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Effect of Doxorubicin on Locomotion Stimulation, Depression and Catechol Amines (Dopamine and 5HT)

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Abstract

Aim: To determine the effect of doxorubicin on locomotion, stimulation, depression and catechol amines (dopamine and 5HT).

Methods: Experimental drug doxorubicin was used as an anticancer drug. It was dissolved in saline and injected intraperitoneally to test group of rats. It was observed very little has been reported on the behavioural effects. Three behavioural studies were observed, which include open field activity specific for locomotion, light and dark activity specific for depression, and home cage activity specific for stimulation after one week, two weeks, three weeks and four weeks of doxorubicin treatments. To determine the concentration of dopamine and 5HT in brain, rats were decapitated gradually and analysed by HPLC-EC detector, results were observed very carefully.

Results: Locomotors activity and stimulatory activity were decreased when the concentration of doxorubicin increased gradually and depression was markedly appeared. The mean value of dopamine and 5HT were decreased markedly in TG group of rats as compared to CG group of rats.

Conclusion: Doxorubicin has negative effect on locomotion and stimulation, but produces the positive effect on depression. These activities were depending upon the concentrations of catecholamine's (dopamine and 5HT). It was also observed that dopamine and 5HT were also significantly decreased.

Keywords: Doxorubicin; Locomotion; Stimulation; Depression; Dopamine; 5HT

Introduction

Doxorubicin is a cytotoxic anthracycline antibiotic, closely related to the natural product daunomycin and similar to all anthracycline [1]. It is available in liposome-encapsulated forms as Doxil, Caelyx, and Myocet. It has photosensitive property and containers are often covered by an aluminium bag and/or brown wax paper to prevent light from affecting it. It was commonly used for liver cancer chemotherapy including hematological malignancies, many types of carcinomas and soft tissues sarcomas, as it works by intercalating DNA with the most serious adverse effect being life threatening heart damage [2]. According to the age for children, side effects for vomiting are different. It is common for vomiting to occur from a viral infection food poisoning, milk allergy, motion sickness, overeating or feeding. Side effects and serious toxicity of doxorubicin are including cardiotoxicity and myelosuppression [3]. Causes of cancer-specific drug resistance are currently believed to be linked to the random drug-induced mutational events (genetic hypothesis) to the drug-induced non-mutational alterations of gene function (epigenetic hypothesis), and recently to the drug-induced karyotypic changes (karyotypic hypothesis). In order to reduce the toxic side-effects and to improve the effect of chemotherapy and various tumor-specific drug carrier systems such as modified liposomes, micelles and self-assembled nanoparticles, and dendrimer have been investigated [4,5]. Chemotherapy is often accompanied by side effects whose severity can lead to reduce drug effectiveness or interrupt therapy but still used for the treatment of various types of cancers [6]. Currently, several researchers are working to make chemotherapy more tolerable and to find a system avoiding that treatment compromises the viability of healthy cells. Recent advances in understanding the molecular basis of cancer have provided insight into the network of signalling pathways that regulate cancer cell growth and metastasis [7,8]. Unlike normal cells, cancer cells undergo modifications that help them bypass the normal control of cell growth and proliferation [9,10]. Anthracycline drugs including Doxorubicin (DOX) are also most commonly used for the therapy of leukemia, lymphoma and breast cancer. In response to oxidative stress, changes in signalling pathways caused by carcinogens such as heavy metals, environmental pollutants, cigarette smoke, toxic gasses, and ionizing radiation are pleiotropic and have been well-described to participate in carcinogenesis. Reactive Oxygen Species (ROS) elicit cancer cell growth and proliferation below a certain limit while an excess of ROS induces cancer cell apoptosis [11]. Further, oxidative stress causes a significant increase in Protein Kinase C (PKC), Phosphoinositide 3-Kinase (PI3K), Phospholipases A2 (PLA2) and other enzymes that activate Nuclear Factor Kappa-B (NF-κB) and Activating Protein-1 (AP-1) [12-14].

