Published in final edited form as:

J Neurosci Methods. 2007 June 15; 163(1): 83–91.

Appropriate Dosing Regimens for Treating Juvenile Rats with Desipramine for Neuropharmacological and Behavioral Studies

Megan E. Kozisek¹, Jean D. Deupree¹, William J. Burke², and David B. Bylund¹

*1*Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center Omaha, NE, 68198-5800

*2*Department of Psychiatry, University of Nebraska Medical Center Omaha, NE, 68198-5800

Abstract

The tricyclic antidepressants, including desipramine (DMI), are no better than placebo in treating childhood and adolescent depression, but are effective in adult depression. Animal studies comparing the effects of DMI in juveniles and adults are complicated by age-related variations in elimination rates. Thus, different dosing regimens are needed to achieve similar brain drug levels in juvenile and adult rats. We compared the half-life of DMI as well as the brain and serum concentrations of DMI and its active metabolite desmethyl-desipramine in juvenile and adult rats after various drug administration paradigms. After acute i.p. administration DMI is eliminated from the brain more slowly in postnatal day (PND) 21 and 28 rats as compared to adults. After chronic i.p. administration (for 4-5 days between PND 9 and 28), lower doses of DMI are needed with juvenile rats to obtain the same brain DMI concentrations as adults. By contrast, two weeks of continuous drug delivery (minipump) to PND 21-35 and adult rats result in similar brain DMI concentrations. Thus, the pharmacokinetic properties of DMI varies with the age of the animal and dosing of DMI and needs to be carefully adjusted in order to have appropriate brain levels of the drug.

Introduction

Major depressive disorder (MDD) is a major health problem in adolescents and children. Although the clinical symptoms of adolescent and childhood depression vary with developmental age, overall they are similar to those seen in adults. In adults, depression can be successfully treated with several classes of antidepressant drugs, including selective serotonin reuptake inhibitors (SSRI), and drugs which inhibit the reuptake of both norepinephrine and serotonin such as tricyclic antidepressants (Hazell et al., 1995). In contrast, in children and adolescents, tricyclic antidepressants do not appear to be clinically effective, whereas some SSRIs have been shown to be clinically effective (Hazell et al., 1995; Kratochvil et al., 2006). Due to lack of demonstrable benefit and side-effects, the tricyclic antidepressants are not approved for children and adolescents. One approach to address this issue of the

Megan E. Kozisek University of Nebraska Medical Center Department of Pharmacology and Experimental Neuroscience 985800 Nebraska Medical Center Omaha, NE, 68198-5800 Phone: 402-559-5165, Fax: 402-559-7495 mlavicky@unmc.edu

Jean D. Deupree, Ph.D. Professor University of Nebraska Medical Center Department of Pharmacology and Experimental Neuroscience 985800 Nebraska Medical Center Omaha, NE, 68198-5800 Phone: 402-559-4565, Fax: 402-559-7495 jddeupre@unmc.edu

William J. Burke, M.D. Professor & Vice-Chair, University of Nebraska Medical Center Department of Psychiatry 985580 Nebraska Medical Center Omaha, NE 68198-5580 Phone: 402-354-6591, Fax: 402-354-6896 wjburke@unmc.edu

David B. Bylund, Ph.D. Professor University of Nebraska Medical Center Department of Pharmacology and Experimental Neuroscience 985800 Nebraska Medical Center Omaha, NE, 68198-5800 Phone: 402-559-4788, Fax: 402-559-7495 dbylund@unmc.edu

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

differential effectiveness of classes of antidepressants is to determine how juvenile animals respond to antidepressant administration in comparison to adult animals. In order to appropriately dose juvenile rats for various experimental paradigms, it is first necessary to understand the pharmacokinetic properties of antidepressants in juvenile compared to adult rats.

Desipramine (DMI) is a tricyclic antidepressant that has been studied extensively in behavioral models of depression in adult rats. It is known to inhibit the norepinephrine transporter (NET) and down-regulate beta adrenergic receptors (Bauer and Tejani-Butt, 1992; Hebert et al., 2001; Mandela and Ordway, 2006; Argenti and D'Mello, 1994b; Hebert et al., 2001). In rats (although not in humans) the major metabolite of DMI, desmethyldesipramine (DDMI), is active and has been shown to down-regulate beta adrenergic receptors (Argenti and D'Mello, 1994b).

The focus of these studies was to determine the dose regimens of DMI administration for juvenile rats that would result in similar brain concentration of DMI and DDMI obtained in adult rats using treatment paradigms routinely used in behavioral and receptor regulation studies. We found that DMI and DDMI are distributed uniformly throughout the brain of juvenile and adult rats and therefore the concentrations of DMI and DDMI in the cerebellum can be used as a measure of DMI and DDMI concentrations in other brain regions. We also found that juvenile rats eliminate DMI and DDMI differently than adult rats and therefore the dose of drug required to produce a given concentration of drug in the brain will vary with age. Guidelines for dose regimens for juvenile rats of various ages are given.

Materials and Methods

Materials

Desipramine was purchased from Sigma-Aldrich (St. Louis, MO, USA) and was dissolved in water or 35% DMSO/65% saline. Desmethyldesipramine (DDMI) was obtained by the National Institute of Mental Health's Chemical Synthesis and Drug Supply Program. Isoflurane was obtained from Hospira, Inc. (Lake Forest, IL, USA). Osmotic minipumps were purchased from Durect Corporation (Cupertino, CA).

