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KU-32 Prevents 5-Fluorouracil Induced Cognitive Impairment

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Abstract

Chemotherapy induced cognitive impairment (i.e. Chemobrain), involves acute and long-term deficits in memory, executive function, and processing speed. However, animal studies investigating these cognitive deficits have had mixed results. Chemotherapy treatment such as 5-Fluorouracil (5-FU) breaks down myelin integrity corresponding to hippocampal neurodegenerative deficits and mitochondrial dysfunction. There is little evidence, however, on pharmacological treatments that may target mitochondrial dysfunction. Using a differential reinforcement of low rates (DRL) task combining spatial and temporal components, the current study evaluated the preventative effects of the pharmacological agent KU32 on the behavior of rats treated with 5-FU (5-FU+Saline vs. 5FU+KU32). DRL performance was analyzed the day after the first set of injections (D1), the day after the second set of injections (D7) and the last day of the experiment (D14). The 5FU+KU32 group had earned significantly more reinforcers on the DRL task at D7 and D14 than the 5FU+Saline group. Further, the 5FU+KU32 group showed significantly better temporal discrimination. The 5FU+KU32 alone showed within-group improvement in temporal discrimination from D7 to D14. No significant differences were observed in spatial discrimination or amount of responding were observed between the groups or as a whole across treatment points. Future implications are discussed.

Keywords

chemobrain; cognitive impairment; temporal discrimination; Post-chemotherapy cognitive impairment (PCCI)

1.0 Introduction

Chemotherapy-induced cognitive impairment, commonly referred to as chemobrain, affects between 17–75% of chemotherapy recipients (1). Human participants show deficits in memory, executive function, and processing speed (2). Deficits in spatial memory, spatial learning, and spatial recognition tasks tend to occur with rodents (3, 4), although results are mixed (4, 5). Unfortunately, there is little existing communication between human and

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animal chemobrain research (4, 6), thus obscuring findings and hampering the potential for effective treatment.

Another barrier to effective treatment is the dearth of animal studies evaluating multi-dimension tasks similar to those used in human studies (4, 6). Using multiple dimensions in behavioral tasks may more reliably produce frontal cortex dysfunction in rodents (7, 8). Often, operant procedures are used to incorporate multiple dimensions of behavior, however, there are only a few examples in the chemobrain literature (3, 7). Incorporating multiple dimensions into behavioral tasks (e.g. spatial and temporal) using operant procedures may increase the cognitive difficulty of the paradigm and enhance opportunities to isolate behavioral parameters. Further, operant tasks may more closely resemble functional characteristics of human behavioral paradigms and real world cognitive processes (6). Operant paradigms may allow for more robust independent variable parameters in conjunction with the ability to link different behavioral parameters to corresponding neurochemical processes.

There are no effective treatments for chemobrain at this time, in part because the underlying mechanism of chemobrain is unknown (9, 10). The etiology of chemobrain is often investigated using the drug 5-fluorouracil (5-FU) because 5-FU is one of the most commonly used chemotherapeutic agents in treatment (11). Exploratory treatments such as physical activity (12), cognitive training (13), and pharmacological agents (14) have shown promise but results have been mixed (4, 15). Existing evidence suggests 5-FU causes degeneration of myelin integrity in animal subjects (16) and that this breakdown (e.g. demyelinated axons in the corpus callosum) corresponds to hippocampal neurodegenerative deficits in white matter that progressively grow worse over time. Proposed mechanisms are oxidative stress (17), mitochondrial dysfunction (18) and hippocampal neurodegeneration (19, 20).

The small molecule drug KU-32 may provide neuroprotective effects during 5FU treatment. KU-32 is a C-terminal inhibitor of the molecular chaperone of heat shock protein 90 [(Hsp 90) (21)], and has neuroprotective effects on electrophysiological, bioenergetic, and morphologic systems related to diabetic neuropathy (22, 23). KU-32 repairs mitochondrial dysfunction preventing myelin degradation, an underlying condition of a variety of neurodegenerative disorders (10). The drug produces a series of reactions initiated by inherent ATP and molecular chaperones Hsp90 and Hsp70 in order to induce a cytoprotective heat shock response. KU-32 decreases neurodegeneration through multiple related mechanisms (23, 24) and may protect cells from a variety of damaging mechanisms induced by 5-FU. One purpose of this study was to determine if KU-32 + 5-FU would result in neuroprotective effects as observed in a multi-dimensional evaluation of cognitive functioning.

