Tamoxifen Effects upon Body Weight, Water Intake, and Several Behaviors, and Tamoxifen’s Unconditioned Stimulus Properties in the Conditioned Taste Aversion Paradigm

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TAMOXIFEN EFFECTS UPON BODY WEIGHT, WATER INTAKE, AND SEVERAL BEHAVIORS, AND TAMOXIFEN'S UNCONDITIONED STIMULUS PROPERTIES IN THE CONDITIONED TASTE AVERSION PARADIGM

by

Steven J. Anderson

A Thesis
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Faculty of The Graduate College
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The antiestrogen tamoxifen citrate (Stuart Pharmaceuticals) is indicated for treatment of breast cancer. Tamoxifen (TMX) is frequently employed because of demonstrated effectiveness in tumor reduction and a low incidence of debilitating side effects compared to similar agents. Even though side effects are uncommon with use of TMX, medical and behavioral sequelae have been noted. The present study investigated several behaviors in female rats administered TMX in two separate studies. In the first investigation, the effects of 25 daily treatments of TMX upon body weight, water intake, and several behaviors were assayed. In the second study, aversion to a saccharin solution (CS) temporally paired with TMX (UCS) was assessed. Results from Experiment I indicated that TMX exerted a decremental effect upon body weight; differences in water intake were also noted while behaviors recorded during the behavioral assays were not affected.

In Experiment II, following five pairings of saccharin solution with TMX, subjects did not evidence avoidance of saccharin across treatment trials. It is suggested that TMX's selective effect upon body weight mimicked the effects of estrogen-induced weight loss affecting receptor sites of the hypothalamus, anterior pituitary, liver, and adipose tissue.
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Most of all, I would like to thank my parents, David and Joan Anderson for their support, encouragement, and faith.

Steven J. Anderson
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CHAPTER I

EXPERIMENT I

Introduction

Breast cancer is the most prevalent malignancy in North American and European women (Legha, Davis, & Muggis, 1978). Of this population, two thirds undergo ablative treatment in late-stage development of breast neoplasm (Legha et al., 1978). Such treatments include adrenalectomies, oophorectomies, and hypophysectomies. Moreover, various concentrations of chemotherapeutic agents, and hormonal treatment in conjunction with the surgical treatment have been shown to be most effective controlling breast cancer (Young, Lippman, Devita, Bull, & Tormey, 1977).

Even though hormonal manipulation via surgical and adjuvant hormonal treatment results in the greatest efficacy for breast cancer reduction, iatrogenic complications are numerous. Undesirable effects of estrogen treatment frequently include gastrointestinal intolerance and associated anorexia (Legha et al., 1978). Androgen therapy typically produced virilization which may lead to noncompliance (Heusen, 1974; Kennedy, 1965). Loss of life following oophorectomy, adrenalectomy, and hypophysectomy occurs with significant frequency (Moore, Woodrow, Aliapoulis, & Wilson, 1967; Veronesi, Pizzocaro, & Rossi, 1975).
In an effort to eliminate such medical sequelae, therapeutic trials involving new additive hormonal treatments have begun (Legha et al., 1978). One such treatment agent is the antiestrogen tamoxifen citrate (Nolvadex). Tamoxifen (TMX) has shown promise in treatment of estrogen-positive breast neoplasms (Young et al., 1977). Tamoxifen is typically administered orally twice per day at doses of 10 mg or 20 mg (Legha, Powell, Buzdar, & Blumenschein, 1981). Tamoxifen is frequently used due to unequivocal effectiveness in suppressing tumor growth (Katzenellenbogen, Norman, Eckert, Peltz, & Mangel, 1984; Legha et al., 1981; Lerner, Band, Isreal, & Leung, 1976; Tormey, Simon, Lippman, Bull, & Charles, 1976; Wilson, Tehrani, & Baum, 1982). In addition, relative to similar therapeutics, TMX yields fewer toxic side effects (Cole, Jones, & Todd, 1971; Heusen, 1976; Roberts et al., 1976). However, adverse side effects reported by patients administered TMX include: nausea and vomiting (Agrawal & Zelkowitz, 1981; Kaing & Kennedy, 1977; Legha et al., 1978, 1981; Tormey et al., 1976), hypercalcemia and associated osseous lesions (Agrawal & Zelkowitz, 1981; Minton, Cantwell, Knight, Rubens, & Hayward, 1978; Kaing & Kennedy, 1977; Legha et al., 1981; Lane, Besa, & Joseph, 1980), hematologic changes (Lerner et al., 1976; Tormey et al., 1976) depression, mental confusion, and weight changes. Even though use of TMX has demonstrated clear clinical effectiveness in reducing tumor growth with relative toxic safety, oncologists are concerned about TMX's reported side-effects (Agrawal & Zelkowitz, 1981; Lane et al., 1980; Legha et al., 1981; Minton et al., 1978).
Of particular concern for the present investigation are TMX's behavioral effects. Alterations in calcium metabolism induced by TMX have been proposed as a mechanism contributing to the development of psychological complications (Legha et al., 1981). Hypercalcemia produced by TMX may occur rapidly and serum levels can increase to toxic levels (Agrawal & Zelkowitz, 1981; Lane et al., 1980; Minton et al., 1978). Serum calcium levels assayed from certain patients receiving TMX have been similar to levels found correlated with the occurrence of psychological abnormalities within psychiatric populations (Petersen, 1968). Therefore, behavior change and psychological risk may well occur for the unmonitored TMX recipient. However, behavioral effects of TMX are not well known. Studies of the effects of TMX upon various behaviors are limited. Existing studies assessing the effects of TMX upon behavior have been concerned with the alterations in sexual behavior (Adkins, Pickett, & Koutnik, 1982; Etgen, 1981).