Animals

Male Sprague-Dawley rats from Harlan Industries (Indianapolis, IN) were used in all experiments. The animals were maintained under standard laboratory conditions with a 12 h light/dark cycle and with free access to water and standard rat chow. The animals were allowed to acclimate to their surrounding for one week prior to experiments and were also handled daily to become familiarized to the investigators. All juvenile rats were obtained from Harlan Industries and shipped in with the dam either on PND 1 or one week prior to experiments. Pups were weaned from the dam at PND 21. Juvenile rats were allowed to adjust to the surroundings for a week prior to experiments. Procedures were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the local Animal Care Committee.

Dosing Paradigms

Acute Half-life of Elimination—Adult (PND 63) and juvenile (PND 11, 21, 28) rats (n=3-5 per time point) were given an i.p. injection of DMI. The PND 11 rats were injected with 3 mg/kg of DMI; all other ages were given 10 mg/kg of DMI. At various time points (2-16 h) after the single injection rats were euthanized and the cerebellum was removed from all animals. Trunk blood was collected from the adult and PND 28 rats. The DMI and DDMI concentrations in the cerebellum and serum were determined.

Three i.p. injections in 24 hours—An injection schedule similar to those used in behavioral studies, such as the forced-swim test and the tail suspension test (Porsolt et al., 1977 and Steru et al., 1985) was implemented. Adult (n=4-7) and PND 21 (n=4-6) rats were injected i.p. with DMI (1, 3, 10 or 20 mg/kg/injection) three times (25, 7 and 2 h) prior to euthanizing. Two to three hours after the last injection animals were killed. The cerebellum was isolated and stored frozen at -80°C for determination of DMI and DDMI concentrations.

Twice daily i.p. injections for four days—Adult (PND 63; n=12) and juvenile (PND 9; n=3-7) rats were given an i.p. injection of DMI for 4 days, twice a day. Adult rats were given 10 mg/kg/injection of DMI while the PND 9 rats were injected with various doses of DMI (1 to 20 mg/kg/injection). All rats were euthanized 12-14 h after the last injection (PND 13 for the juvenile rats). The brains were promptly removed and the cerebellum was isolated and saved at -80°C for determination of DMI and DDMI concentrations.

Twice daily i.p. injections for 5.5 days—Injections similar to those used in behavioral studies, such as the learned helplessness model of depression (Seligman, 1972; Seligman et al., 1975; Seligman and Beagley, 1975) were used in this experiment. To mimic conditions used in the learned helplessness paradigm, adult (n=5), PND 16 (n=6) and PND 23 (n=6) rats were given an i.p. injection of DMI (10 mg/kg) for 5.5 days, twice a day. Animals were sacrificed 1.75 h after the last injection, PND 21 or PND 28, respectively for the juvenile rats. Brains were promptly removed and the cerebellum was isolated and saved at -80°C for determination of DMI and DDMI concentrations.

Minipump Delivery (2 weeks)—Adult (PND 63; n=5) and juvenile (PND 21; n=6) rats were anesthetized with isoflurane and subcutaneously implanted with osmotic minipumps containing either vehicle (35% DMSO/65% saline) or desipramine (10 mg/kg/day) using Alzet pump 2ML2, calibrated to release 5 $\mu\text{l/h}$ with a capacity; 2 ml. In the juvenile rats, pumps were changed on PND 28, due to a significant change in body weight over the first week. For the first week, Alzet pump 1007D was used which releases 0.5 $\mu\text{l/h}$ with a capacity of 100 μl . For the second week Alzet pump 2001 was used releasing 1.0 $\mu\text{l/h}$ with a capacity of 200 μl . The dose of drug was based on the estimated weight of the animal half-way through drug delivery interval. After pump implantation the animals were monitored closely and were housed in separate cages. After fourteen days of drug treatment, the animals were euthanized under isoflurane anesthesia and decapitated (on PND 35 for the juvenile rats) and trunk blood was collected. At the time of death there was still drug remaining in the pumps indicating that drug was still being delivered to the animal. The rat brains were immediately removed and kept at -80°C until used. The prefrontal cortex (Bregma 6.70 to 4.20 mm), thalamic (Bregma -1.80 to -2.56 mm and -3.60 to -4.52 mm) regions and cerebellum from brain slices were saved at -20°C in order to determine DMI and DDMI concentrations. The serum was also collected and frozen for determination of DMI and DDMI concentrations.

HPLC Determination of DMI and DDMI Concentrations—Brain tissue was kept on ice and was homogenized at room temperature for 30 to 60 s at 16000 rpm using a TissueMizer Ultra Turrax (IKA Works, Inc., Wilmington, N.C.) in 20 volumes of water. An internal standard (McN-JR-1854, chlorohaloperidol) was added to each sample prior to extraction of the DMI to correct for any loss of DMI during the extraction procedure. Serum and brain samples were then alkalinized with 1M sodium carbonate, extracted into a hexane 1% isoamyl alcohol solution for 2 h and the DMI was back extracted from the organic layers into 20 mM phosphoric acid for 1.5 h and then injected onto an HPLC column. HPLC parameters include: absorbance: 252 nm; mobile phase: 50 mM phosphate buffer pH 7.2, 50% acetonitrile, and 10 μl of dimethyloctylamine per 100 ml; column (Alltech Adsorbosphere C18 HS 3u, 150 mm by 4.6 mm, Deerfield, Illinois).