The main purpose of this study was to analyse the temporal course of the antidepressant-like effect of an acute administration of the doxorubicin.

Experimental protocol

Locally bred male albino Wister rats weighing about 180 to 200 g on arrival purchased from Animal House Research Institute of Agha Khan University, Karachi, Pakistan, were used throughout the experiments. Total of twelve rats were divided into two groups: 6 Test Group (TG) and 6 Control Group (CG). These all rates were housed individually in specially designed cages with saw dust cover floor in a quiet room with free access to cubes of standard rat's food and water for at least 3 to 4 days before starting the experiment, so that the rates could adapted them to the new environment.

Injection of doxorubicin to the rats: Doxorubicin was dissolved in saline. It was injected by 30 mg/kg body weight, intraperitoneally (i.p) for four weeks to test group of rats. After 1st, 2nd, 3rd and 4th weeks of treatment different behavioural experiments were performed for the observation of stimulatory, locomotory and depression activity.

Behavioural activities: The open filed apparatus used in this experiment consists of a square area (76 × 76 cm) with walls 42 cm high. The floor divided by lines into 25 equal squares. The experiments were performed under normal day light in a very quiet room to avoid any noise effect. An animal taken out from the specialized cage and placed in the center square of the open filed apparatus, rats move from the center square, crossing with all four paws corner sittings, grooming, gnawing. These all activities scored for 5 min. Light and dark activity is specific for anxiety. This apparatus consists of small square area (26 × 26 × 26 cm) with an access (12 × 12 cm) walls of one compartment were transparent and other dark. Under normal day light, rats were placed on the dark side of the apparatus then observed that how many times takes to rats move in the light portion within 5 min. Specially designed Perspex home cage (26 × 26 × 26 cm) with saw dust covered floor was used for this purpose. These activities were monitored for rats as the number of cage crossing and 0.4 scales of increasing intensities of grooming and gnawing.

Decapitation of rats and brain collection technique: After 4 weeks of injections, rats were decapitated by a specialized design apparatus (guillotine). Then brains were collected very quickly from the cranial cavity within 30 s of decapitation. The skull covered by the skin was cut along the midlines by a very fine scissor, which exposed the whole brain and the whole brain was collected by very fine forceps. Brain which was covered by a membrane was removed, fresh brain was dipped in a cold saline (0.9% w/v) and then kept in plastic bags and stored at low temperature (-70°C) until analysed by HPLC-EC detector.

Preparation of extraction medium: 0.4 M perchloric acid containing 0.1% sodium met bisulphate 0.1% EDTA and 0.1% cystine, DH_2O was added to makes up the volume up in 1000 mL.

Homogenization of brain: Frozen brains samples were homogenized in 8 mL volume of 0.4 M per chlorate containing 0.1% sodium metabisulfite, 0.01% EDTA and 0.1% cystine. Glass homogenizer with Teflon pestle was used. The homogenates were placed in a refrigerator for 20 to 30 min to down the precipitate and were removed by centrifugation at 10,000 × g at 4°C in Eppendorf centrifuge tubes for 20 min, supernatant was kept very slowly in another Eppendorf tub, which were used for HPLC analysis.

HPLC analysis: HPLC analysis takes place by two phases, stationary and mobile phase. Stationary phase was used 511 shima-pack ODS separation column of 9.0 mm internal diameter and 150 mm length, separation takes place by mobile phase containing menthol 14% acetyl sodium sulphate 0.023%, EDTA 0.0035% in 0.1 M phosphate buffer pH 2.9.

Standard solution: Chromatogram of standard solution containing Non-Epinephrine (NE), Dopac, Hydroxy Venalic Acid (HVA), 5-Hydroxy Indole Acetic Acid (5HIAA), 5-Hydroxy Tryptamine (5HT) made solutions of these standards were used before analysing the sample to identify their specific retention times. Concentrations of standards NE, 82 mg/mL, 5HT 86.5 mg/mL, HVA 199 mg/mL, Dopac 100 mg/mL, 5H1AA 100 mg/mL and dopamine 100 mg/mL.