Since the behavioral effects of TMX are largely unknown, the behavior of laboratory rats in several tests (Nanry, Sewell, Gallus, Vaneczek, & Poling, 1983; Sewell, Gallus, & Nanry, 1982) was selected to study the effects of TMX. It was reasoned that employment of several behavioral assays would increase understanding of TMX's behavioral effects. In addition, if behavioral alterations could be produced in laboratory settings, future analyses might uncover effective antagonists for the behavioral alterations. Specifically, in Experiment I, the effects of daily administration (Clarke & Peck, 1976) of several doses of TMX on fluid intake, body weight, wheel
running, drinking, feeding, grooming, and general activity were assayed.

Method

Subjects

Twenty-eight, mature, female, Sprague-Dawley rats (mean body weight in grams = 251; SE = 5.2), bred and raised in the colony with no prior experimental history, served as subjects. The rats were individually housed in the colony, under conditions of constant illumination and temperature (24 degrees C, ca). Purina Laboratory Chow (Rat Chow 5012, Ralston Purina Co., St. Louis, MO) and water were available continuously in home cages throughout the study.

Apparatus

Fluid consumption was determined by use of inverted graduated cylinders which served as fluid reservoirs. The cylinders were plugged with No. 5 rubber stoppers holding drinking spouts. A reservoir was attached to each stainless steel home cage (32 x 24 x 20 cm; Unifab Corp., Kalamazoo, MI). Individual body weights were also measured daily using a top-loading scale (Pelouze, Model 1000). Motor activity was assessed for each subject, using one of three standard Wahmann Running Wheels (Wahmann Co., Baltimore, MD). Each running wheel (35 cm diameter x 11 cm wide) activated a microswitch upon one revolution in either direction. Running wheels were secured separately in sound attenuated chambers (61 x 61 x 61 cm). Chambers were equipped with masking white noise (80 dB), forced air ventila-
tion, and illumination (7.5 watt G. E.).

Procedure

Water intake and body weight measurements were taken at the same time daily. Water intake was determined by noting the milliliters absent from initial volume. Fluid reservoirs were then filled with fresh tap water and placed on home cages. Next, body weight, in grams, was measured by removing each subject from the home cage and placing it on a top-loading scale, noting weight in grams, and then returning it to the home cage. A 6-day acclimation period to these procedures occurred. Following this, there occurred 25 daily (chronic) TMX or vehicle treatments. Each subject received one administration each day, immediately following the daily water intake and body weight determinations.

After 23 chronic drug treatments, additional behavioral tests were conducted. On Treatment Day 24, behavioral activity was observed by trained observers. Observation periods occurred 30 minutes after treatment. Observers watched animals in each home cage individually for a period of 10 seconds before observing behavior in the next cage. Observation occurred for approximately 1 hour; thus, each subject was observed on 12 occasions for 10 seconds. Specific behaviors recorded were drinking, feeding, and grooming. Observers did not record frequency of occurrences, but simply placed a single mark on a checklist which denoted the occurrence or occurrences of the above behaviors. On the final day of study (Day 25), locomotion was measured. Each subject was placed in a running wheel 30 minutes follow-
ing drug treatment for a 30-minute period. Revolutions for each session were then recorded.

Drug Preparation and Administration

Stock preparations of tamoxifen citrate (Stuart Pharmaceuticals, Wilmington, DE) were suspended (10 mg/ml) in sesame seed oil. Subjects were randomly assigned to 5 groups of 6 rats per group (however, after group assignments, 2 subjects in the 0.5 mg/kg group were discovered to have been pregnant and were thus dropped from study). Each group was then randomly assigned to a dose level of TMX. Levels consisted of 0.0, 0.25, 0.5, 1.0, and 2.0 mg/kg (Bowman, Jones, Leake, & Morris, 1983; Bowman, Leake, & Morris, 1982; Messiha, 1981). The group assigned to the 0.0 dose level received the drug vehicle only (sesame seed oil). Subjects received treatment via intragastric-gavage in 1.0 ml/kg volumes.