Statistics

Data were analyzed by analysis of variance followed by Tukey's test; $p < 0.05$ was assumed to be the minimal level for significance. Half-life was determined using non-linear regression analysis of the natural logarithm of the serum or brain concentrations versus time. Statistical analysis was conducted using SPSS (SPSS, Inc., Chicago, IL).

Results

DMI and DDMI Brain Distribution in Adult and Juvenile Rats

Drug concentrations were measured via HPLC in the prefrontal, thalamic and cerebellar regions in adult and juvenile rats after 2 weeks of DMI continuous (minipump) treatment. Although the brain concentrations of DMI and DDMI varied from animal to animal, there was a significant positive correlation (0.87 to 1.00) between the concentration of DMI and DDMI in the cerebellum and in the prefrontal and thalamic regions in the adult and PND 35 rats (Fig. 1; Table I). The cerebellar concentration of DMI and DDMI in the adult and PND 35 rats were similar ($p > 0.05$). These results indicate that concentrations of DMI and DDMI in the cerebellum are representative of other brain regions. In the studies reported here, the concentration of DMI and DDMI in the cerebellum was used as a measure of concentration of these drugs in the brain.

Elimination Half-Life of DMI and DDMI from Brain and Serum

Adult and PND 11, 21 and 28 rats were given a single i.p. injection of DMI and euthanized between 2 and 16 h later to determine elimination rates for DMI (Fig. 2) from the brain and serum. Serum DMI and DDMI concentrations were not measured in the PND 11 and 21 rats because the low amount of serum that could be collected was insufficient for measuring drug levels. All groups except the PND 11 rats received an injection of 10 mg/kg. The PND 11 rats received a lower dose (3 mg/kg) because preliminary studies indicated this dose produced similar brain concentrations to that seen in adult rats.

As indicated in Table II, adult rats eliminate DMI faster from both the brain and serum ($t_{1/2} = 3.3$ and 4.6 h, respectively), than juvenile rats. PND 21 rats have the slowest elimination half-life ($t_{1/2} = 8.4$ h) of DMI from the brain. Elimination of DMI from brains of PND 28 rats ($t_{1/2} = 4.6$ h) was slower than that of either PND 11 or adult rats. Elimination from the serum of PND 28 rats was similar to that of adult rats. The rank order of elimination of DMI from the rat brain was: adult < PND 11 < PND 28 < PND 21. Ninety-six percent of DMI was eliminated 16 h after a single injection in the adult rat, whereas 71% and 93% was eliminated from PND 21 and PND 11 rats, respectively.

The general trend for DDMI elimination was the opposite of DMI, because DDMI is formed as DMI is metabolized. In the PND 21 and 28 rats DDMI levels increased during the time that the DMI levels were decreasing (Fig. 3). In adults rats DDMI levels were stable and did not start to decrease until roughly 12 h after the injection of DMI. PND 11 rats had the lowest DDMI levels among the four groups, most likely due to the lower dose of DMI injected.

Brain DMI and DDMI Concentrations in Adult and Juvenile Rats Following Standard Treatment Paradigms

To determine the appropriate dose of DMI for juvenile animals for receptor regulation or behavioral studies, it is necessary to compare the brain concentrations of DMI and DDMI in juvenile and adult rats following the dosing regimens routinely used for these studies in adult rats. Changes in receptor density have been studied using twice daily injections for four days, which results in the drug levels fluctuating with time (Argenti and D'Mello, 1994b) and also following drug delivery via osmotic minipumps where drug levels are relatively constant

(Goodnough and Baker, 1994). For behavioral testing using the forced-swim test drugs are usually given 24, 6 and 1 h before the test. In the learned helplessness model of depression, the drugs are often given twice daily for 5.5 days with testing occurring within one to two hours after the last dose.

Receptor binding treatment paradigms

To mimic the four-day treatment studies, DMI and DDMI concentrations were measured in rat cerebellum after four days of twice daily i.p. injections of DMI in adult and PND 9-12 rats (Table III). The rats were euthanized 12 h (PND 13 for the juveniles) after the last injection. Increasing the dose of DMI in the juveniles resulted in a non-linear increase in brain concentrations of DMI and DDMI. A dose of 2 mg/kg/injection in PND 13 rats produced a brain concentration (1.04 $\mu\text{g/g}$) that is half of that seen in adult rats (2.28 $\mu\text{g/g}$) receiving 10 mg/kg per injection. When the dose in juvenile rats was increased to 3.5 mg/kg/injection, the DMI brain concentrations (4.92 $\mu\text{g/g}$) increased nearly 5-fold to approximately twice that seen in adult rats. Not all the PND 13 rats receiving 10 mg/kg/injection survived and the brain concentrations of those that did were 13 times higher (29.7 $\mu\text{g/g}$) than for adult rats. The DDMI concentration was highest in adult rats, followed closely by the 10 mg/kg/injection in PND 13 rats, and then levels proceeded to decline with decreasing doses. Four days of DMI (10 mg/kg/injection) treatment in adult rats produced similar levels of DMI and DDMI (ratio 1.02) in the brain. The ratio of DDMI/DMI in the PND 13 rats decreased with increasing dose between 2 and 10 mg/kg/injection.

