Possible enhancement of long-term fear conditioning performance by cisplatin administration in rats

Possível facilitação do desempenho em condicionamento aversivo por administração de cisplatina em ratos

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Abstract

Background: neurocognitive deficits associated with chemotherapy represent an increasing concern, and the development of animal models to investigate chemotherapy-induced alterations in memory is warranted.

Aims: to examine the effects of systemic injection of cisplatin on formation of fear-motivated memory in rats.

Methods: male Wistar rats were given an intraperitoneal (i.p.) injection of saline or cisplatin followed by inhibitory avoidance (IA) training. Memory retention was tested 1 and 7 days after training. Control experiments using an open field were carried out to confirm the specificity of the cisplatin-induced alteration in IA performance.

Results: cisplatin induced a unexpected enhancement of IA performance measured 7 days after drug injection and training. Control experiments suggested that the effect could not be attributed to sensorimotor alterations or toxic effects.

Discussion: the findings are discussed in the light of previous preclinical evidence that cancer chemotherapy can, under some conditions, lead to memory enhancement.

Keywords: cisplatin; cancer chemotherapy; memory; cognitive function; animal models

Resumo

Introdução: é crescente a preocupação com disfunções cognitivas associadas ao uso de quimioterapia para tratamento de câncer. É necessário o desenvolvimento de modelos experimentais que permitam avaliar alterações na memória induzidas por antineoplásicos.

Objetivos: avaliar os efeitos da administração sistêmica de cisplatina sobre a formação de memória motivada por medo em ratos.

Métodos: ratos Wistar machos receberam uma injeção intraperitoneal (i.p.) de solução salina (controles) ou cisplatina antes de uma sessão de treino em esquiva inibitória (EI). A retenção da memória de EI foi avaliada em testes realizados 1 e 7 dias depois do treino. Experimentos controle em um campo aberto foram usados para confirmar a especificidade das alterações induzidas por cisplatina no desempenho em EI.

Resultados: a administração de cisplatina levou a um inesperado aumento do desempenho de EI medido 7 dias após o treino. Os experimentos controle indicam que esse efeito não deve estar relacionado à toxicidade ou alterações em funções sensoriais e motoras.

Discussão: os resultados são discutidos em relação a estudos prévios que indicam que, em algumas condições, quimioterápicos antineoplásicos podem levar a uma facilitação da memória.

Palavras-chave: cisplatina; quimioterapia anticâncer; memória; função cognitiva; modelos animais

Cancer chemotherapy has been increasingly associated with neurocognitive deficits, which can include subtle or severe memory impairment (1,2). This chemotherapy-induced cognitive dysfunction, which has been called "chemobrain" or "chemofog", has been described primarily in breast cancer survivors (3), but is also an increasing concern in adults with other types of cancer (4), as well as in childhood cancer survivors (5,6). Research investigating the neural basis of "chemobrain" is required for the development of preventive and therapeutic interventions to alleviate this problem.

In recent years, several studies have aimed to characterize cognitive dysfunction in cancer survivors. However, these studies are often confounded by the possible influence of factors other than chemotherapy that are also likely to affect cognition (e.g., the malignancy itself, radiotherapy, opioid analgesics, corticosteroids). Therefore, the development of animal models to investigate chemotherapy-induced memory dysfunction is warranted (2,3,7,8). Recent studies using different treatment protocols in rodents have shown memory-impairing effects of chemotherapeutics including cyclophosphamide (9,10), doxorubicin (10,11), methotrexate (12-14), 5-fluorouracil (12,14), adriamycin, and cytoxan (15). Although anticancer therapy with the platinum compound cisplatin has been related to cognitive deficits in patients (7), previous studies have not examined its effects on memory in animal models. In the present study, we aimed to verify the effect of acute systemic administration of cisplatin on fear memory formation in rats.

Methods

Animals

Eighty-three adult male Wistar rats (80-97 days of age, 250-370 g) from the local university center for breeding and supply of experimental animals (CREAL-UFRGS) were housed five to a cage in a temperature-controlled colony room with food and water available ad libitum, and maintained on a 12-h light/dark cycle (lights on at 7:00 A.M.). Experimental procedures were conducted during the light phase of the cycle between 10:00 and 17:00. All procedures were conducted in accordance with the NIH Guide for Care and Use of Laboratory Animals (NIH publication No. 80-23 revised 1996), and the experimental protocols were approved by the institutional animal care committee (GPPG-HCPA 07-009). All efforts were made to minimize the number of animals used and their suffering.