Data Calculation and Statistical Analysis

Daily changes in body weight were analyzed by calculating mean difference scores ascertained by subtracting daily group means from respective baseline means. Baseline values for each group consisted of the last weight value on the final day (6th) of acclimation.

Relative water intake was measured, as follows, to eliminate weight fluctuations as a possible confounding factor. Relative water intake (RH₂O) difference scores were calculated by dividing daily milliliters of intake (mlH₂O) by the subject's weight (Wt) upon that day and subtracting from that quotient, the quotient obtained by
dividing baseline milliliters intake (BLmlH₂O) by baseline body weight (BLWt) (see formula below). Baseline milliliters of intake for each subject were obtained by calculating average intake over the last 3 days of acclimation. Baseline averages of milliliters of intake for each group were then calculated.

\[
\text{RH}_2^0 = \frac{(\text{mlH}_2^0)}{(\text{Wt})} - \frac{(\text{BLmlH}_2^0)}{(\text{BLWt})}
\]

The daily weight and relative water intake differences scores were then analyzed by use of repeated measures analysis of variance (RMAOV). If a day-by-treatment interaction or main effects were indicated, a one-way ANOVA procedure was used to detect the specific day upon which significant differences occurred. A protected LSD pair-wise comparison procedure was then used to indicate the dosage group(s) exhibiting significant differences. Tamoxifen effects upon locomotion, drinking, feeding, grooming, and general activity were analyzed by the use of a one-way analysis of variance (ANOVA). The alpha level for all statistical analyses throughout was .05.

Results

The Effects of Daily Administration of Tamoxifen Upon Body Weight

Figure 1 shows the effects of daily treatments of TMX upon body weight change from baseline for each dose group. A repeated-measures RMAOV indicated a significant day effect \( F(4, 23) = 188.47, \ P < .0001 \), a treatment effect \( F(4, 23) = 2.50, \ P < .0001 \), and a
Figure 1. Weight Changes From Baseline as a Function of TMX and Vehicle
day-by-treatment interaction $F(4,23) = 1.61$, $P < .0006$. The changes in body weight for the 1.00 and 2.00 mg/kg TMX groups are significantly different from weight changes in the vehicle control group. A one-way ANOVA procedure showed that these effects began to occur on Day 5 for the 1.00 mg/kg group and Day 6 for the 2.00 mg/kg group. The effects occurred on the majority of days thereafter (closed triangles signify significant differences compared to the vehicle group and are illustrated on Figure 1). For the remaining dose groups (0.5 and 0.25 mg/kg), with one exception, significant effects were not seen on any day when compared to control group data. Figure 1 also shows a dramatic effect of TMX upon body weight when comparisons are made between the 0.5 and 1.00 mg/kg TMX-treated groups. These results indicate a toxic effect of TMX on body weight for only the 1.00 and 2.00 mg/kg dose groups.

The Effects of Daily Administration of Tamoxifen on Relative Water Intake

Figure 2 displays relative water intake over 25 days for TMX administration for each dose group. This figure shows that for all groups no systematic increases or decreases across days were evidenced. A repeated-measures RAMOV showed a significant main effect for days $F(4, 23) = 19.47$, $P < .0001$ and a significant main effect for treatments $F(4, 23) = 10.77$, $P < .0001$. A day-by-treatment interaction was not observed $F(4, 23) = 1.02$, $P < .4357$.

A one-way ANOVA and protected LSD pair-wise comparison test indicated that on Days 9 and 10, the 2.00 mg/kg group consumed
Figure 2. Relative Water Intake Change From Baseline Per 100 Grams of Body Weight as a Function of TMX and Vehicle.
more water compared to the vehicle control group. (Day 9: \( P < .0195 \); Day 10: \( P < .0404 \)). Additionally, on Day 21 the 0.25 and 0.50 mg/kg groups consumed significantly less water on this day than did the vehicle group generating probability values equaling: \( P < .0037 \) and \( .0457 \), respectively. These results indicate that relative water intake for the groups employed differed significantly throughout the study. Also, the magnitude of difference in relative water intake among the groups did not change over days. Furthermore, the results from the one-way ANOVA and protected LSD pair-wise comparison test suggest that the 2.0 mg/kg TMX-treated group consistently consumed more water than the remaining groups, followed by the vehicle control groups and the 1.00 mg/kg TMX-treated group, while the groups that received 0.25 and 0.50 mg/kg of TMX consistently consume the least amount of water.