DMI and DDMI cerebellar concentrations were measured following two weeks of continuous drug delivery via osmotic minipumps to adult (10 mg/kg/day) and PND 21 (starting age) rats (10 and 15 mg/kg/day; Table IV). In this study the animals were euthanized while drug was still being delivered. Two weeks of continuous 10 mg/kg/day DMI treatment produced similar brain concentrations of DMI and DDMI in adult and PND 35 rats. At this dose both adult and PND 35 rats eliminate DMI and DDMI from the brain at the same rate (i.e. there is no significant difference between brain DMI and DDMI concentration levels in the two groups). The ratio of DDMI/DMI after 2 weeks of 10 mg/kg/day treatment in adult and PND 35 rats was similar indicating that adult and PND 35 rats metabolize DMI and DDMI in the same fashion in this treatment paradigm. Two weeks of 15 mg/kg/day of DMI in PND 35 rats resulted in a greater than proportional increase in DMI and DDMI concentrations as compared to 10 mg/kg/day.

Behavioral treatment paradigms

To mimic the conditions of the forced-swim test paradigm adult (10 mg/kg/injection) and PND 21 (1, 3, 10 or 20 mg/kg/injection) rats were injected three times with DMI. The second injection was 18 h after the first injection and the third injection 5 h after the second. The rats were euthanized 2 (adult) or 3 (PND 21) h after the last injection. In the PND 21 rats, there was a linear increase in brain DMI and DDMI concentrations ($r^2 = 0.95$ and 0.97 , respectively) with increasing dose of DMI (Table V). When adult and PND 21 rats received the same dose of DMI (10 mg/kg/injection) similar DMI and DDMI concentrations were obtained. The ratio of DDMI/DMI in the brain of adult and PND 21 rats were comparable to each other, except at the dose of 1 mg/kg/injection in PND 21, for which no DDMI was detected.

In order to mimic the normal treatment paradigm for the learned helplessness model of depression, rats received 5.5 days of twice daily i.p. injections (a total of 11) of DMI (10 mg/kg/injection) and were euthanized 1.75 h after the last injection. The DMI concentrations were the lowest in the adult rats (5.90 $\mu\text{g/g}$), followed by PND 28 (9.58 $\mu\text{g/g}$) and PND 21 rats (21.3 $\mu\text{g/g}$) (Table VI). The PND 21 and PND 28 rats had similar DDMI/DMI ratios (0.14 and 0.13, respectively) that were lower than the ratio for adult rats (0.24).

Discussion

To better understand the differential effects of antidepressant drugs in children as compared to adults, behavioral and pharmacological models need to be developed in juvenile animals. As a preface to these important studies, it is first necessary to determine the appropriate dose of drug to administer to juvenile rats in order to obtain brain concentrations that were comparable to those used in adult rats. Our studies show that because DMI and DDMI are distributed uniformly throughout the brain, DMI and DDMI concentrations in the cerebellum can be used as an estimate of their concentrations in other brain regions. Because juvenile rats eliminate DMI and DDMI differently than adult rats, the dose of DMI given to juvenile rats will often be different than that given to adults in order to have comparable brain concentrations.

DMI and DDMI Distribution in the Brain

Assessment of the concentrations of drug in the cerebellum is a valid method of determining the amount of drug the animal was exposed to because: (1) concentration in the cerebellum changed linearly with that in the serum, and (2) there was a positive correlation with levels in the cerebellum with other areas of the brain at pharmacological doses. Other studies have also shown a uniform distribution in the brain among several brain regions after DMI treatment (Biegon and Samuel, 1979; Hrdina and Dubas, 1981). Use of cerebellum drug concentrations rather than serum drug concentrations overcomes the difficulty of getting enough serum from juvenile animals to obtain accurate blood concentrations. Furthermore, in most pharmacological studies the receptor regulation is usually measured in areas of the brain outside of the cerebellum. This means the cerebellum can be removed from the brain to measure brain concentrations without changing the procedures for the assessment of receptor densities in other areas of the brain. Because the brain is the site of action of antidepressants, it is more important to know the concentration of drug in the brain than in serum.

One concern with this method of assessment is that the concentration of DMI in the cerebellum compared to the concentration of DMI in the other brain regions will not follow a positive correlation pattern at high doses (Figure 1). At higher doses of DMI the thalamic region will have a higher DMI concentration compared to the prefrontal region. Conversely, the DDMI concentration will be greater in the prefrontal region compared to the thalamic region at higher doses. This may pose a potential problem when using the cerebellum as a measure for brain drug concentrations when higher doses are used.

Comparison of DMI concentrations of drug in the brain with various age groups

Both the acute and chronic studies show differences in brain drug concentrations at the different age groups. There are several possible reasons why brain drug levels in juvenile animals are higher than in adult animals at the same dose/body weight. During early postnatal development body weight and brain weight increase at a constant rate, but after three weeks of age, body weight increases at a more rapid rate (Happe and Murrin, 1990), possibly causing drugs to distribute differently in adult and juvenile brains. Juvenile animals would have the smallest ratio between brain weight and body weight, as the animal ages the body weight increases at a faster rate than the brain causing the ratio between the two to become larger. Another important factor in differences in brain drug concentrations with age is that the changing lipid composition of the brain could alter how drugs distribute throughout the brain. This may not play an important role in the DMI distribution because the juvenile animals have less lipid content in the brain than adult animals and yet they have higher brain DMI concentrations when given similar doses based on body weight. It is also probable that juvenile animals metabolize the drug at different rates than adult rats.

