Journal of the Neurological Sciences 360 (2016) 66–71

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Cabazitaxel causes a dose-dependent central nervous system toxicity in rats

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ARTICLE INFO

Article history:
Received 6 October 2015
Received in revised form 11 November 2015
Accepted 17 November 2015
Available online xxxx

Keywords:
Cabazitaxel
Central nervous system
Neurotoxicity
Rat

ABSTRACT

Background: Chemotherapeutic agents may lead to serious neurological side effects, which in turn can deteriorate the quality of life and cause dose limiting. Direct toxic effect or metabolic derangement of chemotherapeutic agents may cause these complications. Cabazitaxel is a next generation semi-synthetic taxane derivative, which is effective in both preclinical models of human tumors sensitive or resistant to chemotherapy and in patients with progressive prostate cancer despite docetaxel treatment.
Aim: The primary aim of this study was to investigate the central nervous system toxicity of Cabazitaxel. Secondary aim was to investigate the safety dose of Cabazitaxel for the central nervous system.
Methods: A total of 24 adult male Wistar-Albino rats were equally and randomly divided into four groups as follows: *group 1* (Controls), *group 2* (Cabazitaxel 0.5 mg/kg), *group 3* (Cabazitaxel 1.0 mg/kg) and *group 4 (*Cabazitaxel 1.5 mg/kg). Cabazitaxel (Jevtana, Sanofi-Aventis USA) was intraperitoneally administered to groups 2, 3 and 4 at 0.5, 1.0 and 1.5 mg/kg (body-weight/week) doses, respectively for four consecutive weeks. Beside this, group 1 received only i.p. saline at the same volume and time. At the end of the study, animals were sacrificed and bilateral brain hemispheres were removed for biochemical, histopathological and immunohistochemical examinations.
Results: Intraperitoneal administration of Cabazitaxel has exerted neurotoxic effect on rat brain. We have observed that biochemical and immunohistochemical results became worse in a dose dependent manner.
Conclusion: Our findings have suggested that Cabazitaxel may be a neurotoxic agent and can trigger apoptosis in neuron cells especially at high doses.

1. Introduction

Neurologic side effects of chemotherapeutic agents can be serious. Neurologic complications such as acute encephalopathy, seizures, visual loss, dementia, neuropathy, vasculopathy and stroke are associated with chemotherapeutic drug treatment [1,2] These side effects can deteriorate the quality of life and can be dose limiting. Direct toxic effect or metabolic derangement of chemotherapeutic agent may cause these complications. Taxanes are well-known antitumor agents and commonly used in treatment of adult solid tumors. Cabazitaxel is a next generation semi-synthetic taxane derivative, which is effective in both preclinical models of human tumors sensitive or resistant to chemotherapy. Also Cabazitaxel is used in patients with advanced prostate cancer which exhibits progression after docetaxel treatment [3]. Phase I and Phase II studies conducted in recent past have also indicated that Cabazitaxel has antitumor activity in advanced solid tumors in context of safety profile [4,5]. While first generation taxanes, docetaxel and paclitaxel have limited ability to pass through the blood brain barrier (BBB) and penetrate into the brain, Cabazitaxel can pass the BBB and diffuse into the brain [6,7]. Interestingly, it has been reported that the neurotoxic effect of Cabazitaxel was less than other taxanes such as docetaxel and paclitaxel [8]. Although Cabazitaxel is widely used with gradually increasing rates in current clinical practice, there is not enough information about the side effects and toxicity profile of Cabazitaxel administration. The primary aim of this study was to investigate the central nervous system toxicity of Cabazitaxel. Secondary aim was to investigate the safety dose of Cabazitaxel for the central nervous system.

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http://dx.doi.org/10.1016/j.jns.2015.11.033
0022-510X/

2. Materials and methods

Study protocols and experimental methods were approved by the local Institutional Ethics Committee of Experimental Animals and the experiments were performed according to the guidelines (NIH, UCSF) on animal use. A total of 24 adult male Wistar-Albino rats weighing 260 ± 20 g were included in this study. Animals were equally and randomly divided into four groups as follows: *group 1* (Controls), *group 2* (Cabazitaxel 0.5 mg/kg), *group 3* (Cabazitaxel 1.0 mg/kg) and *group 4* (Cabazitaxel 1.5 mg/kg). Cabazitaxel (Jevtana, Sanofi-Aventis USA) was intraperitoneally administered for four consecutive weeks to the rats in groups 2, 3, 4 at 0.5, 1.0 and 1.5 mg/kg (body-weight/week) doses respectively. On the other hand, group 1 was given only i.p. saline at the same volume and time. Whole rats were maintained in a 12-h light/dark cycle environment (lights on 7:00–19:00 h) at 22 ± 1 °C temperature and 50% humidity. Rats had access to food and water ad libitum. At the end of the experimental protocol, animals were sacrificed and bilateral brain lobes were removed for biochemical, histopathological and immunohistochemical analyses.

2.1. Biochemical analysis

Tissue samples were immediately weighed and washed with 0.9% NaCl solution, homogenized (2000 rpm/min for 1 min, 1:10 w/v) using a stirrer (Stuart SHM 1, UK) in 1.15% KCl solution in an ice bath. Then homogenate was centrifuged at 5000 ×*g* for 60 min at 4 °C. The resultant supernatant was used at the analyses. Protein analysis in homogenate and supernatant was performed according to the Lowry method [9].

Malondialehyde (*MDA*) level in tissue homogenates was determined via using the single heating method described by Yoshioka et al., which is mainly based on thiobarbituric acid (TBA) reactivity [10]. MDA concentration was calculated by the absorbance coefficient of MDA–TBA complex (absorbance coefficient ε = 1.56 × 105/M/cm) and was described as μmol/mg tissue protein.