The Effects of Tamoxifen Upon Locomotion

Measurement of locomotion occurred on Day 25 of chronic administration of TMX. Subjects were administered their assigned drug dosages 30 minutes prior to assay. They then were placed in the running wheels for 30 minutes. Figure 3 displays resultant wheel revolutions as a function of dose level. Results from a one-way ANOVA indicated that group means did not differ significantly \( F(4, 23) = .92, P < .4700 \).
Figure 3. Effects of Dose Level of 25 Daily THX and Vehicle Treatments Upon Wheel Running.
The Effects of Tamoxifen Upon Drinking, Grooming, Feeding, and General Activity

Figure 4 presents the effects of the dose level of TMX on drinking, feeding, grooming, and a sum of these behaviors termed general activity. Observation occurred in the home cage 30 minutes following treatment. A one-way ANOVA indicated that no dose group drank significantly more or less water during observations: $F(4, 23) = .69, P < .6070$. Similarly, a one-way ANOVA procedure assessing average occurrences of grooming indicated these results were also not statistically significant, $F(4, 23) = .43, P < .7840$. For the third behavior observed, feeding, a one-way ANOVA procedure again failed to show significant difference across dose groups: $F(4, 23) = 1.54, P < .2240$. When occurrences of these behaviors were summed for each dose group, and then averages calculated by dividing total activity by group size; no group was significantly different: $F(4, 23) = .7, P < .5864$. Thus, these data suggest that TMX did not have an effect upon the behaviors observed.

Discussion

Daily administration of TMX resulted in body weight decrements in two groups (1.00 and 2.00 mg/kg) while the remaining groups (0.5 and 0.25 mg/kg) did not show significant changes when compared to the control group. A day effect, a treatment effect, and a day-by-treatment interaction were observed. Additionally, a marked difference in drug effect upon body weight is observed when comparisons are made between the body weight changes noted in the 0.5 mg/kg group.
Figure 4. Effects of Dose of 25 TMX and Vehicle Treatments Upon Drinking, Feeding, Grooming and a Sum of These Behaviors Termed "General Activity."
to weight changes observed in the 1.00 mg/kg group (Figure 1).

Significant differences in behaviors recorded during the behavioral
and assays were absent. Relative water intake data showed a main
effect for days and treatments.

In light of TMX effects upon body weight, it was hypothesized
that this effect may have occurred as a result of a state of general
illness. Specifically, anorexia may have occurred as a result of
illness induced by daily treatment of TMX. Another possibility is
that decrements in body weight may have occurred as a function of
conditioned taste avoidance (CTA) of rat chow. Assuming the exis­
tence of illness induced by TMX, gustatory stimulation correlated
with illness could have led to avoidance of that substance (Garcia,
For example, in the experiment of Garcia et al. (1967), cyclophospha­
mide, a chemotherapy agent, followed a period during which food-
deprived animals consumed chow. Over repeated trials, the rats con­
sumed less chow. (Garcia et al. (1967) concluded that subjects con­
sumed less chow as a function of CTA to the chow. Conditioned taste
aversion has been repeatedly demonstrated in various experiments
involving a variety of laboratory animals (Ader, 1973; Bolles, Riley,
& Laskowski, 1973; Cheornton & Amit, 1984; Grote & Brown, 1971;
Dragoin, Hughes, Devine, & Bentley, 1973; Riley & Baril, 1976;
Stolerman & D'Mello, 1977). Agents which have produced conditioned
taste aversion include radiation (Garcia, Kimeldorf, & Keolling, 1955;
Smith, Hollander, & Spector, 1981) and several cancer-chemotherapy
drugs (Dragoin et al., 1973; Garcia et al., 1967). In general, CTA
appears to occur in accordance with the principles of classical conditioning. Specifically, gustatory stimulation (conditional stimulus or CS) when temporally paired with a treatment which produces nausea (unconditional stimulus or UCS) eventually produces nausea in the absence of the UCS. In order to evaluate TMX's effectiveness in the paradigm in Experiment II, TMX was 5 times paired with a saccharin solution.
CHAPTER II

EXPERIMENT II

Introduction

Of the side-effects reported with the use of TMX, nausea and vomiting are among reported side-effects and occur most frequently (Agrawal & Zelkowitz, 1981; Kaing & Kennedy, 1977; Lane et al., 1980; Legha et al., 1978, 1981; Lerner et al., 1976; Tormey et al., 1976). Gastrointestinal disturbances as a consequence of chemotherapy provide conditions under which conditioned taste aversion may be acquired. Systematic analyses of chemotherapy recipients have evidenced this occurrence (Bernstein & Webster, 1980; Kutz, Borysenko, Come & Bensen, 1980; Nesse, Carli, Curitis, & Kleinman, 1980). Messiha (1981) showed that experimental animals receiving TMX developed aversion to ethanol; however, Messiha did not employ the CTA paradigm, but simply administered TMX to animals who had developed a preference for ethanol. The development of CTA associated with TMX appears probable, yet, sufficient supportive data are lacking. Therefore, in Experiment II, TMX's function as a UCS in the CTA paradigm was assessed.

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Method

Subjects

Thirty, mature, female, Sprague-Dawley rats (Mean body weight in grams = 225; SE = 4.2). bred and raised in our colony with no prior experimental history, served as subjects. The rats were housed and maintained identically to those in Experiment I with the exception that fluid was restricted as described in the following procedure section.

Apparatus

Fluid consumption and body weights were measured with the same devices used in Experiment I.

