

An investigation of the value of the *Tetrahymena pyriformis* as a test organism for assessing the acute toxicity of antidepressants

Uma S.^a, Kelly J.P.^b and Rajasekaran S.K.^c

Department of Physiology & Endocrinology^a, Department of Pharmacology & Clinical Therapeutics^c, St. Matthew's Medical University, Grand Cayman, Cayman Islands, Department of Pharmacology & Therapeutics^b, National University of Ireland Galway, Ireland.

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Correspondence: usenthilkumar@smucayman.com

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Abstract

Antidepressants differ greatly from class to class and within individual members of a class in terms of their adverse drug profile and their toxicity in acute overdose conditions. To reduce the use of animals, a number of *in vitro* tests are now under investigation to determine the acute toxicity of drugs. The objective of this study was to investigate fresh water protozoa *Tetrahymena pyriformis* with regard to the acute toxicity of antidepressants. The cytotoxic effects of a range of currently marketed antidepressants were evaluated using the MTT assay, and the IC₅₀ values were determined for each. These IC₅₀ values did not correlate with the FTI (Fatal Toxicity Index) values in humans. Thus it can be concluded that the *Tetrahymena pyriformis* cytotoxicity assay is a poor predictor in evaluating the acute toxicity of antidepressants in humans, which is in keeping with other *in vitro* methodologies using mammalian cells. It can be concluded that, in the absence of credible *in vitro* tests, acute toxicity in intact animals will continue to be the method for predicting the acute toxicity of antidepressants.

Introduction

Most of our current understanding about the toxicity of various chemicals comes from animal data, and as Andrew Rowan notes in his critical evaluation of animal research, *Of Mice, Models & Men*, "there is no doubt that our knowledge of the risks to humans of most chemicals is very inadequate" [1]. In recent years the practice of acute toxicity testing in animals has been criticized by animal right activists and antivivisectionists, as well as moderate organizations, e.g. FRAME (Fund for the Replacement of Animals in Medical Experimentation). Serendipitously, Russell and Burch's proposal of the three R's, i.e. Replacement, Reduction and Refinement alternatives has coincided with the development of molecular biology [2], as well as with vast improvements in technique for such things as propagating cells in culture and measuring molecular processes. However, some form of acute toxicity testing in animals is still deemed to be necessary in evaluating a test compound's safety. Such investigation can provide useful information on signs of intoxi-

cation, target organs of toxicity and cause of death. This can be achieved if the experiments are performed by well trained personnel, using all available techniques to monitor physical and pathomorphological changes of the organs [3]. However, there are many potential advantages of *in vitro* systems in toxicity testing are numerous. *In vitro* tests are usually quicker and less expensive. Experimental conditions can be highly controlled and the results are easily quantified. However, the relative simplicity of non whole-animal testing results in limitations as well. Cells or tissues in culture often cannot predict the effect of a toxin on a living organism with its complex interaction of nervous, endocrine, immune, and hematopoietic systems [4]. Although tissue and cell culture assays are currently the most popular *in vitro* tests for evaluating acute toxicity, other techniques are gaining prominence. These techniques may involve the use of bacteria and lower life forms for the determination of toxicity [5]. In the past decade, some laboratories have shown a significant correlation between *in vivo* or *in vitro* toxicity using mammalian cells and *in vitro* test systems using bacteria and pro-

tozoa [6], [7]. At present the *in vitro* methods available for determining sensitizers principally involve the use of cell mono layers, co-cultures or isolated skin explants. There are other fields of research which could lead to the development of more complex test systems. These are able to more accurately mimic the events which lead to skin sensitization *in vivo* and include structure activity relationships (SAR), quantitative structure activity relationships (QSAR) and expert systems. It is hoped to investigate the immunology and cell biology underlying the phenomenon of skin sensitization, to determine the most realistic opportunities for successful development of non-animal alternatives [8]. Protozoa are the simplest eukaryotic organisms but despite this contain almost the same metabolic systems as higher animals [9]. In addition they are appropriate organisms for toxicological studies, owing to their ease of culturing, short life cycle and large surface contact with the environment. The most common protozoan model used in toxicological studies is *Tetrahymena pyriformis* [10]. Antidepressants represent a major achievement in the treatment of patients suffering from depression. Unfortunately, reports of lethal overdoses of antidepressants started appearing early in the course of their clinical use, demonstrating their potential for harm as well as their therapeutic effects [11]. Antidepressant drugs are one of the most frequently ingested substances for accidental poisoning or a suicide attempt. The American Association of Poison Control Centers reported that antidepressants were the most frequent cause of death from drug ingestion in the years 1983 and 1984 [12].

