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Chemotherapy-induced long-term alteration of executive functions and hippocampal cell proliferation: Role of glucose as adjuvant

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ABSTRACT

In patients, cancer and treatments provoke cognitive impairments referred to "chemofog". Here a validated neurobehavioral animal model, the unique way to explore causal direct links between chemotherapy used in clinical practices and brain disorders, allowed investigation of the direct long-term impact of colo-rectal cancer chemotherapy on cognition and cerebral plasticity. Young and aged mice received three injections every 7 days during 2 weeks of 5-fluorouracil either alone (5-FU, 37.5 mg/kg) or in combination with oxaliplatin (3 mg/kg) or with glucose (5%). The long-term effects (from day 24 to day 60) of chemotherapy were tested on emotional reactivity, learning and memory, behavioral flexibility and hippocampal cell plasticity.

5-FU (in saline)-treated aged and also young mice exhibited specific altered cognitive flexibility and behavioral hyper-reactivity to novelty, whereas the combination 5-FU (in saline)/oxaliplatin (in glucose) did not provoke any cognitive dysfunction. We thus observed that glucose counteracted 5-FU-induced altered executive functions and hippocampal cell proliferation *in vivo*, and protected neural stem cells *in vitro* from toxicity of 5-FU or oxaliplatin. In conclusion, these data suggest that the lasting chemotherapy-induced selective impairment of executive functions, whatever the age, and associated with a reduced number of hippocampal proliferating cells, can be counteracted by co-administration with glucose.

1. Introduction

Cancer survivors treated with anticancer agents display long-term cognitive impairments referred as "chemobrain" or "chemofog", including deficits of information processing speed, attention and concentration, verbal and visual memory, and mental flexibility, which can dramatically affect quality of life, return at work or autonomy of elderly (Vardy and Tannock, 2007), even several years after the treatment completion (Ahles et al., 2002; Schagen et al., 1999).

The majority of studies dealing with the cognitive dysfunctions induced by anticancer agents concerns patients younger than 65 years (Brezden et al., 2000; Collins et al., 2009; Schagen et al., 1999; van Dam et al., 1998) whereas cancers are most frequent in patients older than 65-years old. In cancer out-patients older than 65 years (Minisini et al., 2008), the chemotherapy group showed no cognitive decline from baseline (before treatment) to T6 (6 months after

Abbreviations: 5-FU, 5-fluorouracil; aCSF, artificial cerebral spinal fluid; Area 35 PRh, area 35 of the perirhinal cortex; Area 36 PRh, area 36 of the perirhinal cortex (ectorhinal cortex); BLa, basolateral amygdaloid nucleus; BrdU, 5-bromo-2-deoxyuridine; CA1, field CA1 of the hippocampus; CA3, field CA3 of the hippocampus; CO, cytochrome oxidase; DG, dentate gyrus of the hippocampus; DP, dorsal peduncular cortex; DS, dorsal subiculum; EPC, endothelial precursor cell; FrA, frontal association cortex; FST, forced swim test; GCL, granule cell layer; Glu, glucose; Ins, insulin; i.p., intraperitoneal; La, lateral amygdaloid nucleus; LD, laterodorsal thalamic nucleus; LSD, least significant difference; M, motor cortex; MD, mediodorsal thalamic nucleus; ML, molecular layer; MTX, methotrexate; NE, north-east; NSC, neural stem cell; NW, north-west; PrL, prelimbic cortex; Pir, piriform cortex; Re, reuniens thalamic nucleus; RS, retrosplenial cortex; SE, south-east; SFM, serum free medium; SGZ, subgranular zone; SVZ, subventricular zone; SW, south-west; WFI, water for injection.

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treatment completion) whereas in breast cancer patients (mean age of 70 years), subjective cognitive alterations have been reported before and 6 months after treatment (Hurria et al., 2006). Thus, it is unclear whether the low cognitive reserve of elderly would finally constitute a risk factor for chemotherapy induced cognitive deficits.