Procedure

Body Weight and Fluid Intake Measurement

Body weights and fluid intake were determined daily. Body weight, in grams, was measured by the same means as in Experiment I. Fluid intake was determined by restricting fluid for 22 hours. Fluid reservoirs were then placed in each cage. Milliliters of water consumed were recorded following a 2-hour access period. All subjects were exposed to a 6-day acclimation period to this procedure before treatment began.
Conditioned Taste Aversion and Choice Procedures

The conditioned taste aversion procedure involved repeated pairings of a novel gustatory stimulus (CS), 10% saccharin in tap water, with tamoxifen (UCS). For control, a group received as UCS lithium chloride (LiCl), an established toxicosis agent (Smootherman, Hennessy, & Levine, 1976; Suarez & Barker, 1975). The LiCl group was employed to assure that experimental conditions and procedures were amenable to the occurrence of CTA. For additional control, a group received vehicle only. This group was included to eliminate the possible contribution of the vehicle substance to the production of CTA rather than TMX alone. Specifically, a treatment trial involved reservoirs, containing the saccharin (Sprinkle Sweet, Pillsbury Co., Harrisburg, PA) solution on each home cage for a 2-hour period; (2) recording milliliters of solution consumed; (3) injecting subjects with their respective dose of TMX, LiCl, or vehicle. Five such treatments occurred (every third day) over a 25-day period. Water intake and body weight measurements continued over the 2 days preceding each treatment trial.

Two days after the fifth and final treatment trial, a choice procedure occurred. This entailed determining body weights followed by the placement of two fluid reservoirs on each home cage: One containing fresh tap water and the other, the saccharin solution. To maintain consistency, the tap water was fastened on the experimenter's left, immediately adjacent to the saccharin reservoir placed to the
experimenter's right. Both adjacent reservoirs were placed in the same location on the home cage as when reservoirs were attached singly and were approximately 1 cm apart. Following a 2-hour access period, milliliters consumed from each reservoir were noted.

Drug Preparation and Administration

Stock preparations of tamoxifen citrate (10 mg/ml) were mixed as stated in Experiment I. Subjects were randomly assigned to 5 groups of 6 rats each. Three groups were assigned to 3 different dose levels of TMX. Dosage levels included: 1.25, 2.50, or 5.00 mg/kg (Bowman et al., 1983; Bowman et al., 1981; Messiha, 1981). The remaining 2 groups were assigned to either a dose level of LiCl (127 mg/kg) or vehicle (sesame seed oil) as control measures. The dose of LiCl was used with prior success in the laboratory in producing CTA without debilitating side-effects. All drug administrations were given intraperitoneally (IP) in volumes of 1.0 ml/kg. As can be noted, administrations of test agents were not routed via intragastric-gavage as found in Experiment I. This procedural alteration was performed to control against production of compound gustatory stimulation.

Statistical Analysis

Daily body weight and relative fluid intake were calculated and analyzed by the same statistical procedure found in Experiment I. Fluid intake for the preference test was analyzed by use of a one-way
ANOVA. Saccharin intake across treatment trials was assessed by a within-group paired t-test.

Results

Effects of Repeated Saccharin and Tamoxifen Pairings Upon Saccharin Consumption

Figure 5 shows the effects of repeated intermittent saccharin (CS) and TMX (UCS) pairings upon saccharin intake. Also shown are the effects of vehicle and LiCl pairings with saccharin upon saccharin intake. As shown, saccharin intake over trials for the TMX-treated groups receiving 1.25 mg/kg and 2.50 mg/kg and the vehicle-treated group did not show the systematic decreases evident for the LiCl group. The group that received 5.00 mg/kg of TMX did show a small decrease in consumption followed by partial recovery. Systematic decrements in saccharin intake after each trial are evidenced by the LiCl group. A one-way ANOVA and a protected LSD pair-wise comparison test indicated significant differences in saccharin intake for the LiCl group on each treatment trial following the third trial. During the third trial, results from the protected LSD pair-wise comparison test showed a significant decrement in saccharin intake for the LiCl group when compared to the vehicle group (P < .0099) and the TMX-treated group receiving 2.50 mg/kg (P < .0295). On the fourth trial this statistical evaluation resulted in significant decrement in saccharin consumption when the LiCl group was compared to all groups (LiCl vs 0.0 mg/kg, P < .0002; LiCl vs 1.25 mg/kg, P < .0025; LiCl vs 2.5 mg/kg, P < .0005; LiCl vs 5.00 mg/kg, P < .0038.

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Figure 5. Effect of Every-Third-Day Saccharin (CS)—TMX, LiCl, and Vehicle (UCS) Pairings Upon Milliliters of Saccharin Intake.
Following the final treatment trial, the LiCl group drank significantly less saccharin than the vehicle and the 1.25, 2.50, and 5.00 mg/kg TMX-treated groups (P < .0010; P < .0037; P < .0017; and P < .0011, respectively). Furthermore, the protected LSD pair-wise comparison test indicated the absence of significant differences in saccharin consumption when comparisons were made between the vehicle and TMX-treated groups.

