

BRIEF REPORTS

Determination of Fluoxetine and Norfluoxetine Concentrations in Cadaveric Allograft Skin

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Fluoxetine hydrochloride is the sixth most prescribed drug in the United States and is administered to treat major depression. A cadaveric skin donation was obtained from a 46-year-old woman who died as a result of a fluoxetine overdose. Due to the potential penetration of the drug and its major metabolite, norfluoxetine, into skin, the safety of using the skin as an allograft was questioned. Our evaluation showed that mean concentrations in skin were 2304 ± 175 and 1353 ± 102 ng/g of skin, respectively. The skin:plasma ratio was 0.41. Clinically, the amount of fluoxetine that can be transferred to an allograft recipient depends on many factors. Based on penetration of drug and metabolite into skin, one would have to evaluate carefully the risk:benefit ratio of using allografts from a donor who died from a fluoxetine overdose.

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Although clinical trials with fluoxetine hydrochloride (Prozac; Eli Lilly and Co., Indianapolis, IN) have been conducted since 1976, disposition of the drug and its major metabolite, norfluoxetine, have not been fully elucidated. Both are lipophilic and have large volumes of distribution in humans (20-45 L/kg), suggesting that they are present in fluids and tissues outside the plasma compartment.¹ Limited pharmacokinetic data suggest that they are widely distributed in body tissues, with highest concentrations in the lungs and liver. However, it is unknown if either drug or metabolite distributes into skin.

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Cadaveric allograft skin is used to treat second- and third-degree burns, abrasions, frostbite, infection, ulcers, and autoimmune diseases.² It is usually rejected by the recipient within 7-21 days, but until rejection, it performs many functions of healthy skin. Currently, no guidelines prohibit the use of allograft skin from donors who were taking agents including fluoxetine.

Knowledge of drug penetration into skin may be important when assessing the safety of cadaveric skin allografts from patients who die of drug overdoses. Unfortunately, there are no data regarding penetration of fluoxetine into skin. Theoretically, skin allografts obtained from patients who die of a fluoxetine overdose could contain sufficient quantities of drug and serve as a drug reservoir, resulting in appreciable concentrations in the recipient.

A donation was received by the University of Michigan skin bank from a patient who died as a result of a fluoxetine overdose. We conducted this study to determine if fluoxetine and norfluoxetine distributed into the patient's skin

and assess the potential correlation between concentrations in the donated skin and in suprathereapeutic plasma.

Skin Donation

Cadaveric skin was obtained from a 46-year-old Caucasian woman who died as a result of an apparent myocardial infarction. An organ-donation agreement was signed by her spouse, and the skin was harvested and sent to the skin bank for preparation, storage, and eventual allografting. According to protocol, the skin was kept under quarantine until the final report of the autopsy was available. The autopsy revealed that the patient had died as a result of a fluoxetine overdose, with concentrations of drug and metabolite in peripheral blood of 5566 and 3265 ng/ml, respectively (normal range 47–469 and 52–446 ng/ml, respectively). No other substances were detected. As a precautionary measure, due to lack of information regarding the penetration of fluoxetine and norfluoxetine into skin, the skin was determined to be unfit for use as an allograft and was dedicated to research.

Methods

The investigation was approved by the University of Michigan institutional review board. The cadaveric skin measured approximately 2.5 square feet and was divided into 10 packets, control rate frozen, and stored at -75°C for 10 months before the investigation. Skin from one packet was used in the experiment. The packet was thawed in a water bath (Cryosan Inc., Newton, MA) at 42°C for 15 minutes and opened, and the skin was rinsed with sterile saline to wash off glycerin used in the storage process. To improve the ability to section each sample finely, the skin was meshed with the aid of a Bioplasty Mesh (Bioplasty Inc., Minneapolis, MN) at a setting of 1:1. With a scalpel, three sections, approximately 1 square inch, were cut and their weight recorded. To determine if any constituents in the skin interfered with the assay, cadaveric skin from a drug-free donor was prepared in the same manner.

Each piece of skin was prepared as follows. The skin was finely sectioned into small pieces with a scalpel and transferred to a Pyrex Tenbroeck tissue grinder (Corning, Corning, NY). It was ground for 2 minutes, after which methanol 1 ml was added and the material ground for an additional 5 minutes. Two additional milliliters of methanol were added to

the grinder and the entire grinder was placed on ice in a sonicator (Fisher Scientific, Pittsburgh, PA) for 10 minutes. The skin-methanol mixture was drawn off with a borosilicate glass pipette and placed into a glass centrifuge tube. The process of methanol addition, grinding, and sonification was repeated until all skin was reduced to fine particles suspended in methanol. A total of 7 ml of methanol was used in the extraction of each piece of skin.

