by standard transgenic technology involving micro-injection of a rat AAG genomic clone (containing the entire coding region along with 4·7 kB of 5′ flanking sequences) into the pronuclei of (C57BL/6 × DBA/2) F_2 embryos. The transgenic founders were identified by Southern analysis and mated to non-transgenic, C57BL/6 mice. Positive transgenic offspring from this cross were subsequently crossed with each other to produce homozygous mice among the offspring. Mice homozygous for the transgene were identified on the basis of the intensity of the signal in Southern analyses and confirmed by progeny testing. Two of such homozygotes were bred and the line was maintained by sequential brother-sister mating. Among the resulting transgenic lines, AGP 9·5-5 was observed to express serum AAG levels 15·2-fold greater than normal (Dewey et al 1990; Yoo et al 1996). This study was performed with (AGP 9·5-5 × C57BL/6) F_1 hybrids which expressed serum AAG levels 8·6-fold greater than normal ($2{\cdot}24 \pm 0{\cdot}16$ compared with $0{\cdot}26 \pm 0{\cdot}05\ mg\ mL^{-1}$) and control mice (pure strain C57BL/6). All mice were maintained in a temperature-controlled animal facility with a 12 h–12 h light-dark cycle; they had free access to food and water.

Serum imipramine protein binding

The extent of serum imipramine protein binding was determined by equilibrium dialysis. Methanolic imipramine stock solutions were dried under nitrogen gas and the drug residue was reconstituted with either transgenic or control serum to yield final serum concentrations of 10, 50, 100, 500, 1000 and 5000 $\mu g\ mL^{-1}$. The serum was then dialysed against phosphate buffer (0·133 M, pH 7·35) at 37 °C for 7 h ($n = 3$ for each concentration). At equilibrium, serum and buffer samples were collected in borosilicate tubes and stored at −20 °C until drug analysis. The unbound fraction was calculated as the ratio of the concentration of the drug in the buffer to that in the serum.

Drug administration

To determine the brain and serum levels of imipramine and its metabolite desipramine, transgenic and control mice ($n = 5$ for each group) were injected intraperitoneally ($30\ mg\ kg^{-1}$) with imipramine ($3\ mg\ mL^{-1}$ as free base in saline). Thirty minutes after injection whole blood was collected by cardiac puncture and left to stand for 1 h. Serum samples were harvested after centrifugation and kept in borosilicate tubes at −20 °C until drug analysis. In addition, whole brains were removed

promptly after cardiac puncture. Each brain was accurately weighed, placed in perchloric acid (0·4 M; 10 mL) containing 10^{-5} M EDTA and homogenized for 60 s. All brain homogenates were stored in glass scintillation vials at −20 °C until drug analysis.

To determine the effects of fluoxetine on imipramine disposition, transgenic and control mice (20–30 g, male and female, 3–4 months old; n = 4 for each group) were administered fluoxetine (20 mg kg^{-1}) daily by intraperitoneal injection (2·0 mg mL^{-1} in water) for 5 consecutive days. On day six, each mouse received a single intraperitoneal injection of imipramine (30 mg kg^{-1}). Thirty minutes after imipramine administration whole-blood samples were harvested by cardiac puncture and whole brains were promptly removed. Serum and brain samples were stored at −20 °C until HPLC analysis.

Microsomal protein incubations

To determine the extent of in-vitro imipramine metabolism, imipramine (0·5–1000 μM) was incubated with hepatic microsomes obtained from transgenic and control mice. Whole livers were taken from transgenic and control mice (n = 4 each group), washed with saline (60 mL), snap-frozen in liquid nitrogen, accurately weighed, and stored at −70 °C until used. Microsomal proteins were prepared in chilled phosphate buffer (0·067 M, pH = 7·4) by standard differential ultracentrifugation techniques. The microsomal protein content of each preparation was determined by the Bradford (1976) protein-dye method using bovine serum albumin as internal standard. All microsomal protein suspensions were stored in plastic vials and kept at −70 °C until used.

All microsomal protein-drug incubations were performed in polypropylene tubes open to ambient air, with a total incubation reaction volume of 1·0 mL. Each reaction tube contained microsomal protein (2 mg), 1 mM NADPH, 10 mM $MgCl_2$ 100 μL drug solution (concentration range 0·5–1000 μM) and phosphate buffer (0·067 M, pH 7·4) to a total volume of 1·0 mL.