Operant research utilizing differential reinforcement of low rates (DRL) of responding in conjunction with a spatial discrimination task may be an ideal avenue to incorporate multi-dimensional components (i.e. spatial and temporal) in an animal chemobrain paradigm. DRL procedures require subjects to wait a minimum amount of time between successive responses to receive reinforcement (25). A target response that occurs prior to the end of the

interval resets the interval. Such a procedure may allow for particularly sensitive measurement of drug-related treatments, as the animal must simultaneously incorporate timing and spatial skills to earn food rewards. The current study therefore evaluated the protective effects of KU-32+5FU in comparison to 5FU alone using a group design across three treatment points. Multiple time points were used to compare more acute versus longer-term effects within and across groups.

2.0 Method

2.1 Subjects

Eleven Wistar rats from Charles River (Raleigh, NC) were maintained on a 22-hr deprivation schedule. Rats earned food pellets (45mg, Bio-Serv, Frenchtown, NJ) during 1-hr experimental sessions and then received ad libitum food for the remainder of the 2-hr access period beginning approximately 10 minutes after session. Rats were housed and fed in pairs but were monitored and fed individually in cases wherein dominance relations developed. The rats were 5 months old at the beginning of the experiment, had previous experience earning food on schedules of reinforcement, water was freely available in the home cages, and cages were located in a colony room with a 12h:12h light–dark cycle. Sessions occurred during the light phase of this cycle. All procedures were in accordance with the guidelines established by the University of Kansas Institutional Animal Care and Use Committee.

2.2.1 Apparatus—Behavioral sessions were conducted in standard MedPC operant chambers (30.5cm long, 24.1cm wide, 29.2cm high; Med Associates, Inc., St. Albans, VT) illuminated by 28-V houselights centered on the back wall (26.7cm from the floor). Centered on the front wall, 1cm above the floor grid was a pellet receptacle (3cm×4cm) into which a pellet dispenser could dispense grain-based pellets. On the curved, rear wall were five side-by-side, nose-poke access openings (2.54cm×2.54 cm), each illuminated by a cue light and featuring infrared response recording. Nose-poke openings were 2.54cm apart and 2cm from the floor. Chambers were housed in sound attenuating cabinets with white noise fans.

2.2 Procedure

Behavioral testing was performed a minimum of six out of every seven days, at approximately the same time each day, for four weeks. Pre-training was not conducted because rats had previous experience with schedules of reinforcement. Rats experienced 12–14 consecutive sessions prior to the experimental phase and received saline intraperitoneal (i.p.) injections and saline oral gavage administrations immediately following behavioral sessions. For the experimental phase, rats were randomized into one of two groups (KU32+5-FU or SAL+5FU). The experimental phase for each rat began with its first administration of 5-FU and KU32/SAL. A second administration occurred one week later.

2.2.1 Behavioral Testing—Rats began behavioral sessions on a differential reinforcement of low-rate 20-second schedules of reinforcement (DRL20). Food pellets were contingent on successive nose-poke responses in the center nose-poke opening (NP3) with inter-response times (IRTs) greater than or equal to 20-s. A nose-poke response with an IRT

less than 20-s reset the interval, such that the IRT between the resetting response and the subsequent response needed to be 20-s in order to earn a reinforcer. Nose-poke responses on the other four nose-poke openings were recorded but did not reset the interval or earn reinforcement. DRL20 schedules moved to DRL40 schedules if rats met stability criteria. Stability occurred if the mean reinforcers earned over the first 3 sessions (of the final 6 sessions) and the last 3 sessions did not deviate from the mean reinforcers for all 6 sessions by more than 10% and there was no visual evidence of a monotonic trend. The DRL40 schedule was identical to the DRL20 schedule but the minimum IRT was 40s. Nine of the 11 rats met stability criteria prior to the treatment phase and consequently were exposed to one day of the DRL-40s schedule prior to treatment.