In addition to altered basal cognitive reserve and aging, factors such as stress, anxiety and depression associated with the disease announcement, the cancer itself, hormonal changes and/or inflammation are potentially involved in cancer-related cognitive troubles (Ahles and Saykin, 2007; Joly et al., 2011), but the nature and long-term impact of neurobiological mechanisms are not clearly characterized. Previous studies reported cerebral histological and functional alterations potentially associated with cognitive dysfunctions after chemotherapy. At short-term, patients exposed to chemotherapy showed a reduced volume of gray and white matter in the prefrontal, parahippocampal gyrus and precuneus correlated with altered attention/concentration and/or visual memory performances (Inagaki et al., 2007). Similarly, white matter integrity alterations in frontal, parietal and temporal brain regions (Deprez et al., 2010) or disrupted regional network characteristics in frontal, striatal and temporal areas (Bruno et al., 2012) have been correlated with cognitive impairments in breast cancer patients. Moreover, a persistent reduced gray matter density following chemotherapy has been shown in frontal and temporal cortex, thalamus and cerebellum one year after chemotherapy completion (McDonald et al., 2010), and long term electrophysiological modifications of brain activity have been recorded in chemotherapy patients (Kreukels et al., 2005; Schagen et al., 2001). Finally, a reduced rest metabolism in the frontal cortex and an altered cerebral blood flow during a short-term memory task in frontal cortex and cerebellum, appear correlated with poor memory performances in patients treated 5–10 years ago with chemotherapy (Silverman et al., 2007).

Thus, the characterization of the cognitive impairments induced by chemotherapy, the mechanisms that sustain these deficits and the use of preventive strategies are particularly important to balance the benefices and risks of the treatment, and to improve the quality of life of patients, especially elderly patients. In this context, the development of animal models is particularly useful to evaluate the direct impact of anticancer agents on cognitive functions, the influence of emotion and aging, and the underlying neurobiological mechanisms. It has been previously shown that methotrexate evoked deficits in conditioning (Madhyastha et al., 2002; Yanovski et al., 1989) and long-term alterations of spatial and novel object recognition memories, as well as decreased hippocampal cell proliferation (Seigers et al., 2008). A short delay between cyclophosphamide administration and behavioral tests, led to memory retention impairments in a step-down inhibitory avoidance-conditioning test in mice (Reiriz et al., 2006). In addition, 5-fluorouracil (5-FU), a thymidylate synthase inhibitor that blocks DNA synthesis (Benz et al., 1980; Kovach and Beart, 1989), induced cognitive impairments when administered alone (ElBeltagy et al., 2010; Fardell et al., 2012; Foley et al., 2008) or in combination with other anticancer agents (Fardell et al., 2012; Gandal et al., 2008; Walker et al., 2011; Winocur et al., 2011, 2006). One study evaluating the cognitive effect of oxaliplatin administered alone in rat indicates object recognition memory impairments (Fardell et al., 2012). However, the variety of protocols used, the number of anticancer agents, animal species, age, treatment doses, behavioral tasks, and mainly the delay between treatment and cognitive assessments, have led to discrepancies on conclusions about the nature of the functions altered by chemotherapy and the involvement of hippocampal cell plasticity. Indeed, chemotherapeutic agents inhibit tumoral cell proliferation but may also have adverse effects on non-tumoral cells in the brain (Dietrich et al., 2006; Rzeski et al., 2004; Seigers et al., 2008). Some studies have reported that 5-FU does not modify cell proliferation in the dentate gyrus (Mignone and Weber, 2006; Mustafa et al., 2008) whereas other studies described that 5-FU and/or methotrexate are able to provoke short or long-lasting defects of cell proliferation (Dietrich et al., 2006; ElBeltagy et al., 2010; Lyons et al., 2011; Mondie et al., 2010; Seigers et al., 2008), and increased cell death (Dietrich et al., 2006). More recently, it has been shown that the antidepressant fluoxetine and the cholinergic agent donepezil prevent 5-FU- and/or methotrexate-induced memory impairments (ElBeltagy et al., 2010; Lyons et al., 2011; Winocur et al., 2011) and that fluoxetine reverses 5-FU- or methotrexate-evoked inhibition of hippocampal cell proliferation in rodents (ElBeltagy et al., 2010; Lyons et al., 2011).