Results from a within-group, paired t-test assessing mean differences across trials (Ho: \( u_2 - u_1 = u_3 - u_2 \ldots u_5 - u_4 = 0 \)) indicated absence of significant increments or decrements in saccharin consumption for the vehicle group, 1.25 mg/kg and 2.50 mg/kg TMX recipient groups. A significant decrease in saccharin intake was observed for the 5.00 mg/kg TMX group when mean saccharin intake on Trial 3 was compared to mean intake on Trial 2. No additional significant changes in saccharin intake were thereafter evident. Results obtained for the LiCl group indicated significant decrements in saccharin consumption on every trial up to Treatment Trial 5. Consecutive probability values from Treatment Trial 1 to Treatment Trial 5 were: P < .0020, P < .0051, P < .0210, and P < .5133. The above results indicate absence of CTA for the groups that received 0.0, 1.25, 2.50, and 5.00 mg/kg of TMX; yet instatement of conditioned aversion to the saccharin solution for the LiCl group.

Fluid Consumption During the Preference Test

Figure 6 shows milliliters of water and saccharin consumed by each group when both solutions were simultaneously presented. The
Figure 6. Consumption of Water or Saccharin Solution When Simultaneously Presented as a Function of Dose Level of TMX Vehicle and LiCl, 5 Times Paired with the Saccharin Solution.
preference test occurred two days following the final treatment trial. Visual inspection of Figure 9 indicates that for the vehicle and TMX groups, a greater volume of the saccharin solution was consumed over water; the reverse was true for the LiCl control group. A protected LSD pair-wise comparison analyzing mean differences in consumption indicated that the LiCl-treated group drank significantly less saccharin than the other groups (P < .0001 for each group compared). Additionally, the 1.25 mg/kg group consumed significantly less saccharin as compared to the 2.50 and 5.00 mg/kg groups (P < .0410 and P < .0295, respectively). A protected LSD pair-wise consumption of saccharin over water for the 0.0, 0.25, 0.50, 1.50, and 2.50 mg/kg TMX-treated groups (P < .0007, P < .0033, P < .0001, and P < .0001, respectively). A protected LSD pair-wise comparison of saccharin over water for the 0.0 mg/kg, 0.25, 0.50, 1.50, and 2.50 mg/kg TMX-treated groups (P < .0007, P < .0033, P < .0001, and P < .0001, respectively. Also significant was the difference in consumption of water rather than saccharin solution for the LiCl control group (P < .0007). These results further substantiate the absence of CTA following repeated vehicle and TMX treatment trials as well as the well-known development of CTA after repeated LiCl treatments.
The Effects of Intermittent Administration of Tamoxifen on Body Weight

Figure 7 displays mean differences in body weight from baseline across days, and the occurrence of the 5 intermittent treatments of 0.0, 1.25, 2.50, 1.0 mg/kg of TMX, and 127 mg/kg of LiCl. Visual inspection indicates rather systematic weight increases for all groups. A repeated-measures RMAOV analysis did not indicate a day effect, a treatment effect, a day-by-treatment interaction, nor a main effect. These results indicate the absence of a toxic effect upon body weight following intermittent TMX treatments.

The Effects of Intermittent Administration of Tamoxifen Upon Relative Fluid Intake

Figure 8 shows the differences in relative water intake from baseline for the vehicle group and TMX dose groups. Relative fluid intake of vehicle and TMX-treated groups showed no systematic changes across days and treatments. Results from a repeated-measures RMAOV did not indicate a day effect, treatment effect, a day-by-treatment interaction, or main effect. Thus, tamoxifen did not alter fluid intake on treatment trials or intake on days which intervened between trials.
Figure 7. Weight Change From Baseline as a Function of Days with Intermittent Administrations of TMX, LiCl, or Vehicle-Only.
Figure 8. Relative Fluid (Water and Saccharin on Treatment Trials) Intake Change, in Milliliter, From Baseline as a Function of Intermittent Treatments of TMX, Vehicle, or LiCl.
CHAPTER III