2.2.3 Drug Administration—All injections took place within two hours following behavioral sessions. All rats received an i.p. saline injection and an oral gavage-administered dose of saline twice (one week removed) prior to the treatment phase to reduce the potential for vehicle-specific effects on behavior during the treatment phase. Rats received drug administration twice over the course of treatment, with the second administration occurring exactly one week removed from the start of treatment. 5-FU was administered by i.p. injection and KU-32 or saline control was administered via oral-gavage. Oral gavage (KU-32 or SAL) was administered immediately prior to 5-FU injections. All injections occurred after behavioral sessions for that particular day.

2.3 Data Analysis

Data were taken from the first day following the first set of injections (i.e. Day 1), the first day following the second set of injections (i.e. Day 7), and exactly one week following the second set of injections (Day 14). To derive overall DRL performance, reinforcers at each time point for each rat were summed and then averaged for each group. For measurement of temporal discrimination, total NP3 responses was divided by total reinforcers at the mean was calculated at each time point for each group. Two-way ANOVAs were used to compare both reinforcers earned and temporal discrimination. Sidak's multiple comparison tests were used as needed to test individual time point differences at treatment time points (i.e. Day 1, Day 7, & Day 14).

Level of responding and degree of spatial discrimination across the treatment phase (i.e. D1, D7, & D14) were evaluated. Level of responding was calculated by taking mean nose-pokes in relation to each nose-poke target for both groups at each time point. Degree of spatial discrimination was measured by taking the average nose-pokes data to plot Gaussian functions for each group and each time point. The Gaussian function can be summarized into one index measure by taking the width of the curve at the points when the y-axis is half of the maximum height (i.e., full-width-half-maximum, FWHM):

$$FWHM=2 \sqrt{2\ln 2} \sigma \cong 2.355 \sigma$$

The standard deviations of Gaussian distributions for each group per time point were averaged and multiplied by 2.355 to derive FWHM (i.e. degree of spatial discrimination). Two-way ANOVA tests were used for both level of responding and degree of spatial

discrimination to evaluate differences between the two-groups and across the three time points. Sidak's multiple comparison tests were used as needed to test individual time point differences at treatment time points.

3.0 Results

Figure 1 shows mean reinforcers earned across the first, seventh, and fourteenth treatment experimental sessions for rats in the 5-FU+KU32 group (squares) and 5-FU+Saline (circles). A Two-way ANOVA revealed significant main effects of group ($F=10.5$, $p=0.003$) and non-significant effects for time point ($F=0.286$, $p=0.75$). A significant interaction effect ($F=3.92$, $p=0.029$), however, was found between group and time point. Post-hoc comparisons revealed that the 5-FU+KU32 group earned significantly more reinforcers than the 5-FU+Saline group at the second ($p=0.033$) and third time ($p=0.031$) points of treatment (i.e. immediately following injection-2 and one week following injection-2).

Figure 2 shows mean reinforcers earned divided by total NP3 responses across the first, seventh, and fourteenth treatment experimental sessions for rats in the 5-FU+KU32 group (squares) and 5-FU+Saline (circles). There were significant main effects of group ($F=13.3$, $p=0.001$), time point ($F=3.5$, $p=0.046$), and the interaction of group and time point ($F=5.9$, $p=0.008$). Post-hoc comparisons revealed that those rats in the KU32+5FU group were significantly more efficient in their reinforcers/total NP3 responses ($p=0.0001$). There were no other significant differences between the groups at Day 1 or Day 7. Within groups, there were no significant differences across time points for the 5FU group, however, those rats in the 5FU+KU32 group were significantly more efficient in their reinforcers/NP3 responding during their D14 session than either Day 1 ($p=0.002$) or Day 7 ($p=0.002$).

Figure 3 shows mean nose-poke responses per nose-poke target (x-axis) for both 5FU (open squares) and 5FU+KU32 rats (open circles) for Day 1 (top panel), Day 7 (middle panel), and Day 14 (bottommost panel). Gaussian curves are fit to each group's mean responses across each target at a given time point (i.e. D1, D7, and D14). To determine level differences in responses between the two groups, Two-way ANOVAs comparing mean responses across nose-poke targets were used. Significant differences were only observed between the groups on D14 such the 5FU+Saline group exhibited more responses than the 5FU+KU32 group. A post-hoc comparison revealed that the only significant difference in mean responses between the groups on D14 was at NP3 ($p=0.006$). An index of the Gaussian gradient (FWHM) was derived for each group at each time point. A two-way ANOVA revealed non-significant findings for group ($p=0.23$) and time point ($p=0.26$).

