HOSTED BY

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

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

Graduation dissertation doi: 10.1016/S2222-1808(15)61038-6

Genotoxic effect of the tricyclic antidepressant drug clomipramine hydrochloride in somatic and germ cells of male mice

Samia Ahmed El-Fiky[1], Fouad Afifi Abou-Zaid[2], Ibrahim Mohamed Farag[1], Maha Aly Fahmy[3*], Naira Mohamed El-Fiky[1]

[1]*Cell Biology Department, National Research Centre, 33 Bohouth St., Dokki, P.O. 12622, Giza, Egypt*

[2]*Zoology Department, Faculty of Science, Tanta University, Tanta, Egypt*

[3]*Department of Genetics and Cytology, National Research Centre, 33 Bohouth St., Dokki, P.O. 12622, Giza, Egypt*

ARTICLE INFO

Article history:
Received 10 Feb 2016
Received in revised form 22 Feb, 2nd revised form 21 Mar 2016
Accepted 10 Apr 2016
Available online 18 Apr 2016

Keywords:
Clomipramine
Mice
Chromosome aberrations
Sperm abnormalities
Histopathology
Liver cells

ABSTRACT

Objective: To assess the genotoxic potential of the antidepressant drug clomipramine hydrochloride (CH) through different mutagenic end points.

Methods: The study included chromosomal aberration analysis of bone marrow cells, primary spermatocytes, morphological sperm abnormalities and histopathological changes of liver cells using both light and electron microscopy. Three doses (0.195, 0.26 and 0.65 mg/20 g body weight) were tested. Each dose was given orally to mice for different periods of time (the doses are equivalent to the recommended daily intake doses in man).

Results: The tested doses of CH applied for 5 and 30 days increased the frequencies of chromosomal aberrations with dose and time dependant manner. The two high doses, 0.26 and 0.65 mg/20 g body weight revealed significant effect in comparison to the control. Dose -dependent increase in morphological sperm abnormalities and decrease in sperm count were recorded after CH treatment for 5 consecutive days. Pathological changes in liver tissue reached to sever damage were recorded after treatment with the medium and the high doses for 30 days. Ultrastructural examination showed that the low dose had little differences in liver histological architecture as compared to the control group, while prominent pathological changes in nuclei as well as dilated rough endoplasmic reticulum were observed in mice treated with the medium or the high dose of the drug.

Conclusions: It is concluded that CH has genotoxic effect in somatic and germ cells of mice as well as damaging effect on liver tissue after treatment with the medium and the high doses. However, the usage of low dose (especially for short time, 5 days) can be utilized as a safe therapeutic dose.

1. Introduction

Depression is one of the most common diseases around the world. It represents a significant burden for both individuals and the society. The prevalence rate of the disease in a population may reach 14%. Depression episode may occur at any age from childhood to elderly. At best to date, only 50% of the depressed patients undergo treatment and among them less than 50% fully recovered with the existing drugs[1,2].

Tricyclic antidepressants (TCAs) are some of the most commonly prescribed drugs worldwide[3]. Although, in some cases the efficacy of TCAs in the acute-phase of depression was lower than initially thought[4], the continuation of therapy in patients reduces the risk of relapse[5].

However, some research studies pointed on the side effects that accompanied the continued use of TCAs, *e.g.* cardiac diseases, heart failure[6] and the risk of type 2 diabetes[7]. Seizures and inhibition of monoamine oxidase (in brain) have been reported to occur in patients with TCA medications. Also, the drugs are known to produce a number of toxic effects on organs containing self renewing cell population such as bone marrow, skin and gastrointestinal tract[8].

Clomipramine hydrochloride (CH) is one of the most used TCA drugs. It contains two benzene rings in its chemical structure.

*Corresponding author: Prof. Maha Aly Fahmy, Department of Genetics and Cytology, National Research Centre, 33 Bohouth St., Dokki, P.O. 12622, Giza, Egypt.
Tel: +201069937580
E-mail: maha_sadky@yahoo. com

All experimental procedures involving animals were conducted in accordance to the ethical guidelines of the Medical Ethical Committee of the National Research Centre in Egypt and approved by Medical Ethical Committee of the National Research Centre in Egypt.

Foundation Project: Supported by the Student Research Committee, Tanta University, Egypt (Grant No. 000453).