Total antioxidant capacity (*TAC*) of the supernatant was determined by using an automated measurement method with a commercially available kit developed by Rel (Total Antioxidant Status Assay kit, Rel Assay Diagnostics, Turkey). The antioxidative effect of samples against potent-free radical reactions initiated by reduced hydroxyl radical was measured via using this method. The results were expressed as millimoles of Trolox equivalent per mg tissue protein.

Superoxide dismutase (*SOD*) estimation was based on the generation of superoxide radicals produced by xanthine on xanthine oxidase, which interact with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazon dye. The SOD activity was then measured by calculating the inhibition degree of this reaction. SOD was performed according to the Sun. et al. method [11] and established results were presented as percent inhibition/mg tissue protein.

Apoptotic DNA fragments — the measurement of cytoplasm histone-associated DNA fragments (nucleosomes) was performed with Cell Detection ELISA plus device (Manheim, Germany). Briefly, tissues were homogenized in cold lysis buffer for 3 min and centrifuged at 20,000 ×*g* for 10 min at 4 °C. Cytoplasm lysates (supernatants) from tissues were transferred to a streptavidin coated plate supplied by the manufacturer. A mixture of antihistone-biotin and anti-DNA-POD (anti-DNA antibody conjugated with peroxidase) were added to tissue lysates and incubated 2 h at room temperature. The complex was then simultaneously conjugated with the peroxidase substrate (ABTS) to form an immune complex on the plate, whose the absorbance was read at 405 nm using an ELISA-reader (Thermo Multiskan FC, USA).

2.2. Histopathological and immunohistochemical analyses

Removed brain tissues were fixed in 10% neutral buffered formalin solution, were processed and embedded in paraffin blocks. Blocks were cut at 5–6 μm thickness in sagittal plane and four sections were taken from each sagittal block face. Slides were then stained with hematoxylin–eosin for morphological examination. Scoring and evaluations of the neurological changes in the brain tissue were hemorrhagic areas, heterochromatic nuclei, eosinophilic necrosis, pyknotic cell and vacuolization level. The scores were derived semiquantitatively using light microscopy with modified Quick score method [12–13], and were reported as follows: (−) negative, (+1) weak, (+2) moderate, (+3) severe, and (+4) very strong.

In immunohistochemical analysis, the cerebral cortex was stained with TdT-mediated dUTP-biotin nick end labeling (TUNEL), Bcl-2 (diluted to 1:200) and inducible nitric oxide synthase [(iNOS, NOS 2, diluted to 1:50]. Slides were examined under high-powered light microscopic (Olympus CX41). Apoptotic cell were detected by using the TUNEL method (ApopTag Peroxidase In Situ Apoptosis Detection Kit, S7100; Chemicon International, USA). The tissue sections are deparaffinized in xylene, rehydrated in a graded series of alcohol solutions. Thereafter, sections were incubated with 3% H_2O_2 (5 min), equilibration buffer (at least 10 min) and then with working-strength TdT enzyme (60 min at 37 °C), at which point the sections were agitated (15 s at room temperature). Then sections were incubated with working strength stop/wash buffer (10 min), antidigoxigenin conjugate (30 min) and 3,3′-diamino benzidine (DAB) solution.

In immunohistochemical analysis, Bcl-2 and iNOS positive cells were stained by streptavidin biotin peroxidase method. These sections were incubated with primary antibodies (Bcl-2: N-9 and iNOS: NOS2, Santa Cruz Biotechnology) and biotinylated secondary antibody and streptavidin biotin-HRP for 1 h and then DAB substrate kits for 3–5 min to obtain immunolabeling. Finally, nuclei were counterstained with Mayer's hematoxylin.

Image analysis was performed using a personal computer, a camera, software (Olympus SC30) and an optical microscope. Tissue sections were evaluated in histopathological and immunohistochemical aspects. The distribution of iNOS, Bcl-2 and TUNEL-positivity has been assessed using the modified Quick score method [12,13]. In each slide, 5 different areas were counted (under 400×), in which the total number of stained cells per 1000 μm^2 was calculated. In the current study, the amount of stained cells in each section was scored based on the study published by Kara et al. [13] as (−) negative = non-reactive, (+1) weak = individualized positive cell reactivity in <25% of neurons, (+2) moderate = positive reactivity in 25–50% of neurons, (+3) severe = positive reactivity in 50–75% of neurons, and (+4) very strong = positive reactivity in >75% of neurons.

2.3. Statistical analysis

Established data were analyzed by using SOFA statistics open source software (www.sofastatistics.com). Results for descriptive statistics were expressed as mean ± standard deviation (SD) or median (range (minimum–maximum)). Statistical comparisons of continuous variables were performed using one-way analysis of variance (ANOVA) or Kruskal–Wallis test based on the number of cases. In cases where Kruskal–Wallis test yielded statistical significance, Mann–Whitney U-test was used to determine the different groups. p values less than 0.05 were considered as statistically significant.

3. Results

3.1. Biochemical analysis

–*MDA levels* were 7.19 ± 1.05 μmol/mg protein in the group 1, 9.97 ± 0.46 μmol/mg in the group 2, 11.19 ± 0.75 μmol/mg in the group 3, 12.65 ± 0.31 μmol/mg in group 4. When these results are compared between all groups; *MDA levels* were significantly lower in group 1 compared with the other all groups ($p < 0.001$), and significantly lower in group 2 compared with the group 3 and group 4 ($p < 0.001$),