Half-Life of Elimination

The half-life of elimination of DMI from the brain is different in juvenile as compared to adult rats. PND 21 and 28 rats eliminate DMI more slowly than adult rats (Table II). Even though the brain elimination half-life values are significantly different ($p < 0.05$) in the different age groups compared to each other and to the adult rats, the most biologically relevant change after an acute (single) injection of DMI was in the PND 21 and 28 rats, which eliminated DMI from the brain at a much slower rate than the other age groups. In agreement between brain and serum (Table II) the adult rats eliminate DMI at a faster rate than PND 28 rats, although this is probably not a biologically significant difference. These findings help explain why adult and juvenile animals respond differently to antidepressant administration.

DMI and DDMI Levels in Receptor Regulation Study Paradigms

Two drug administration paradigms have been used to demonstrate down-regulation of adrenergic receptors with desipramine: i.p. injections and minipump implantation. From a pharmacokinetic standpoint, once or twice daily injections result in peaks and valleys in the brain drug concentrations, whereas with continuous infusion steady-state conditions are reached within about 2 days. Twice daily injections of DMI results in down-regulation of beta-adrenergic receptors in four days (Argenti and D'Mello, 1994a) whereas studies with continuous infusion are usually done for two to four weeks, in order to more closely mimic the time course for clinical improvement frequently seen in humans. In comparing the two dosing regimens in juvenile rats we found that PND 9-12 rats needed far less drug (2 - 3 mg/kg/injection) to obtain the same brain concentrations at the adult rats (10 mg/kg/injection). PND 21 to 35 rats appear to need the same amount of drug as adult rats when delivered by continuous infusion.

In PND 13 rats, following 4 days of twice-daily injections, the brain drug concentrations increase in a non-linear fashion with dose. Possible explanations for this phenomenon include: a) the younger rats cannot eliminate DMI efficiently; b) the lipid content of the brain is higher in juvenile compared to adult rats; c) juvenile rats do not metabolize DMI with the same isozyme as adult rats; and d) the activity of metabolizing enzymes is lower in the juvenile rats than adult rats. The most likely explanation is that there is a decreased activity of the enzyme responsible for converting DMI to DDMI in the juvenile rats. Following acute injections, the half-life of elimination of DMI from the brain is the same in the juvenile rats as in the adult rats, so the differences in brain concentrations are not in the rates of movement of drug out of the brain. The increase in brain concentration of DMI in juvenile rats compared to the adult rats is probably not related to the lipid content of the tissue. Happe and Murrin (1990) have demonstrated that the lipid content of the brain increases with age and the water content decreases. Based on this, one would expect DMI to accumulate more in the adult brain than the brain of the younger animals based on its lipid solubility ($\log P$ (octanol-water) of 4.9).

Studies on acute injections of DMI suggest that the rate at which the drug is being metabolized is similar at both age groups and that the same enzyme is involved in metabolizing DMI in juvenile and adult rats. However, with multiple injections it is possible that the drug concentration exceeds the capacity of the enzyme to metabolize it. At this point the rate at which the drug is entering the body will exceed the rate at which it is leaving the body and the plasma levels and brain concentrations would be expected to increase exponentially rather than linearly with dose. The data presented here show exponential increases in brain concentrations with dose in the PND 9-12 rats suggesting that the amount of enzyme is limited in these rats and even at 2 mg/kg/injection, repeated injections result in saturating the metabolizing enzymes. Furthermore, DDMI concentrations in the brain are far less in the juvenile rats than in the adult rats at similar DMI brain concentrations, suggesting that rate limiting step is not in the elimination of DDMI.

The brain DMI and DDMI levels for adult rats are quite different between the 4-day twice daily injections and continuous drug delivery via osmotic minipump. The two main differences in these experiments are a) animals receiving drug by twice daily i.p. injections received twice as much drug as the animals receiving continuous drug delivery via osmotic minipumps and b) there are peaks and valleys in the drug concentrations with i.p. injections, but the brain concentrations are constant after day two of continuous drug delivery (unpublished observation) via osmotic minipumps. Because animals were sacrificed twelve hours after the last dose in the 4-day paradigm, the brain DMI concentrations represent their minimum and the DDMI concentrations are closer to the maximum concentrations. This is probably why the ratio of DDMI/DMI is so much higher in these animals. In the continuous delivery paradigm, the animals were sacrificed when drug was still being delivered to the animal and brain drug concentrations represent steady-state levels. The fact that there is 4.5 times as much total drug (DMI + DDMI) in the brains of rats receiving 15 mg/kg/day than those receiving 10 mg/kg/day, suggest that the elimination process for DMI may be saturating at the higher dose of drug.