Inhibitory avoidance conditioning

We used inhibitory avoidance (IA) to assess fear memory in rats treated with cisplatin. IA is a widely used rodent model of fear conditioning in which the animals learn to associate a location in the training apparatus with an aversive stimulus (footshock). IA training and memory retention test procedures were carried out as previously described (11,16). The IA apparatus was a 50 X 25 X 25-cm acrylic box (Albarsch, Porto Alegre, Brazil) whose floor consisted of parallel caliber stainless steel bars (1 mm diameter) spaced 1 cm apart. A 7-cm wide, 2.5-cm high platform was placed on the floor of the box against the left wall. On the training trial, rats were placed on the platform and their latency to step down on the grid with all four paws was measured with an automatic device. Immediately after stepping down on the grid, rats received a 0.5 mA, 2 s footshock and were removed from the apparatus immediately afterwards. Memory was assessed in retention test trials carried out 1 and 7 days after drug injection and training. The retention test trial was procedurally identical to training, except that no footshock was presented. Step-down latencies (s) on the retention test trial (maximum 180 s) were used as a measure of IA retention. In a control experiment designed to verify whether cisplatin by itself produced alterations in the time rats spent on the platform, rats were put on the platform and allowed to step down in the absence of footshock, then again placed on the platform 1 and 7 days after the first exposure, and the animals' latencies to step-down were recorded.

Open field behavior and habituation

In order to verify whether cisplatin affected sensorimotor function or other behavioral parameters, an open field behavior assay was used to evaluate locomotor and exploratory activity and anxiety in a separate set of rats as previously described (11). The open field behavior test was carried 7 days after cisplatin or saline injections. The open field was a 50 X 25-cm arena, surrounded by 50-cm high walls, made of brown plywood with a frontal glass wall. The floor of the open field was divided into 12 equal squares by black lines. Rats were put in the open field, placed on its left rear quadrant, and left to freely explore the arena for 5 min. Crossing of the black lines and rearings performed during arena exploration, and latency to start locomotion were used as measures of locomotion, exploration, motivation, and anxiety. Habituation to the open field was measured 24 after the first exploration session by allowing the animals to again explore the arena for 5 min. The number of rearings performed was recorded and used as a measure of habituation.

Drug treatments

Twenty minutes before IA training, or 7 days before open field exploration, rats were given a single intraperitoneal (i.p.) 0.1 ml/kg injection of saline (NaCl 0.9%, control group) or cisplatin (0.1, 0.3, or 1.0 mg/kg) dissolved in saline. The doses of cisplatin were chosen on the basis of previous studies (17,18). It has been proposed that the acute administration of high doses of chemotherapeutic agents is better for the initial

characterization of cognitive deficits in animal models than the use of chronic or sub-chronic treatment protocols (2).

Statistics

Data are shown as mean + standard error of mean (S.E.M.). Because a ceiling is imposed in IA retention test trials, nonparametric statistics is more adequate for the analysis of IA data. Thus, comparisons of IA performance among groups were done with a Kruskal–Wallis analysis of variance followed by Mann–Whitney U tests, two-tailed when necessary (10,11,16). Results for open field behavior were analyzed with a one-way analysis of variance (ANOVA) as previously described (11). In all comparisons, P<0.05 was considered to indicate statistical significance.

Results

Inhibitory avoidance conditioning

Results for the IA conditioning are shown in Fig. 1. Administration of cisplatin 20 min before training did not affect the animals' performance in the training (H=2,87, df=3, P=0,41) or 1-day retention test trial (H=2,28, df=3, P=0,52). Rats treated with cisplatin at either 0.1 or 1.0 mg/kg showed higher step-down latencies when tested 7 days after training compared to control rats (both Ps<0.05), suggesting a possible memory-enhancing effect. The intermediate dose of cisplatin (0.3 mg/kg) did not affect 7-day IA performance (P=0.18 compared to controls).

