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Investigating the effects of homeopathic preparations on human cancer cell lines.

Introduction

There has been considerable interest in a recent paper by Frenkel *et al* entitled "Cytotoxic effects of ultra-diluted remedies on breast cancer cells"[1]. The aim of this newsletter is to outline the main findings of this article, describe the methods used and put this article in the right scientific context, while making it accessible to non-specialists.

The authors report a toxic effect of homeopathic preparations on human cancer lines. Furthermore, this effect is seen to be specific to cancer cell lines, not affecting non-cancerous cells.

Methods

This paper reports on experiments performed at the University of Texas on human cancer cell lines (so-called *immortalised cell lines* commonly used for cancer and other types of biological research) using homeopathic preparations.

The preparations used were four homeopathic preparations commonly used in the 'Banerji protocol', a homeopathic cancer treatment protocol designed by Indian homeopath Dr Banerji for the treatment of a wide variety of cancers: *Phytolacca* 200C, *Carcinosin* 30C, *Conium* 3C and *Thuja* 30C (for reference a 30C dilution corresponds to 30 successive 1:100 dilutions with vigorous succussion at each stage in the dilution process).

The authors of this article used a variety of 'state of the art' biological research tools to investigate the specific effects of homeopathic preparations on human cancer lines (Box 1). The authors also investigated the effects of the homeopathic preparations on the internal biochemical machinery of the cells, investigating cellular processes affected by the homeopathic preparations.

Box 1: Methods used

Methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay

MTT is a standard toxicology test used to measure the toxicity of compounds on cell cultures. MTT is degraded to a compound with a specific colour; the amount of this compound can then be measured, giving an indication of the level toxic load on the cells. This test was used to measure the toxicity of the homeopathic preparations on the different cell lines used.

Fluorescence in situ hybridization (FISH)

This technique is used to quantify the amount of material in certain portions of chromosomes. Here they used this technique to quantify telomere length - telomeres being the sections of non-coding DNA found at the ends of chromosomes.

Flow cytometry or fluorescence activated cell sorting (FACS)

Flow cytometry is used to separate out and measure cell populations according to the presence or absence of certain markers. In this case, markers for apoptosis (cell death) were used to quantify the number of cells dying.

Western blot

Western blot uses gel electrophoresis to separate proteins of different sizes as they migrate through a gel. This technique is used to detect the presence or absence of certain specific proteins in a sample.

Results

The effect of four homeopathic preparations was tested on three different cancer cell lines: two breast cancer cell lines and one non-cancerous breast tissue cell line. The authors first investigated the toxicity of these homeopathic preparations on these cell lines using the MTT protocol (see Box 1 for details).

Despite an effect of the solvent control (87%

alcohol), these homeopathic preparations showed some additional cytotoxic effect.

The fact that the controls had cytotoxic effects is to be expected as alcohol itself has a toxic effect on cells in general. However, they found that homeopathic preparations showed increased toxicity on the cancer cell line but not on the control cells. *Phytolacca* 30C and *Carcinosin 30C* were identified as being more potent than *Conium* 30c and *Thuja 30C*, so in the following experiments only *Phytolacca* 30c and *Carcinosin 30c* preparations were used.

The researchers used the FACS and Western blot techniques (see Box 1) to quantify the portion of cells undergoing apoptosis (cell death). They found that these two homeopathic preparations increased the amount of apoptotic cells, specifically in cancer cell lines.

The group also performed a number of experiments designed to elucidate the exact mechanisms by which the cells were dying. These experiments outlined below - although interesting - yielded less significant results.

Using FACS and Western blot techniques they investigated the progression of the cells through their growth/division cycle. The progression through the cell-cycle is essential for the cell-lines to continue proliferating; any arrest in this cell-cycle would ultimately lead to cell-death.

Another experiment consisted in quantifying the amount of non-coding material at the end of chromosomes (telomeres) using a technique called FISH (See Box 1 for details). The length of this material is linked with chromosome integrity and hence is a factor in cell survival.

The results from these investigations revealed some interesting preliminary findings but were overall not convincing; more work remains to be done to elucidate the exact mechanisms by which cell death is triggered in these cell lines by the homeopathic preparations.

Discussion

The authors showed that two homeopathic preparations (*Carcinosin* 30c and *Phytolacca 30c*) induce a toxic load on cancer cell lines leaving normal cells intact. They showed this toxic load to be possibly linked with lowered genetic stability in the treated cancer cell lines, leading to cell cycle arrest and subsequent cell-death.

Other studies have claimed that homeopathic preparations have cytotoxic activity on cancer cell lines[2,3]. However, it is the first time that this effect has been shown to be specific to cancer cells, leaving normal cell lines intact. Furthermore, it is the first time a study has started to investigate the specific effects of the homeopathic remedies on the biochemistry of the cells, thereby starting to shed light on the biological mechanisms of action of homeopathic preparation.

An important point to notice when considering Frenkel *et al*'s paper is that it is aimed at an expert audience and was never intended to be accessible to a wider audience. Moreover, the findings reported in this paper are not definitive and are to be considered as work-in-progress. The study suffers from a number of flaws which will have to be resolved in order for the results to be fully trusted. In particular, the experiments were not repeated a sufficient number of times, leading to poor statistical validity. Also, blinding was not performed, leaving open the possibility of bias. That being said, blinding is not commonly performed in these types of biological experiments and the purpose of this 'expert' paper was obviously not to provide definitive answers but rather to report on their interesting explorative work. This paper opens the way for further studies to confirm or disprove, as the case may be, the interesting preliminary results reported.

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References

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