Drug analysis

Concentrations of imipramine and desipramine in the serum and brain were determined by a modification of a previously published HPLC procedure for assay of imipramine (Yoo et al 1995). Briefly, analysis was performed with Shimadzu (Columbia, MD) equipment fitted with a Microsorb MV octadecyl column (Rainin, Woburn, MA). The mobile phase was 60 : 40 acetonitrile-distilled water containing 10 mM aqueous triethylamine, with the pH adjusted to 3·0 by dropwise addition of 85% phosphoric acid. The flow rate was set at 1·0 mL min^{-1} and the effluent monitored for UV absorption at 260 nm. Duplicate serum (50 μL) or brain homogenate (100 μL) samples were extracted and assayed for drug content using clomipramine as internal standard.

Data analysis

Data obtained from microsomal enzyme incubations were analysed by means of the non-linear computer program GraphPad Prism. The velocity of substrate metabolism was expressed as nmol substrate metabolized (mg microsomal protein)$^{-1}$ min^{-1} and substrate concentrations were expressed in μmol. The mean apparent microsomal metabolic capacity (V_{MAX}) and affinity (K_M) values for the metabolism of each drug were determined as the means of results from three microsomal incubations. Microsomal protein content was expressed as mg protein (g liver)$^{-1}$. Brain drug concentrations were expressed as ng drug (g brain)$^{-1}$. The brain-to-serum (B/S) drug concentration ratios were calculated. For statistical analysis the unpaired Student's *t*-test was used to test the significance of differences between brain and serum drug concentrations in transgenic and control mice. The significance level was set at $P < 0·05$.

Results

The mean unbound fraction of imipramine was significantly lower in transgenic mice than in control mice (3·7% compared with 10·2%). The unbound fraction of imipramine was constant over the concentration range studied (10–5000 μg mL^{-1}). Before pre-treatment, serum concentrations of imipramine 30 min after an intraperitoneal injection of imipramine were significantly higher in transgenic mice than in control mice (983·7 ± 239·7 compared with 357·4 ± 41·5 ng mL^{-1}) (Table 1). Conversely, brain levels of imipramine were significantly lower in transgenic mice (10 056·4 ± 1085·6 compared with 15 639·2 ± 2554·1 ng g^{-1}). A similar trend was found for desipramine levels in the serum and brain (Table 2).

Five days of fluoxetine pre-treatment resulted in increased serum imipramine levels in both groups of mice and the extent of increase was significantly greater in transgenic mice (4·5-fold elevation compared with 3·1-fold). A similar trend was found in serum desipramine levels between the groups of mice after fluoxetine administration. Fluoxetine pre-treatment also resulted in an increase in the brain levels of imipramine and desipramine. Again,

Table 1. Serum and brain levels of imipramine 30 min after a single intraperitoneal injection of imipramine (30 mg kg^{-1}) before and after pre-treatment with fluoxetine in transgenic and control mice.

Parameter	Transgenic	Control
Before pre-treatment		
Serum concentration (ng mL^{-1})	983·7 ± 239·7*	357·4 ± 41·5
Brain concentration (ng g^{-1})	10 056·4 ± 1085·6*	15 639·2 ± 2554·1
B/S ratio	10·2 ± 2·7*	43·8 ± 4·6
After pre-treatment		
Serum concentration (ng mL^{-1})	4514·1 ± 499·4*	1125·0 ± 135·9*
Brain concentration (ng g^{-1})	30 328·7 ± 1673·7*	31 847·7 ± 2704·6*
B/S ratio	6·7 ± 0·7*	26·7 ± 1·6*

* $P < 0.05$ compared with corresponding control.

Table 2. Serum and brain levels of desipramine 30 min after a single intraperitoneal injection of imipramine (30 mg kg^{-1}) before and after pre-treatment with fluoxetine in transgenic and control mice.

Parameter	Transgenic	Control
Before pre-treatment		
Serum concentration (ng mL^{-1})	232·1 + 16·2*	41·1 ± 13·0
Brain concentration (ng g^{-1})	654·5 ± 83·9	762·4 ± 112·4
B/S Ratio	2·8 ± 0·3*	18·5 ± 7·5
After pre-treatment		
Serum concentration (ng mL^{-1})	1310·5 ± 105·2*	248·2 ± 50·5*
Brain concentration (ng g^{-1})	1111·3 ± 131·2*	1010·3 ± 185·9
B/S ratio	0·8 ± 0·05*	4·4 ± 0·7*

* $P < 0.05$ compared with corresponding control.

the extent of the increase was greater in transgenic mice than in control mice (3·0-fold compared with 2·0-fold). Therefore, serum imipramine and desipramine concentrations did not correlate with brain drug levels in the presence of elevated serum AAG levels both before and after pre-treatment. No difference was found between the total microsomal protein content of transgenic and control mice (Table 3). Further, no strain differences were noted between total microsomal metabolic capacity (V_{MAX}) or affinity (K_M) for imipramine for the two groups of mice (Table 4).