In the era of growing concerns of animal rights and the human safety issues related to exposure to biological samples, there is a huge need for developing alternative methods of toxicological evaluation of drugs. In this study we made an attempt to investigate the possibility of using *Tetrahymena pyriformis* as an alternative for studying the drug's effect on cell proliferation. This study concentrated on one particular *in vitro* cytotoxicity test using *Tetrahymena pyriformis*, and investigating the effects of antidepressants. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used for measuring cytotoxicity. The tetrazolium salt reduction method is widely used as a means of examining cell proliferation and viability. The internal environment of proliferating cells is more reduced than that of non-proliferating cells [13]. The tetrazolium salts are reduced at sites in the mitochondrial electron transport system and are a test for succinate dehydrogenase activity. The reaction converts yellow salts to blue crystals that are dissolved and can be read spectrophotometrically. The most frequently used of these are MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), XTT (sodium 3'-[1-phenylamino]-carbonyl]-3,4-tetrazolium]-bis (4-methoxy-6-nitro) benzene-sulfonic acid hydrate, and MTS. Tetrazolium salt reduction, as an indicator of cell growth, has been

used in models for screening cytotoxic chemicals [14], [15] or cell growth promoting factors and cytokines [16].

Materials and Methods

All the antidepressant compounds tested were obtained from Sigma Aldrich, Dublin, Ireland. All assays are conducted in axenic conditions. 18–24 h cultures of *Tetrahymena pyriformis* used were strain GL, ref. CCAP/1630/1F from Strains of Culture Collection of Algae and Protozoa, Scotland, United Kingdom. The cells were grown to exponential phase at room temperature in Proteose Peptone Yeast Extract Medium (PPY) which consisted of 2% w/v protease peptone and 0.25% w/v yeast extract at pH 7.0–7.5. The density of *T. pyriformis* cultures was adjusted in fresh PPY in order to obtain 10^4 cells/ml. The cells were initially counted using a Sedgwick Rafter counting chamber.

A volume of 80 μ l of suspended *T. pyriformis* was added to each well of a 96-well flat-bottom microtitre plate along with 10 μ l of the antidepressants which were tested at a range of concentrations. The well plate was then incubated at 28°C under 5% CO₂ for 1 hr. Then, 10 μ l of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma, Chemical Co., Dorset, UK) stock solution (10 mg/ml in distilled water) were added and time 0 readings were taken at 540 nm using the spectrophotometer and the plates were incubated at 28°C for 4 hrs. The MTT reduction was stopped with 200 μ l of a solution containing 50% dimethylformamide and 10% sodium dodecyl sulfate-(DMF/SDS) without removing the medium. The formazan production due to succinate dehydrogenase activity was measured 30 min after DMF/SDS incubation.

Concentration-response graphs were generated for each antidepressant using GraphPad Prism software. These graphs were analysed using a curve fit for sigmoid dose-response, and IC₅₀ values were derived. Results are expressed as mean IC₅₀ with the 95% confidence intervals.

Results

Concentration response curves were obtained for each antidepressant when incubated with *Tetrahymena pyriformis* and the IC₅₀ values determined. A typical concentration response is illustrated for desipramine (Figure 1). The results for each antidepressant are summarized in Table 1. As can be seen from the table, The IC₅₀ of the tested compounds in *Tetrahymena pyriformis* ranged from 0.24 mM for trimipramine to 5.33 mM for imipramine using the MTT assay.