DMI and DDMI Levels in Behavioral Study Paradigms

The two paradigms used to mimic the conditions used in behavioral studies to evaluate the effects of desipramine administration were based on the forced-swim test and the learned helplessness model. The forced-swim test is a rat or mouse behavioral test used frequently to evaluate the potential efficacy of antidepressant drugs (Porsolt et al., 1977; Porsolt et al., 1978). In the forced-swim test, the usual paradigm consists of three injections in 24 h with 10 mg/kg/injection producing a behavioral effect (Connor et al., 1998). Three injections of 10 mg/kg DMI in adult and PND 21 rats yield comparable DMI and DDMI cerebellum levels. This implies that 3 injections of 10 mg/kg DMI is an appropriate dose for PND 21 rats in the forced-swim test. The forced-swim test treatment paradigm in PND 21 rats produced a linear increase in DMI brain concentrations with increasing dose (1 to 20 mg/kg/injection). This suggests that PND 21 rats metabolize DMI in a linear fashion and the rate limiting step for drug elimination has not been saturated in this paradigm.

In the learned helplessness model, animals demonstrate escape deficits following repeated uncontrollable shocks that are reversible by antidepressant drugs (Cryan et al., 2002). The normal injection paradigm used in learned helplessness model in adult rats is 5.5 days of twice daily injections of 10 mg/kg of DMI (Besson et al., 1999). This dosing regimen appears to saturate the elimination system in PND 21 and 28 rats because the brain concentrations were four and two times higher, respectively, in these rats than in the adult rats. These results are also in agreement with the fact that the half-life of elimination of DMI from the brain is greater for PND 21 rats than for PND 28 or adult rats.

The peak and valley concentrations of DMI and DDMI may be estimated from the receptor binding (4-day injection) and the learned helplessness model (5.5-day injection) treatment paradigms. In the learned helplessness paradigm, the brain concentrations are measured two hours after the last dose when the DMI levels will be high and DDMI concentrations will be low because they have not had time to build up. In the receptor binding paradigm, the brain concentrations are evaluated twelve hours after the last injection at the time when the animal would receive the next dose of drug. At the twelve hour time point the DMI concentration will be low, however the DDMI concentration may still be elevated since DDMI is eliminated after DMI.

Based on the data presented here, the recommended doses of DMI for various ages of juvenile rats that produce brain concentrations of DMI comparable to those used in adult rats for receptor regulation and behavioral studies are given in Table VII. Extrapolating from the twice a day i.p. injection paradigm to daily dosing for the chronic treatment we would predict that at the higher doses, the same daily dose given in a single injection would result in toxic brain

concentrations shortly after giving the drug and a non-linear increase in brain concentrations of DMI and DDMI in juvenile rats as the result of the metabolizing enzymes being saturated. A lower dose of DMI may be needed in juvenile animals receiving daily injections for the levels of the metabolizing enzymes not to be saturated.

The present study is the first to compare the pharmacokinetic properties of juvenile and adult rats treated with desipramine, a tricyclic antidepressant drug. From brain distribution experiments it was established that cerebellum concentrations of DMI and DDMI can be used as an estimate of drug concentrations in the brain at various time points after drug delivery. DMI and DDMI levels in receptor regulation study paradigms were examined using two different drug administration paradigms, i.p. injections and osmotic minipumps. In comparing the adult and PND 9-12 rats after 4 days of twice daily i.p. injections, we found that PND 9-12 rats need far less drugs (2-3 mg/kg/injection) to obtain the same brain concentrations as the adult rats (10 mg/kg/injection). Two weeks of continuous DMI (10 mg/kg/day) result in similar brain DMI and DDMI levels in adult and PND 35 rats. The administration paradigm used in the forced-swim test produced similar DMI and DDMI cerebellum levels in adult and PND 21 rats. Both the PND 21 and 28 rats had higher DMI and DDMI levels compared to adult rats following the learned helplessness paradigm, indicating that juvenile animals should be administered a lower dose of DMI to obtain the same brain concentrations of DMI and DDMI obtained in adult rats.

Acknowledgements

The authors would like to thank Dan Jacques for his work on determining drug levels in the brain and serum using HPLC analysis and Rebecca Whisler for assistance with the animal treatments. This research was supported by NIH grant MH64772.

References

- Argenti D, D'Mello AP. Design of a desipramine dosing regimen for the rapid induction and maintenance of maximal cortical beta-adrenoceptor downregulation. *Neuropharmacology* 1994a;33:1117-1124. [PubMed: 7838325]
- Argenti D, D'Mello AP. The pharmacodynamics of desipramine and desmethyldesipramine in rats. *J Pharmacol Exp Ther* 1994b;270:512-519. [PubMed: 7915311]
- Bauer ME, Tejani-Butt SM. Effects of repeated administration of desipramine or electroconvulsive shock on norepinephrine uptake sites measured by [³H]nisoxetine autoradiography. *Brain Res* 1992;582:208-214. [PubMed: 1327403]
- Besson A, Privat AM, Eschalier A, Fialip J. Dopaminergic and Opioidergic Mediations of Tricyclic Antidepressants in the Learned Helplessness Paradigm. *Pharmacology Biochemistry and Behavior* 1999;64:541-548.
- Biegon A, Samuel D. The in vivo distribution of an antidepressant drug (DMI) in male and female rats. *Psychopharmacology (Berl)* 1979;65:259-263. [PubMed: 117497]
- Connor TJ, Kelly JP, Leonard BE. Forced swim test-induced endocrine and immune changes in the rat: effect of subacute desipramine treatment. *Pharmacol Biochem Behav* 1998;59:171-177. [PubMed: 9443552]
- Cryan JF, Markou A, Lucki I. Assessing antidepressant activity in rodents: recent developments and future needs. *Trends Pharmacol Sci* 2002;23:238-245. [PubMed: 12008002]
- Goodnough DB, Baker GB. 5-Hydroxytryptamine₂ and beta-adrenergic receptor regulation in rat brain following chronic treatment with desipramine and fluoxetine alone and in combination. *J Neurochem* 1994;62:2262-2268. [PubMed: 8189233]
- Happe HK, Murrin LC. Tritium quench in autoradiography during postnatal development of rat forebrain. *Brain Res* 1990;525:28-35. [PubMed: 2245324]
- Hazell P, O'Connell D, Heathcote D, Robertson J, Henry D. Efficacy of tricyclic drugs in treating child and adolescent depression: a meta-analysis. *BMJ* 1995;310:897-901. [PubMed: 7719178]