4.0 Discussion

The current study is the first to our knowledge to demonstrate maintained prevention of chemotherapy induced cognitive impairments in a temporal-spatial discrimination task, and to do so using a pharmacological treatment. Rats from the 5FU+KU32 group and the 5FU+Saline group exhibited nearly identical overall DRL performance on all measures immediately following the first set of injections (D1). Rats who received 5FU+KU32, however, earned significantly more reinforcers than the 5FU+Saline group when evaluated

immediately following their second set of injections (D7) and one week following their second set of injections (D14). In addition, within the 5FU+KU32 group, rats earned significantly more reinforcers at D14 than at D7. Similarly, rats who received 5FU+KU32 significantly improved their temporal discrimination from D7 to D14 when compared to the 5FU+Saline group. Lastly, there were no significant differences in the spatial discrimination between the two groups, however, the 5FU+Saline group. There are three further points we wish to note.

First, the current study on chemobrain is the first to our knowledge to use simultaneous temporal and spatial requirements within an operant procedure. The current study adds to the small literature on temporal deficits in animals induced by 5FU by using a DRL schedule (20, 26). Using operant paradigms to evaluate chemobrain in rodents may allow for more consistent and sensitive procedures in part because the use of positive reinforcers can allow for more congruence in the functional similarities between tasks (6). Further, operant tasks may be more likely to elicit frontal cortex mechanisms (7, 20, 26) found to be relevant in human studies on chemobrain. Operant procedures also preclude the necessity to use paradigms that include stressful events (e.g. spatial memory water-maze test) which may unduly influence dependent variables (6).

Second, the current study provides additional utility by measuring behavioral performance prior to and throughout the multiple drug treatments. Specifically, demonstrating stable baseline performance in conjunction with measurement of behavior throughout the course of the current treatment matches human testing contexts wherein preexisting skills are tested (4, 6). In contrast, animals in the chemobrain literature are typically exposed to novel behavioral tasks after prolonged exposure to a chemotherapy regimen, thus further separating the functional characteristics of performance across human and animal paradigms.

There are limitations of the current study to note. First, there was no untreated group in the current study, therefore, statements regarding KU32 promoting the recovery of DRL performance cannot be made at this time. Second, the sample size of the two groups were relatively small for the 5FU+Saline group (n=5) and 5FU+KU32 group (n=6), however, significant effects in overall performance were observed following the second treatment and even larger differences were observed one week from the second treatment. Further, the current study evaluated behavior in the experimental phase for longer than is typical and variability within groups was considerably small (see Figures 1–3). Lastly, two rats in the KU32+5FU group did not experience DRL40 schedules; however, this did not affect the statistical significance of any tests. Future research, however, implement a baseline phase, followed by a 5FU+Saline phase for all rats, and then implement the experimental phase of the present experiment.

5.0 Conclusion

The current study found that the novel drug KU32 prevented temporal discrimination deficits induced by 5FU following the second injection and one week following the second injection. We further showed that there were no differences in spatial discrimination

performance between the two groups at any point or in general across treatment points. Whether relative differences between the two groups exemplifies recovery effects is unknown at this time, however, the current behavioral paradigm may allow for improved sensitivity to measure treatment effects derived from novel pharmacological agents such as KU-32.

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Fig. 1.
Mean number of reinforcers earned (y-axis) for the 5FU + Saline group (circles) and 5FU + KU32 group (squares) across D1, D7, and D14 (x-axis).

Fig. 2.
Mean reinforcers earned divided by total NP3 responses (y-axis) across D1,D7, and D14 (x-axis) for rats in the 5-FU + KU32 (squares) and 5-FU + Saline (circles) groups.

Fig. 3.

Top, middle, and bottom panels represent D1, D7, and D14 respectively. Mean responses (y-axis) per nose-poke target (axis) are plotted for 5-FU + KU32 rats (squares and dotted gradient) and 5-FU + Saline (circles and non-dotted gradient).