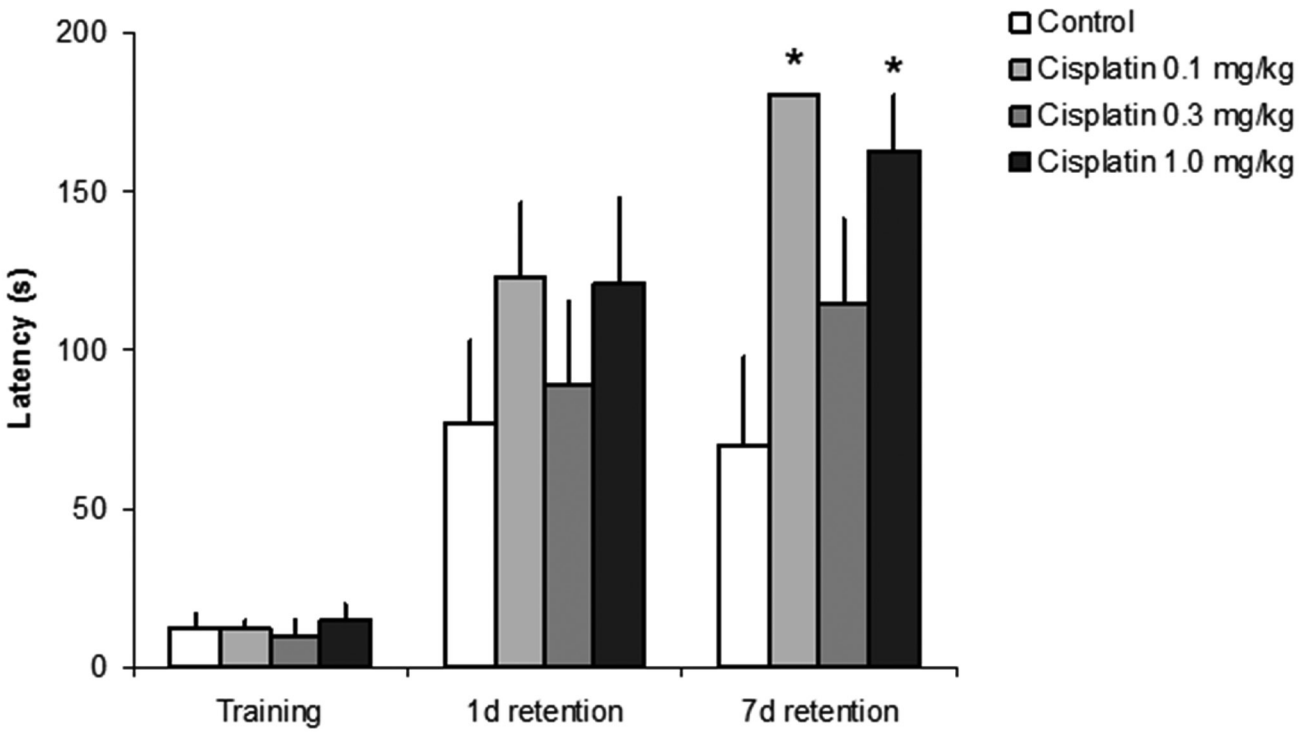

Figure 1: Inhibitory avoidance (IA) performance of rats given a single systemic injection of saline (controls, n=9) or cisplatin at 0.1 (n=8), 0.3 (n=10), or 1.0 mg/kg (n=9) 20 min before training. Retention test trials were carried out 1 or 7 days (d) after training. * P<0.05 compared to control rats given saline.

The higher latency displayed in the IA test 7 days after cisplatin injection, observed in the first experiment, could be related to a late enhancement of memory retention. Alternatively, cisplatin could have produced long-lasting toxic effects, or alterations in locomotor activity, motivation, anxiety or sensorial perception, which could account for the higher time latencies taken to step-down during IA test. We thus carried out a control experiment to verify whether cisplatin injection by itself could produce alterations in the time rats spent on the platform 7 days after training that could be explained by toxicity or sensorimotor effects. Rats were given an injection of cisplatin at the dose of 1.0 mg/kg and 20 min later they were placed on the platform and allowed to step-down to the floor. No footshock was given. The animals were placed on the platform again 1 and 7 days after the first session. Cisplatin did not affect behavior in the IA box in this protocol. Mean + SEM latencies (s) in the test carried out 7 days after the first exposure were: control, 6.4+1.6; cisplatin, 9,6+2.7, n=7 rats per group; P=0.26). The result suggests that the higher latency observed 7 days after training in rats given cisplatin was not caused by toxic, sensorimotor, or other nonspecific effects of cisplatin.

Open field behavior and habituation

In order to further control for possible effects of cisplatin on performance that could be unrelated to memory, we carried out an open field behavior test 7 days after cisplatin injection in a separate set of rats as a control experiment to verify whether cisplatin affected behavioral parameters other than memory. Results are shown in Table 1. Cisplatin at the same doses used in the IA experiment did not affect any parameter measured in the open field test [crossings, $F(3,42)=1.64$, $P=0.20$; rearings, $F(3,42)=0.11$, $P=0.95$; latency to start locomotion, $F(3,42)=0.57$, $P=0.64$]. In addition, there was no significant difference among groups in an open field habituation session carried out 1 day after the first exploration session [$F(3,42)=2.81$, $P=0.06$]. These results indicate that cisplatin did not cause visible toxic effects influencing behavior or gross alterations in locomotion, motivation, anxiety, sensorial function, or non-associative memory 7 days after injection.