The mixture was centrifuged at 5000 rpm for 5 minutes, and the methanol supernatant drawn off and placed in polystyrene tubes. The supernatant was then evaporated to approximately 1 ml in a nitrogen analytical evaporator (Organomation, South Berlin, MA). The precise volume of supernatant was measured and recorded.

HPLC Assay

Fluoxetine and norfluoxetine concentrations in the methanol extraction were determined by high-performance liquid chromatography (HPLC) at Warde Laboratories, Ann Arbor, MI. The assay methodologies were based on a modification of a published method.³ Briefly, deionized water 1 ml, 1 M carbonate buffer (pH 10) 0.2 ml, protriptyline 1000 ng/ml 0.1 ml, and trimipramine 1000 ng/ml 1 ml as internal standards were added and mixed with methanol extraction 0.5 ml. Hexane 5 ml was then added, and each sample was mixed at high speed on a tube rotator for 10 minutes and centrifuged for 5 minutes at 3500 rpm. Of the resulting hexane layer, 4.5 ml was transferred to a glass autosampler vial and evaporated under nitrogen to dryness at $35\text{--}55^{\circ}\text{C}$. The residue was reconstituted with 0.4 ml of mobile phase and injected into the HPLC system, which consisted of a Supelcosil LC-PCN 5- μ column (Supelco, Inc., Bellefonte, PA) and an ultraviolet detector at 225 nm. The mobile phase consisted of 60:15:25 (vol/vol/vol) acetonitrile, methanol, and 0.01 M phosphate buffer.

Quantification was determined by comparing peak height ratios (peak height of drug divided by peak height of internal standard) with the standard curve. Interday and intraday coefficients of variation for both compounds were 5% and 3%, respectively. The assay was linear along the concentrations 25–5000 ng/ml for both fluoxetine and norfluoxetine. The minimum quantifiable concentration for both was 10 ng/ml.

Results

Both fluoxetine and norfluoxetine were

Table 1. Amount of Fluoxetine and Norfluoxetine Obtained in Cadaveric Skin

Specimen	Weight (g)	Amount (ng)		Amount/g skin (ng/g)	
		Fluoxetine	Norfluoxetine	Fluoxetine	Norfluoxetine
1	0.3457	728	427	2106	1235
2	0.4088	998	573	2441	1402
3	0.3997	945	568	2364	1421
Mean \pm SD	0.384 \pm 0.034	890 \pm 143	523 \pm 82.9	2304 \pm 175	1353 \pm 102

detected in the cadaveric skin (Table 1). Mean concentrations were 2304 \pm 175 and 1353 \pm 102 ng/g skin, respectively. The skin:plasma ratio for both compounds was 0.41. There were no detectable drug concentrations in control skin.

Discussion

Both fluoxetine and norfluoxetine were detected in the skin of the woman who died from fluoxetine overdose. Given the large volume of distribution (20–45 L/kg) and extremely high plasma concentrations (5566 and 3265 ng/ml fluoxetine and norfluoxetine, respectively), this was not unexpected.

These concentrations are less than those detected in other organs after fluoxetine overdose.^{4,5} In a 28-year-old woman who died of an overdose of the agent, brain and liver fluoxetine concentrations were 3600 and 12800 ng/g, and norfluoxetine concentrations were 3200 and 8800 ng/g, respectively. In our investigation, mean concentrations in skin were 2304 and 1353 ng/g, respectively.

Postmortem plasma concentrations of drug and metabolite in the donor are the second highest reported in the literature.⁶ It is possible that they were artificially higher than those immediately before death, as postmortem redistribution of drugs leading to falsely elevated blood concentrations is well documented.^{7,8} Due to increased pooling of blood and release of drug-rich blood from the liver into the larger vessels postmortem, drug concentrations obtained at autopsy can be considerably higher than those antemortem. Digoxin, tricyclic antidepressants, and barbiturates are more extensively studied, but fluoxetine also has this type of redistribution.⁴

Fluoxetine and norfluoxetine concentrations in the skin of this patient were 41% of those in peripheral blood. However, if the true antemortem plasma concentrations were lower, the tissue:plasma ratio would be higher (i.e., > 41%). In cases where drugs are known to redistribute postmortem, peripheral blood specimens are

considered to be more reliable. Peripheral blood was obtained for the toxicology screen during the autopsy of this patient and thus the concentrations most likely represent those at the time of death.