- Hebert C, Habimana A, Elie R, Reader TA. Effects of chronic antidepressant treatments on 5-HT and NA transporters in rat brain: an autoradiographic study. *Neurochem Int* 2001;38:63–74. [PubMed: 10913689]
- Hrdina PD, Dubas TC. Brain distribution and kinetics of desipramine in the rat. *Can J Physiol Pharmacol* 1981;59:163–167. [PubMed: 7225943]
- Kratochvil CJ, Vitiello B, Walkup J, Emslie G, Waslick BD, Weller EB, Burke WJ, March JS. Selective serotonin reuptake inhibitors in pediatric depression: is the balance between benefits and risks favorable? *J Child Adolesc Psychopharmacol* 2006;16:11–24. [PubMed: 16553525]
- Mandela P, Ordway GA. The norepinephrine transporter and its regulation. *J Neurochem* 2006;97:310–333. [PubMed: 16539676]
- Porsolt RD, Anton G, Blavet N, Jalfre M. Behavioural despair in rats: A new model sensitive to antidepressant treatments. *European Journal of Pharmacology* 1978;47:379–391. [PubMed: 204499]
- Porsolt RD, Le PM, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. *Nature* 1977;266:730–732. [PubMed: 559941]
- Seligman ME. Learned helplessness. *Annu Rev Med* 1972;23:407–412. [PubMed: 4566487]
- Seligman ME, Beagley G. Learned helplessness in the rat. *J Comp Physiol Psychol* 1975;88:534–541. [PubMed: 1150935]
- Seligman ME, Rosellini RA, Kozak MJ. Learned helplessness in the rat: time course, immunization, and reversibility. *J Comp Physiol Psychol* 1975;88:542–547. [PubMed: 1150936]

Figure 1.

Distribution of DMI and DDMI in the brain of adult and PND 35 rats following two weeks of continuous DMI delivery by osmotic minipumps. The statistical parameters are given in Table I. $n = 4-6$ animals per group.

Figure 2.

A) Elimination of DMI from cerebellum in adult and PND 11, 21 and 28 rats after a single injection. B) Elimination of DMI from serum in adult and PND 28 rats after a single i.p. injection.

Figure 3.
DDMI cerebellum levels in adult and PND 11, 21 and 28 rats after a single i.p. DMI injection.
The DDMI concentrations are from the same rats presented in Fig 2.

Correlation of DMI and DDMI levels of adult and PND 35 rats in the prefrontal and thalamic regions versus cerebellum concentrations after two weeks of continuous DMI delivery.

Table 1

Drug / Region	Slope	Adult r ²	n	p	Slope	PND 35 r ²	n	p
DMI / Prefrontal	1.08 ± 0.25	0.91	4	0.048	0.80 ± 0.11	0.93	6	0.002
DMI / Thalamic	1.57 ± 0.04	1.00	4	0.001	1.22 ± 0.16	0.93	6	0.002
DDMI / Prefrontal	1.61 ± 0.28	0.96	4	0.019	0.93 ± 0.18	0.87	6	0.007
DDMI / Thalamic	0.89 ± 0.13	0.94	4	0.029	0.76 ± 0.13	0.90	6	0.004

Table II

Brain and serum elimination half-life of DMI in adult and juvenile rats

Rat Age	Dose (mg/kg)	Tissue	Half-life (h)
Adult	10	Cerebellum	3.3
PND 11	3	Cerebellum	3.7*
PND 21	10	Cerebellum	8.4*
PND 28	10	Cerebellum	4.6*
Adult	10	Serum	4.6*
PND 28	10	Serum	4.8*

* Significantly different than the other values in the same tissue

Table III

Receptor binding paradigm: Four days of twice daily injections

DMI and DDMI concentrations were measured in rat cerebellum after four days of twice daily i.p. injections of DMI starting on PND 9 for the juvenile animals. Animals were sacrificed 12 h after the last injection of DMI (PND 13 for the juveniles). Drug concentrations are expressed as μg of drug per g wet weight of tissue. Values are means $\pm$ SEM.