Statistical analysis: Results were represented as mean, ± SD (n=6), data was analysed by using one-way ANOVA. Significant difference was considered by student t-test p>0.01 level from TG and CG.

Results

Behavioural technique

Figure 1a represents Doxorubicin treatment on open filed behaviour shows effects on TG and CG treated rats. Statically analysis by student t-test for 1st week was (df2, 12) (t=21.977) (**p>0.01), 2nd week (df2, 12) (t=24.13) (**p>0.01), 3rd week (df2, 12) (t=26.77) (**p>0.01) and 4th week (df2, 12) (f=19.77) (**p>0.01) all results shows that after one month of treatment motor activity were markedly decreased in TG group of rats as compared to CG group of rats.

Figure 1b represents Doxorubicin treatment on light and dark activity (time in seconds) was monitored shows effects on TG and CG treated rats. Statistical analysis by student t-test for 1st week (df2, 12) (t=21.37) (**p>0.01) 2nd week (df2, 12) (t=22.23) (**p>0.01), 3rd week (df2, 12) (t=25.77) (**p>0.01) and 4th week (df2, 12) (f=18.57) (**p>0.01). These results show that after one month of treatment CG group of rats in light portion spend a long time as compared to TG group of rats.

Figure 1c shows entries in light portion were monitored very carefully statistical analysis by student t-test for 1st, 2nd, 3rd and 4th week was observed (df2, 12) (t=11.97) (**p>0.01), (df2, 12) (t=12.213) (**p>0.01), (df2, 12) (t=15.77) (**p>0.01) and (df2, 12) (f=18.27) (**p>0.01) respectively. Results indicate that entries in light portion were markedly decreased in TG group of rats as compared to CG group of rats, rats feel depression.

Figure 1d represents stimulatory activity was monitored after administration of doxorubicin. Statistical analysis by t-test for 1st week (df2, 12) (t=19.77) (**p>0.01), 2nd week (df2, 12) (t=14.43) (**p>0.01), 3rd week (df2, 12) (t=15.97) (**p>0.01) and 4th week (df2, 12) (f=18.93) (**p>0.01) were calculated. Results shows that after one month of treatment stimulatory activity were markedly decreased in TG group of rats as compared to CG group of rats.

Figure 2a shows effect of Dopamine was observed. Statistical analysis by student t-test for 1st week was (df2, 12) (t=13.57) (**p>0.01), 2nd week (df2, 12) (t=15.51) (**p>0.01), 3rd week (df2, 12) (t=14.47) (**p>0.01) and 4th week (df2, 12) (f=17.73) (**p>0.01). Results shows that dopamine activity was decreased in TG group as compared to CG group of rats.

Figure 2b represents Doxorubicin treatment effect of 5HT was analysed. Statistical analysis by student t-test for 1st week was (df2, 12) (t=16.27) (**p>0.01), 2nd week (df2, 12) (t=18.33) (**p>0.01), 3rd week (df2, 12) (t=17.37) (**p>0.01) and 4th week (df2, 12) (f=19.93)

(**p>0.01). Results shows that 5HT activity was decreased in TG group as compared to CG group of rats.

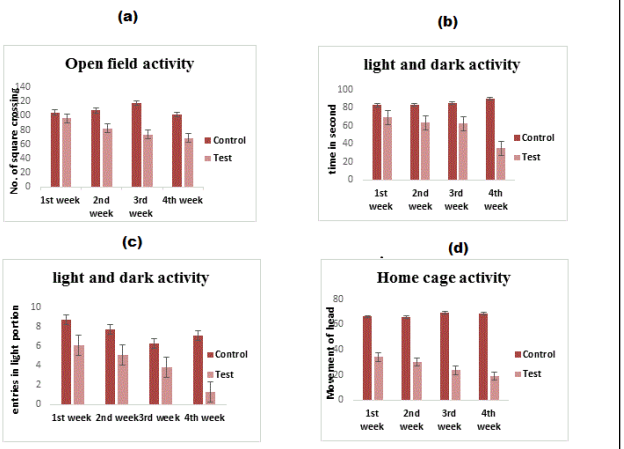

Figure 1: Behavioural studies. Results were represented as mean, ± SD (n=6) significant difference by student t-test p<0.01 level from CG and TG following one-way ANOVA. (a) open field activity (no. of square crossing) (b) light and dark activity (time in second) (c) light and dark activity (entries in light portion) (d) home cage activity (movement of head).