GENERAL DISCUSSION

Experiment I showed that daily administration of the antiestrogen tamoxifen citrate produced decrements in body weight when body weight changes across days were compared to weight changes observed for the vehicle group. This effect occurred only for the higher dosage groups (1.00 and 2.00 mg/kg) (Figure 1). The modulation of this effect as a function of a day-by-treatment interaction was also evident. Administration of estradiol, an estrogen compound, has produced inhibitory effects on food intake and associated weight loss (Devenport & Torres, 1984, Wade & Zucker, 1970). Similar effects upon body weight have been demonstrated by the antiestrogens clomiphene (Bowman, et al., 1981) and nafoxidine (Wade & Blaustein, 1978) and TMX (Bowman et al., 1982). The mechanisms by which these antiestrogens mimic estrogen effects upon body weight regulation has been hypothesized to involve the alteration of the estrogen receptor system of the hypothalamus and anterior pituitary (Wade & Blaustein, 1978). Specifically, antiestrogens, similar to estradiol, may alter hypothalamic RNA and protein synthesis involved in the production of proteins which affect the estrogen receptor sites related to food intake regulation (Luine & McEwen, 1979). Antiestrogens may act similarly to estradiol and produce changes in anterior pituitary RNA
and protein synthesis involved in the manufacturing of proteins which regulate food intake at estrogen receptor sites (Eisenfeld, 1976; Eisenfeld & Axelrod, 1965). Thus, body weight changes induced by estrogen agonism may be controlled via cellular changes in the hypothalamus and anterior pituitary.

Another possible explanation for reported weight loss involves changes in liver activity and adipose tissue. Studies have shown that rat liver contains cytoplasmic estrogen receptors (Aten, Weinberger, & Eisenfeld, 1978). Estradiol treatment has been shown to affect body weight by altering hepatic RNA the synthesis of amino acids which affect estrogen receptor sites that are involved in the regulation of food intake (Kurtz, Sippel, Ansah-Yiadom, & Feigelson, 1976; Menard, Corvol, Poliot, & Raynaud, 1973). Estrogen receptors have also been located in rat adipose tissue (Gray & Wade, 1979). Hamosh and Hamosh (1975) and Kim and Kalkhoff (1975) report that estradiol-binding at estrogen sites in adipose tissue alter lipoprotein lipase, which allows storage of fatty acids in adipose tissue. Therefore, estradiol may produce decrements in adiposity by inhibiting the function of lipoprotein lipase at estrogen receptors in adipose tissue. If TMX acts similarly to estradiol at these peripheral sites, the herein reported body weight decrements may be a function of cellular changes of estrogen receptors located in liver and adipose tissue. The peripheral function of TMX at these particular sites has not been elucidated (Bowman et al., 1983).

A third potential explanation for the observed decrements in body weight following daily administration of the higher TMX doses may
involve the inhibition of bone growth. Estradiol treatments have produced inhibition of bone growth (Devenport & Torres, 1984). If TMX produces the effects of that of estradiol upon bone development, bone growth inhibition may have contributed to the here-reported weight loss.

Yet a fourth plausible account of the weight decrements is that these could have occurred as a result of conditioned taste aversion. The reasoning here is that avoidance of chow led to decreases in body weight. This may be unlikely since CTA was not evidenced in Experiment II. Unfortunately, Experiment II did not reproduce the conditions of Experiment I (weight decreases were not observed) and, thus, did not constitute a test of whether CTA occurred in Experiment I.

As stated above; repeated intermittent pairings of a gustatory stimulus (10% saccharin) followed by TMX treatment did not result in avoidance of this stimulus (Figure 5). In fact, when water and saccharin were simultaneously presented in the choice procedure, subjects consumed significantly more saccharin than water. For the LiCl group, a clear learned avoidance of saccharin is evidenced (Figure 6). Other chemotherapy agents have also produced CTA in experimental animals (Ader, 1973; Bolles et al., 1973; Cheornton & Amit, 1984) and in humans (Bernstein & Webster, 1980; Bernstein, 1978; Kutz et al., 1980; Nesse et al., 1980).

Two procedural variables may have played a role in the absence of CTA demonstration for TMX. First, traditional studies of classical conditioning indicate that the temporal relation between the CS and UCS is a critical parameter. Usually, if the UCS presentation
follows the CS more than several seconds, conditioning is reduced or simply does not occur (Revusky & Garcia, 1970). However, CTA is an exception to this rule—animals learn to avoid the CS even though the UCS may be delayed at least 1 to 2 hours (Revusky and Garcia, 1970). It has been hypothesized that stimulation associated with the CS is still persisting within the organism's system when the UCS is presented, and thus a correlation is produced. Such a correlation may not have existed in the present study due to the plausible delayed onset of TMX as the UCS. Slowed rates of absorption may have delayed critical UCS properties beyond the cessation of gustatory stimulation. A second factor relating to the lack of CTA may be dose level.

The results of Experiment I indicated weight changes for the higher dosage groups which remained throughout the study. This effect appeared to accrue as the number of administrations grew. Subjects in Experiment I received may times greater the amount of TMX than those in Experiment II. Therefore, instatement of CTA may occur if dosage levels are increased to match the amount of TMX received by animals in Experiment I or if TMX was injected daily.