Discussion

Before fluoxetine pre-treatment, serum imipramine levels in transgenic mice were significantly higher than those in control mice, primarily because of a substantially reduced volume of distribution (40% below that for control mice) (Yoo et al 1996). This reduced volume of distribution of imipramine in transgenic mice is consistent with the observed increase in serum-imipramine protein binding as a result of increased serum AAG (Table 1). Although imipramine accumulated extensively in the brain in both groups of mice, the extent of imipramine distribution into the brain was much lower in transgenic mice before fluoxetine pre-treatment (B/S ratio 10·2 compared with 43·8), despite higher serum drug levels.

The systemic clearance of high-extraction-ratio drugs is said to be predominantly a function of hepatic blood flow. Further, changes in the unbound fraction of such a drug should not play a significant role in determining its hepatic clearance (Wilkinson & Shand 1975). However, this classical view has recently been challenged for several high-extraction-ratio drugs, including imipramine, disopyramide and lignocaine (Huang & Øie 1985; Yasuhara et al 1985; Braun et al 1988). We have previously shown that the systemic clearance of imipramine was significantly reduced in transgenic mice with elevated serum AAG levels (Holladay et al 1996). Assuming that cytochrome P450 metabolism of imipramine is dependent on the concentration of unbound drug at the site of metabolism, a significant reduction in the unbound fraction of imipramine might reduce its clearance and thereby result in higher imipramine drug concentrations.

Table 3. Microsomal protein content (± s.d.) in control and transgenic mice.

Mice	Sex	Body weight (g)	Liver weight (g)	Microsomal protein content (mg g^{-1})
Control	M	33·1 ± 2·3	1·47 ± 0·06	25·2 ± 5·1
	F	25·1 ± 1·6†	1·44 ± 0·05	23·8 ± 4·4
Transgenic	M	34·4 ± 4·8	1·52 ± 0·06	24·1 ± 6·0
	F	22·1 ± 3·0†	1·49 ± 0·07	22·7 ± 5·7

n = 8 per group. †Significantly different from male ($P < 0·05$).

Table 4. Michaelis-Menten values (± s.d.) for the microsomal metabolism of imipramine in transgenic and control mice.

Parameter	Transgenic Mice	Control Mice
Affinity (μM)	908·7 ± 145·4	800·3 ± 104·1
Microsomal metabolic capacity (μmol mg^{-1} min^{-1})	13·8 ± 2·8	16·9 ± 5·2

n = 8 per group.

Furthermore, when imipramine is concomitantly administered with fluoxetine, even higher serum imipramine concentrations can result, considering the inhibition of the imipramine hydroxylation pathway and the already reduced serum-unbound fraction of imipramine. In our study, levels of imipramine in the serum of transgenic mice were inordinately higher than values for control mice when imipramine was concomitantly administered with fluoxetine. This discrepancy between the resultant serum concentrations of imipramine for the groups of mice after fluoxetine pre-treatment (i.e. metabolic inhibition) is presumably because of the much lower unbound fraction of imipramine in transgenic mice.

As previously mentioned, imipramine is primarily metabolized by hydroxylation and demethylation (Koyama et al 1997). Theoretically, changes in the intrinsic clearance of a highly extracted drug should have little effect on its total clearance. Hence, the microsomal enzyme activities of transgenic and control mice were examined to seek the presence of any differences in imipramine metabolism which could lead to the observed elevations in imipramine serum levels after fluoxetine pre-treatment. In transgenic mice, the total microsomal protein content was unaltered between the groups of mice. Further, the K_M and V_{MAX} values for imipramine metabolism in transgenic mice were similar to those found for control mice. Therefore, differences in the extent of increases in imipramine and desipramine serum levels after fluoxetine pre-treatment were not because of alterations in drug metabolic capabilities in transgenic mice.

In conclusion, when fluoxetine is co-administered with imipramine during elevated serum AAG states, inordinately large elevations in the serum levels of imipramine and desipramine can result, with disproportionate changes in brain drug levels. This significantly greater increase in serum levels of imipramine and desipramine after fluoxetine pre-treatment might be the result of a significant reduction in unbound imipramine at the site of metabolism (i.e. hepatocytes) in the presence of elevated serum AAG. These findings are crucial to monitoring the therapeutic outcome of this dual antidepressant regimen, because serum concentrations of imipramine can increase in the presence of elevated serum AAG, with potentially no enhancement of pharmacological activity.

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