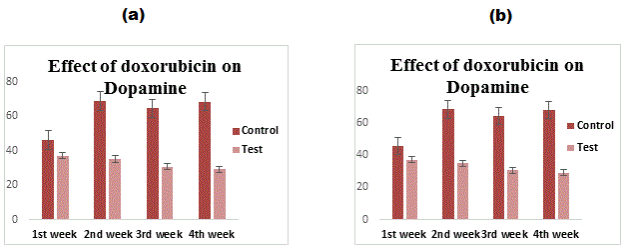

Figure 2: Neurochemical studies. Results were represented as mean ± SD (n=6) significant difference by student t test p<0.01 level from CG and TG following one-way ANOVA. (a) Effect of doxorubicin on dopamine (b) Effect of doxorubicin on 5HT.

Discussion

Doxorubicin and like all anthracyclines work by intercalating DNA [15]. It has negative effect on locomotion, stimulation, mood behaviour and all the functions of body related to the relaxation by decreasing the concentration of dopamine and 5HT. It was also proposed earlier that the hormonal fluctuations are related with the depression at some stages in their life in which estrogen plays an important role [16]. It has been also studied that the therapeutic action of some antidepressants can be improved or decreased by estrogens [17]. Selective serotonin reuptake inhibitors and catecholamine reuptake inhibitors act as an antidepressant, produce initially a blockade of their respective reuptake site, thereby enhancing the neurotransmitter availability [18]. Earlier initiator shows that in several brain areas, estrogens can inhibit the function of the monoaminergic transporter and interact with either 5HT or adrenergic receptors [19]. Previously, it was also studied that the synthetic steroidal compound ethinyl estradiol anticancer drugs such as doxorubicin inhibit with comparable efficacy both the serotonergic and the catecholaminergic transporters [20]. On this basis, the main purpose of the current study was to investigate the possible antidepressant-like effect of doxorubicin by using open field apparatus, light and dark box activity and home cage activity and also the analysis of the neurobiological bases of depression (dopamine and 5HT), which is considered as an index of "behavioural despair" to decrease in immobility. While the selective noradrenergic and dopaminergic reuptake inhibitors like maprotiline and bupropion decrease in mobility accompanied by an increase in climbing behaviour, hence a second purpose of this investigation was to determine the behavioural outline and compare them with those produced by the classic antidepressants [21,22]. In the previous study, it was shown that both the natural and the synthetic adrenergic compounds respectively had antidepressant-like actions while the noradrenergic synthetic compound was inactive and shows antidepressant-like effects. Alcohol, caffeine, and sugar all seem to decrease dopamine activity in the brain [23]. Dopamine is an important adrenergic neurotransmitter in the brain with several potential functions or for brain chronic diseases such as Alzheimer's, Parkinsonism and Schizophrenia [24]. It was also observed previously that the compounds and medicines which are used for the treatment of cancers decline the concentration of dopamine and 5HT in the brain [25]. In the current study, doxorubicin treatment decreases the concentration of dopamine and 5HT. It was the main reason that it was also observed that behavioural activity like locomotion and stimulation was also decreased. Light and dark box activity results show that depression was also produced in rats after doxorubicin treatment. Hence, further research is needed in humans to indicate its contraindications.

Conclusion

Doxorubicin is an anticancer drug. It has negative effect on locomotion and stimulation, but produces the positive effect on depression. These activities were depending upon the concentrations of catecholamine's (dopamine and 5HT). It was also observed that dopamine and 5HT were also significantly decreased.

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