The journal implements double-blind peer review practiced by specially invited international editorial board members.

Benzene is an important toxic material, as it is metabolized in the liver by cytochrome P450 2E1 to various phenolic metabolites which accumulate in the bone marrow[9]. These metabolites can produce reactive free radical species. Redox cycling of these free radicals produces active oxygen that may damage cellular DNA and cause DNA adducts. Consequently this can lead to inducing of chromosome aberrations[10]. Several studies have been demonstrated that benzene and its metabolites might significantly contribute in inducing chromosome aberrations in somatic and germ cells as well as sperm abnormalities[11,12]. Therefore, the present study was designed to evaluate the potential genotoxic effect of CH in male albino mice. This evaluation included cytogenetic assays, sperm parameters and histopathological examination of liver cells.

2. Materials and methods

2.1. Experimental animals

Male Swiss albino mice (*Mus musculus*) aging 3 months old and weighing about 20 g were obtained from Schistosome Biological Supply Centre in Theodor Bilharz Research Institute, Cairo, Egypt. The animals were housed in stainless steel wire mesh cages on a bedding of wood chips. They were kept in an ambient temperature of (25 ± 3) ℃ on a light/dark cycle of 12/12 h and supplied with food and water *ad libitum*.

2.2. Ethics

Handling of animals and the anesthetic procedures were performed according to the ethical guidelines of the Medical Ethical Committee of the National Research Centre in Egypt and being sure that animals did not suffer at any stage of the experiment.

2.3. Chemical drug

CH (Supranil) was a pure powder, purchased from Alpha Chem Advanced Pharmaceutical Industries S.A.E Company.

The drug was dissolved in sterile distilled water and orally administrated at three dose levels of 0.195, 0.26, and 0.65 mg/20 g body weight of mouse. These doses are equivalent to the doses of acceptable daily intake of CH for human (75 mg, 100 mg and 250 mg/70 kg body weight), after modification on the basis of relative surface area between species according to Paget and Barnes formula[13].

2.4. Experimental design

For chromosomal aberration analysis in somatic and germ cells and histopathological examination, mice were divided into six groups (5 mice/group). Three groups were orally administrated the tested doses daily and samples were taken after 5 days of treatment. The other three groups were treated for 30 days.

For sperm shape abnormalities, the method of Fahmy *et al.*[14] was followed and mice were orally administrated CH for 5 successive days. Samples were collected 35 days after the 1st dose of treatment.

In all experiments, a concurrent control group was taken for each treatment. Both treated and control animals were sacrificed by cervical dislocation.

2.5. Cytological preparation

2.5.1. Chromosome aberration analysis

Mice were *i.p.* injected with 0.5 mL of colchicine (0.05%)/kg body weight 2 h before sacrificed. Femurs were removed and the bone marrow cells were aspirated using saline solution. Metaphase spreads were prepared using the method of Fahmy *et al*.[14]. Fifty metaphase spreads per animal were analyzed for scoring different types of chromosomal aberrations. Also, metaphase spreads from spermatocyte cells were prepared according to Al-Ashaal *et al.*[15] and Russo[16] for meiotic chromosomal analysis.

The mitotic and meiotic indices of bone marrow and spermatocyte cells respectively were investigated by recording the number of dividing cells/1 000 cells/ animal.

2.5.2. Sperm shape abnormalities

Sperm were prepared according to the recommended method of Fahmy *et al.*[14] and smears were stained with 1% Eosin Y. At least 600 sperm per animal were assessed for morphological abnormalities. The sperm abnormalities were evaluated according to the standard method of Fahmy *et al.*[14]. Epididymal sperm count was also determined by hemocytometer as described by Pant and Srivastava[17].

2.5.3. Histopathological examination

2.5.3.1. Light microscopy

Specimens of liver from all animals were dissected immediately after death and fixed in 10% neutral buffered formal saline for at least 72 h. Specimens were washed in tap water for half an hour and then dehydrated in ascending grades of alcohol (70%, 80%, 90%, 95% and finally absolute alcohol), cleared in xylene, impregnated in soft paraffin wax at 55 ℃ and embedded in hard paraffin. Serial sections of 6 μm thick were cut and stained with haematoxylin and eosin[18] for histopathological investigation. Images were captured and processed using Adobe Photoshop Version 8.