The decremental effects of TMX upon body weight were not observed in Experiment II, yet were so observed in Experiment I. Results of Experiment II show a fairly systematic weight increase across days. Several factors may have produced this difference. This may have occurred as a result of a larger capacity to gain weight via muscle, bone, and adiposity development since animals in Experiment II were of slightly younger age and weight. Second, animals in Experiment I received test substances through intragastric-gavage while the sub-
jects of Experiment II received test agents via intraperitoneal injection. The former route may have directly affected gastrointestinal tissue and/or produced tissue damage of associated consupptatory structures. Third, Clark and Peck (1979) assert the necessity of daily administrations of antiestrogens to produce biological effects. Thus, it may be that chronicity of the drug is a critical variable; intermittent treatments at those dose levels will not yield weight loss. Additionally, subjects in Experiment I received much more substance than animals in Experiment II. Thus, a fourth possibility is that the overall amount of the drug was relevant. Therefore, weight changes similar to those of Experiment I might have been achieved in Experiment II if larger doses had been used in Experiment II—doses large enough to equalize the total amounts administered across both studies. In summary, differences in initial age and weight, treatment regimen, dosage level, and route of administration may have been critical variables determining presence and absence of weight changes.

Experiment I showed that daily treatment of TMX resulted in differences in relative water intake among the groups. Statistical analysis indicated a main effect for days and a main effect for treatments. Also, these data suggest that the 2.00 and 1.00 mg/kg TMX-treated groups consistently consumed more water than the remaining groups. This effect was not observed in Experiment II where fluid intake was not substantially affected by intermittent injection of TMX. The effect upon relative water intake in Experiment I may have occurred as a result of altered renal function.
Legha et al., (1981) has reported isolated occurrences of edema with TMX recipients. The herein reported differences in water intake may have occurred as a result of different percentages of body fluid to body mass, resulting from fluid retention induced by TMX. Thus, the animals that retained more water required less daily water to maintain homeostasis. If this hypothesis is true, it appears that the 0.5 and 0.25 TMX-treated groups retained more fluid than the 2.00 and 1.00 mg/kg TMX-treated groups.

Another explanation for the differences in relative water intake is that weight changes may have led to these differences. Specifically, the animals may have consumed approximately the same volume of water each day throughout the study, and weight loss alone resulted in the difference in relative water intake values. As stated above, it appears that the 2.00 and 1.00 mg/kg groups consumed more water than the 0.25 and 0.50 mg/kg groups. The 2.00 and 1.00 mg/kg groups also lost weight while the 0.25 and 0.50 mg/kg groups did not. Thus, higher relative water intake values for the 2.00 and 1.00 mg/kg TMX-treated groups could be a result of weight decrements alone and not because of TMX-altered fluid consumption. Analysis of absolute water intake may lend further understanding of TMX's effect upon water consumption.

The data relating TMX to wheel running did not show significant differences when mean occurrences of these behaviors were compared to control group data and when between-group comparisons were made (Figure 3). Gentry, Wade, & Roy (1976) have demonstrated inhibitory effects of antiestrogen on estradiol-induced enhancement of...
wheel running. Additionally, Leshner (1971) hypothesized a body weight regulatory function of wheel running. Leshner (1971) suggests that spontaneous wheel-running activity may decline as a function of lowered systemic ketone and fat levels and increase as a result of elevations in ketone and fat levels. It stands to reason that the TMX-related weight reductions noted herein may have resulted in less wheel running. However, as stated above, no such effects were observed.

Daily treatment of TMX did not result in significant differences in drinking, feeding, grooming, and general activity. The lack of behavioral differences between groups may be related to the time of observation. Behavioral assessment occurred 30 minutes following intragastric-gavage administration. It is plausible that behavioral alteration may have occurred much later as a result of delayed effects due to slow absorption rate, for instance. It is also possible that large group variability may have masked significant difference. The observed high variability for the behavioral assessment may be reduced in future studies by increasing the durations and frequencies of observation or by comparing pre- and post-drug effects upon behavior.

In summary, tamoxifen citrate is frequently employed for treatment of estrogen-positive breast neoplasms (Legha et al., 1978). Tamoxifen is an agent of choice because of its efficacy in reducing tumor growth (Katzenellenbogen et al., 1984; Legha et al., 1981; Lerner et al., 1976; and Wilson et al., 1982) and low incidence of debilitating side-effects when compared to similar agents; (Cole, Jones, & Todd, 1971; Heuson, 1976; Roberts et al., 1876). However, recipients
can experience nausea and vomiting, hypercalcemia, hematological changes, and depression and confusion. Experiment I assessed TMX effects upon relative water intake, body weight, and several behaviors. The results indicate that TMX produced an effect upon when administered daily. This effect upon body weight was hypothesized to occur as a result of an estrogen agonist property of TMX involving estrogen receptor sites located in the hypothalamus, anterior pituitary, liver, and adipose tissue which regulate food consumption and body mass. Differences in relative water intake were hypothesized to result from altered renal function or weight loss alone, without substantial changes in fluid intake. Neither weight loss nor differences in fluid intake were observed when TMX was delivered intermittently. Finally, TMX did not function as an effective UCS when potential CTA effects were assessed. These results lend further evidence regarding tamoxifen's safety, since a general lack of significant behavioral toxicities was observed.


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