Age (dose/injection)	DMI Cerebellum ($\mu\text{g/g}$)	DDMI Cerebellum ($\mu\text{g/g}$)	n	DDMI/DMI
Adult (10 mg/kg)	2.28 ± 0.27	2.32 ± 0.19	12	1.02
PND 13 (1 mg/kg)	0 ± 0^a	0 ± 0^a	7	0.00
PND 13 (2 mg/kg)	1.00 ± 0.13	0.24 ± 0.03^a	10	0.24
PND 13 (3.5 mg/kg)	4.49 ± 0.33^{abc}	0.56 ± 0.04^a	11	0.12
PND 13 (5 mg/kg)	13.6 ± 1.4^{abcd}	1.40 ± 0.06^{abcd}	5	0.10
PND 13 (10 mg/kg)	29.7 ± 2.7^{abcde}	1.98 ± 0.19^{bcd}	3	0.07

^a $p < 0.05$ compared to adult (10 mg/kg)

^b $p < 0.05$ compared to PND 13 (1 mg/kg)

^c $p < 0.05$ compared to PND 13 (2 mg/kg)

^d $p < 0.05$ compared to PND 13 (3.5 mg/kg)

^e $p < 0.05$ compared to PND 13 (5 mg/kg)

Table IV

Receptor binding paradigm: Two weeks of continuous drug delivery

DMI and DDMI concentrations were measured in rat cerebellum following two weeks of continuous drug delivery of DMI to adult and juvenile (between PND 21-35) rats. Rats were euthanized while drug was still being delivered to the animals. Drug concentrations are expressed as μg of drug per g wet weight of tissue. Values are means $\pm$ SEM.

Age (dose/day)	DMI Cerebellum ($\mu\text{g/g}$)	DDMI Cerebellum ($\mu\text{g/g}$)	n	DDMI/DMI
Adult (10 mg/kg)	0.70 ± 0.05	0.22 ± 0.03	10	0.31
PND 21-35 (10 mg/kg)	0.59 ± 0.10^a	0.17 ± 0.03^a	6	0.29
PND 21-35 (15 mg/kg)	1.39 ± 0.18^b	0.54 ± 0.07^b	4	0.39

^a $p < 0.05$ compared to PND 21-35 (15 mg/kg)

^b $p < 0.05$ compared to adult (10 mg/kg)

Table V

Behavioral treatment paradigm: Three injections within 24 h

Adult and PND 20 rats were given three injections of DMI within 24 h and euthanized 2 (adult) or 3 (PND 21) h after the last dose. Drug concentrations in the cerebellum are expressed as μg of drug per g wet weight of tissue.

Values are means $\pm$ SEM.

Age (dose/injection)	DMI Cerebellum ($\mu\text{g/g}$)	DDMI Cerebellum ($\mu\text{g/g}$)	n	DDMI/DMI
Adult (10 mg/kg)	9.29 ± 1.01	1.09 ± 0.16	7	0.12
PND 21 (1 mg/kg)	0.37 ± 0.09^a	$0 \pm 0,^{ab}$	4	0
PND 21 (3 mg/kg)	$2.74 \pm 0.29,^{ab}$	$0.23 \pm 0.02,^{ab}$	6	0.09
PND 21 (10 mg/kg)	$11.6 \pm 1.64,^{bc}$	$1.10 \pm 0.12,^{cd}$	5	0.09
PND 21 (20 mg/kg)	$27.7 \pm 1.54,^{abcd}$	$2.44 \pm 0.10,^{abcd}$	4	0.09

^a $p < 0.05$ compared to adult (10 mg/kg)

^b $p < 0.05$ compared to PND 21 (1 mg/kg)

^c $p < 0.05$ compared to PND 21 (3 mg/kg)

^d $p < 0.05$ compared to PND 21 (10 mg/kg)

Table VI

Learned helplessness treatment paradigm: 5.5 days of twice daily injections

DMI and DDMI concentrations were measured in rat cerebellum after 5.5 days of twice daily i.p. injections of DMI to adult and PND 17-21 and 24-28 rats. The rats were killed 1.75 h after the last dose (PND 21 and 28).

Drug concentrations are expressed as μg of drug per g wet weight of tissue. Values are means $\pm$ SEM.

Age (dose/injection)	DMI Cerebellum Concentration ($\mu\text{g/g}$)	DDMI Cerebellum Concentration ($\mu\text{g/g}$)	n	DDMI/DMI
Adult (10 mg/kg)	5.90 ± 0.26	1.41 ± 0.08	5	0.24
PND 21 (10 mg/kg)	21.3 ± 1.4^a	2.92 ± 0.29^a	6	0.14
PND 28 (10 mg/kg)	9.58 ± 1.3^b	1.27 ± 0.12^b	6	0.13

^a $p < 0.001$ compared to adult (10 mg/kg)

^b $p < 0.001$ compared to PND 21 (10 mg/kg)

Table VII

Brain concentrations and recommended doses of DMI for different administration paradigms in juvenile as compared to adult rats

Injection paradigm	4-day i.p. 2 × day	Osmotic minipump	5.5 day, i.p. 2 × day	3 × in 24 h, i.p.
Adult Brain DMI (µg/g)	2	0.8	6	9
Time after last dose (h)	12	0	1.75	2 to 3
Normal adult dose	10 mg/kg/inj	10 mg/kg/day	10 mg/kg/inj	10 mg/kg/inj
Suggested Doses of DMI for Juvenile Studies				
PND 13	2.4 mg/kg/inj			
PND 21		10 mg/kg/day	~3 mg/kg/inj	10 mg/kg/inj
PND 28			~6 mg/kg/inj	