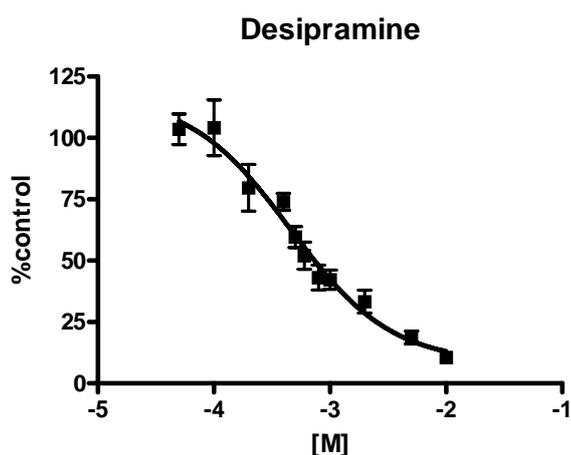


Fig. 1: The cytotoxic effect of desipramine in *Tetrahymena pyriformis*

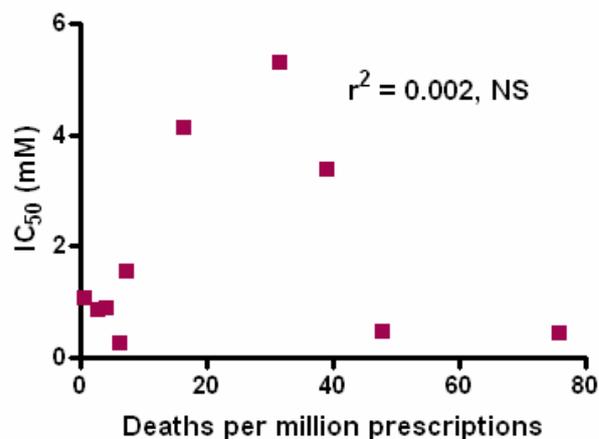


Fig. 2: Correlation between the IC₅₀ values and the human FTI

Table 1 The IC₅₀ values for the cytotoxicity of antidepressants using the *Tetrahymena pyriformis* assay.

Antidepressant	IC ₅₀ values
<i>TCA</i> s	
Amitriptyline	3.4 mM (2.5-4.8)
Desipramine	0.455 mM (0.289-0.717)
Imipramine	5.33 mM (3.6-7.8)
Trimipramine	0.24 mM (0.09-0.58)
Dothiepin	0.482 mM (0.258-0.898)
Clomipramine	1.57 mM (1.08-2.23)
<i>Other</i>	
Maprotiline	4.15 mM (2.42-7.10)
Trazodone	3.36 mM (2.19-5.15)
Mianserin	0.90 mM (0.52-1.56)
Reboxetine	10.1 mM (5.67-18.21)
<i>SSRIs</i>	
Paroxetine	0.89 mM (0.64-1.24)
Citalopram	5.21 mM (3.61-7.54)
Fluoxetine	1.07 mM (0.75-1.54)
Sertraline	0.282 mM (0.229-0.346)

Results are expressed as IC₅₀ and 95% confidence intervals.

The results obtained in this study using *Tetrahymena pyriformis* were then compared and correlated with the human Fatal Toxicity Index (FTI) for antidepressants (Fig. 2). FTI data was taken from Henry et al [17]. The above analysis shows that there is a very poor correlation

between the human FTI and the IC₅₀ values obtained for the antidepressants using *Tetrahymena pyriformis*.

FTI data updated from Henry et al [17].

Discussion

The acute IC₅₀ of the tested compounds in *Tetrahymena pyriformis* ranged from 0.24 mM for trimipramine to 5.33 mM for imipramine using the MTT assay. The general trend in the results obtained shows that, on average, the heterocyclic compounds were determined to have higher IC₅₀ values than the TCA and SSRI compounds, which by inference would make the heterocyclic compounds more toxic to cells. This was a surprising result considering the general consensus that heterocyclic compounds are safer than TCA drugs [18].

Use of human blood cells in the cell proliferation studies is limited because of the safety concerns, vaccination, availability of trained professional to draw blood, compensation for the donor, transport of blood samples to the lab and the handling of blood samples in the lab etc. The use of animal blood cells for such studies is again limited by many of the above said factors plus the ethical issue of animal sacrifice. The least one can do in basic research is to avoid tests which cause severe suffering to animals, as is required in Switzerland and other European countries by binding ethical principles and guidelines. The increasing standard of approval and control procedures has improved the situation over the years. There are many examples of successful alternative methods in basic research. But, the application of such methods is in most cases limited to the laboratories in which they were developed, calling for technology transfer [19].

Tetrahymena pyriformis has been used extensively in the past to perform various toxicological assays and has been consistent in reproducing the results [20]. Hence this organism is being commonly used as a toxicological tool.

The data obtained from this MTT assay shows a good linear regression with the data obtained from assays using rat blood PBMC's, human neutrophils and rat kidney cells [21], [22], [23]. The FTI and the rat LD₅₀ do not however correlate with the results obtained from *Tetrahymena pyriformis*.

It can be concluded from the results and analysis that *Tetrahymena pyriformis* can be considered to perform acute toxicity studies involving antidepressants. MTT assay seems to be a viable test for studies with *Tetrahymena pyriformis*. The human FTI and the IC₅₀ values obtained from this study does not correlate with each other. Its ability to produce toxicity data relevant to human remains questionable. Further studies are warranted to fully establish the role and use of *Tetrahymena pyriformis* in acute toxicity studies.

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Correspondence:

Uma Senthilkumar
 Department of Physiology & Endocrinology
 St. Matthew's Medical University
 Grand Cayman
 Cayman Islands
 e-mail: usenthilkumar@smucayman.com