The present study explored the direct role of colo-rectal cancer chemotherapy on cognitive performances at distance of the treatment, in function of age. We also investigated the underlying biological and physiological mechanisms sustaining chemotherapy-induced potential alterations. 5-FU is one of the major drugs administered with oxaliplatin, a platinum-based drug classified as alkylating agent, and with leucovorin (FOLFOX) for the treatment of colo-rectal cancers (de Gramont et al., 2000). Indeed, a number of *in vitro* and *in vivo* studies (Cressy and Schell, 1966) validated the beneficial impact of the co-administration of oxaliplatin and 5-FU. Considering the Food and Drug Administration recommendations, oxaliplatin should be administered in 5% glucose and before 5-FU to insure oxaliplatin stability Moreover, high dose of glucose exerts a significant potentiation of the antitumor action of 5-FU *in vivo* (Kung et al., 1963; Santelli and Valeriote, 1980). Thus, we assessed the effects of the chemotherapy 5-FU (in saline or in glucose), alone or in combination with oxaliplatin (in glucose). Their impacts were tested in young (8 weeks) and aged (20 months) mice on emotional behaviors, spatial learning and memory, learning plasticity and recognition memory. Moreover, we investigated their effects on *in vivo* hippocampal cell proliferation and *in vitro* neural stem cell (NSC) survival as well as on cerebral metabolic activity in some hippocampal- and frontal-related areas.

2. Materials and methods

2.1. Animals

Young (6 weeks old) and aged (19.5 months old) male C57BL/6J Rj mice (Janvier, Le Genest Saint Isle, France) were housed under controlled standard environmental conditions of temperature (22 ± 1 °C) and light (12/12 h light/dark cycle, light on at 00:00). Animals were housed 5 by cage (Makrolon Cage: $L \times l \times H$: 35 × 19 × 13.5 cm) with water and food available *ad libitum*. Treatments administration began when mice were 8 weeks (young) or 20 months (aged) of age. The number and the suffering of animals were minimized in accordance with the French Ethical Committee and guidelines of the directive 2010/63/EU of the European Parliament and of the Council for the protection of animals used for scientific purposes. Animal manipulations were carried out under the supervision of authorized investigator (H. Castel; authorization no. 76.98 from the Ministère de l'Alimentation, de l'Agriculture et de la Pêche).

2.2. Drug administration

Treatments were administered in a volume of 10 μl/g, as one injection every 7 days for 2 weeks (total of 3 injections; Fig. 1A.a), young and aged mice were intraperitoneally (i.p.) injected with 5-FU (37.5 mg/kg body weight) (Sigma–Aldrich, Ilkirch, France) or 0.9% NaCl (saline). This dosage was chosen on the basis of the conversion from human treatment dosage, previous animal study (Foley et al., 2008) and our pilot study in which doses of 5-FU used were 37.5 mg/kg and 75 mg/kg as three injections every 7 days during 2 weeks (data not shown). As the 75 mg/kg treatment caused drastic body weight loss, we kept the dosage of 37.5 mg/kg, well tolerated by the animals. For this study, groups were set up as followed: Young Saline, $n = 13$; Young 5-FU, $n = 10$; Aged Saline, $n = 12$; Aged 5-FU, $n = 12$. In experiment 2 (Fig. 1A.b), young and aged mice received i.p. injections of oxaliplatin (3 mg/kg), diluted in a 5% glucose in water for injection (glucose/WFI) solution, or 5% glucose/WFI solution only, followed 7 h later by an injection of 5-FU (37.5 mg/kg) or saline. The dose of oxaliplatin was chosen in accordance with the dose used clinically (Gauchan et al., 2009). Oxaliplatin was administered before 5-FU to reduce