Despite every attempt to detect all fluoxetine and norfluoxetine in the cadaveric skin, the possibility remains that the actual concentrations are higher than reported. Thus, these amounts represent only minimum concentrations for several reasons. Specifically, highly bound fluoxetine and norfluoxetine may have remained in residual skin left over from the extraction process. Therefore, the concentrations reflect only the amount that was extracted from the extracellular fluid after extensive maceration and grinding. In addition, variability in the extraction method may have prevented additional drug and metabolite from being detected. Due to the cylindrical nature of the tissue grinder, it was difficult to retrieve the entire methanol fraction. Furthermore, residual methanol containing the compounds that saturated the skin pellet after centrifugation could not be collected.

Finally, the concentrations may be only a conservative estimate due to uneven dermal thickness that is characteristic of cadaveric skin allografts. Thickness varies throughout the length of each section as a consequence of uneven pressure applied to the dermatome when skin is harvested. The thickness of samples obtained for extraction may not represent that of other areas of the allograft. It is conceivable that other areas may have been thicker than those we sampled and thus may have contained larger deposits of drug. However, one could theoretically argue that the opposite is true and the average thickness may have been less than in our samples.

Clearly, many factors may have influenced the recovery of both compounds. However, as indicated, most of them would have led to loss of drug recovery, and thus our estimate of total amount of drug recovered should be viewed as conservative.

When considering passage of a drug through skin into the systemic circulation of healthy

patients, barriers to diffusion must be considered. The three primary barriers to drug absorption through the skin are the stratum corneum, epidermis, and dermis.⁹ Due to the compositional differences of each of these layers, diffusion into the systemic circulation depends on a drug's hydrophobicity, solubility, and diffusion coefficient. In patients requiring skin grafting, these layers are often nonexistent or have been surgically debrided, and the barrier to diffusion is vastly diminished. When a cadaveric allograft skin is saturated with drug, combined with the fact that blood vessels from the recipient supply the skin graft, it is not unreasonable to suspect that drug would cross into the systemic circulation.

Typical skin grafts consist of approximately 2–3 square feet and weigh 140–210 g (i.e., 20% replacement in a 70-kg patient). Based on our conservative estimates of the amount of fluoxetine in the skin of this graft, if a patient were to receive a 3-square foot, 210-g allograft, and assuming all drug deposited in the allograft were transferred to the recipient, the cumulative dose of fluoxetine received would be 483,840 ng, or only 0.484 mg. This small amount is obviously not a therapeutic dose. Since the true concentration of fluoxetine and norfluoxetine could not be determined, it would be prudent to assess the risk:benefit ratio of using the allograft. Theoretically, even small quantities of drug could be transferred and sufficient to cause a hypersensitivity reaction.

The number of organ donations has increased every year since 1984 due to increased awareness of need.¹⁰ Skin donations have increased as well, yet they do not meet the needs of burn centers across the country. Thus, these centers cannot afford to reject a donation, and every attempt is made to use the skin while maintaining the safety of the recipient. These allografts are obtained after careful screens of medical records, and interviews with medical staff and next of kin for medical history and high-risk lifestyles. Samples of the donor's blood are also tested for transmissible diseases such as hepatitis and the human immunodeficiency virus. Although agents that the donor may have taken while alive are documented, they are used as surrogates for disease states that would preclude donation rather than as excluding variables themselves. One would predict that lipophilic drugs with large volumes of distribution and the propensity to cause toxicities may cause a problem on allografting. However, since no published reports

describe drug penetration into skin in cases of overdose, skin banks are forced to discard the skin as unsafe. Ideally, if penetration of drugs into skin were known, it might be possible to use selected donations for allografting.

Fluoxetine is prescribed for the treatment of major depression, bipolar disorders, obsessive-compulsive disorder, obesity, anorexia nervosa, bulimia nervosa, and panic attacks.^{11–17} In 1996 it was the sixth most prescribed drug in the United States.¹⁸ Therefore, it is not surprising that it is frequently listed on autopsy reports of skin donors. Based on our data, it is reasonable to expect that the agent and its metabolite distribute into skin of patients taking traditional dosages with therapeutic concentrations. However, the probability of adverse consequences from using allografts from such patients is most likely small. Even if all deposited drug in the allograft were transferred to the recipient, the quantities would be extremely small and possibly below the limit of detection. Future studies investigating the pharmacokinetics of drug absorption from skin allografts will provide valuable information into the utility and safety of these products.

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