Table 1: Open field behavior and habituation in rats treated with a single systemic injection of saline (controls) or cisplatin (0.1, 0,3, or 1.0 mg/kg) 20 min before arena exploration.

Open field behavior				
Group	**n**	**Crossings**	**Rearings**	**Latency (s)**
Control	13	61.42 + 7.70	33.08 + 3.61	12.73 + 2.91
Cisplatin 0.1 mg/kg	12	58.00 + 8.42	36.18 + 4.28	6.08 + 1.37
Cisplatin 0.3 mg/kg	11	61.18 + 5.36	38.55 + 2.59	6.60 + 1.12
Cisplatin 1.0 mg/kg	11	56.60 + 6.92	33.27 + 2.85	9.95 + 3.44
Habituation				
Group	**n**		**Rearings**	
Control	13		21.08 + 3.85	
Cisplatin 0.1 mg/kg	12		21.95 + 3.30	
Cisplatin 0.3 mg/kg	11		32.32 + 3.16	
Cisplatin 1.0 mg/kg	11		19.55 + 3.04	

Discussion

Our results indicate that rats given an acute systemic administration of cisplatin showed an enhancement in IA performance measured 7 days later. Performance during training and the 1-day retention test trial were not affected by cisplatin, indicating that the pretraining drug administration did not affect shock sensitivity, attention or motivation during training, or short-term memory. In addition, our control experiment using exposures to the IA apparatus in the absence of footshock, as well as an open field behavior test, showed that rats given cisplatin showed normal behavioral performance and habituation to a novel environment 7 days after training. Thus, it is unlikely that the enhanced latency observed in the 7-day IA test was attributable to a nonspecific behavioral alteration such as changes in pain sensitivity, exploratory behavior, locomotion, attention, sensorial perception, anxiety, or short-term memory processing. One possible interpretation for the results of the IA experiment is that cisplatin produced an enhancement of memory retention 7 days after drug injection and learning. However, because cisplatin is highly toxic and there is no known mechanism that could account for a memory-enhancing effect of cisplatin, this interpretation should be taken with caution, and further experiments using other memory tasks and behavioral parameters are required to verify whether cisplatin can enhance cognitive function.

The lower and higher doses of cisplatin used produced the enhancement of IA performance, whereas the intermediate dose had no effect. This inverted U–pattern of dose-response is a common feature of drugs that act by regulating memory storage in conditioning models like IA (19, 20). Thus, the dose-response pattern for the IA experiment is consistent with the possibility that cisplatin might modulate neurochemical mechanisms influencing memory formation.

Most previous studies examining the effects of cancer chemotherapy on memory in rodent models have found that chemotherapy impairs memory (9–15). We have previously shown that acute systemic administration of cyclophosphamide and doxorubicin impaired IA memory in mice and rats, respectively (10,11). However, acute administration of tamoxifen had no effect on retention of a nose-poking response except at behaviorally toxic dose (21), and a recent study found no alterations in recent or remote spatial memory in mice treated with cytosine arabinoside (Ara-C) for five days (22). Sharpe et al. have recently reported the interesting finding that a systemic injection of oxaliplatin did not affect retention or extinction of fear conditioning, but impaired the reinstatement of fear after extinction, in rats (23).

Although unexpected, the present results are not the first evidence suggesting memory enhancement in rodents after treatment with a cytotoxic chemotherapeutic agent. Lee et al. (24) have found that rats receiving injections of cyclophosphamide or 5-fluorouracil every 4 weeks for a total of 18 weeks showed enhanced spatial memory assessed in a water maze between 8 and 10 weeks of recovery from treatment. The enhanced memory associated with chemotherapy in these rats was accompanied by increased long-term potentiation, a cellular mechanism of synaptic plasticity proposed to underlie memory formation. These findings indicate that cancer chemotherapy can lead to neural changes that stimulate the neural mechanisms of memory formation and result in enhanced cognitive function.

The findings reported by Lee et al. (24) described above suggest that, at least under some experimental conditions, cancer chemotherapy can enhance rather than impair behavioral performance neural plasticity and cognitive function in rodent models. Thus, it is possible that the present findings are related to a long-term enhancement of IA memory resulting from cisplatin injection. Further experiments should verify whether the enhanced performance observed in the present report can be explained by behavioral alterations other than memory retention, characterize the experimental conditions under which these effects occur, examine the underlying neural mechanisms, and determine whether these findings are limited to preclinical models or have any implications for alterations of cognitive function in patients treated with chemotherapy.

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