THE EFFECT OF VARYING INCUBATION TIMES FOR HYPOTONIC TREATMENT OF LYMPHOCYTES IN DICENTRIC ASSAY TECHNIQUE

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ABSTRACT

The International Atomic Energy Agency (IAEA) has recommended that incubation time for the hypotonic treatment of lymphocytes in dicentric assay technique to be between 12 to 20 minutes. Incubation time will affect the hypotonic treatment of lymphocytes and thus, the breakage of cytoplasmic membrane. The objective of this study is to examine the effect of varying incubation times for hypotonic treatment of lymphocytes in dicentric assay technique. In this study, we choose to use our standard protocol for dicentric assay technique. However, for the hypotonic treatment of lymphocytes, the incubation times were varied from 10, 15, 20, 25 and 30 minutes respectively. Lymphocytes were then fixed and stained with Giemsa. The cells were then analyzed for clear background that indicates good metaphases. We found that incubation time of 30 minutes gives the best metaphase images. This incubation time is longer than what has been recommended by the IAEA. This may be explained by the fact that our country's climate is of higher humidity compared with the European countries.

Keywords : dicentric assay technique, hypotonic treatment.

ABSTRAK

Agensi Kuasa Atom Antarabangsa (IAEA) telah mencadangkan masa inkubasi untuk rawatan hipotonik limfosit yang digunakan dalam teknik asai disentrik adalah di antara 12 hingga 20 minit. Jangkamasa inkubasi akan mempengaruhi rawatan hipotonik, dan seterusnya menggalakkan pemecahan membran sitoplasma. Objektif kajian ini adalah untuk memeriksa kesan kepelbagaian masa inkubasi untuk rawatan hipotonik limfosit dalam teknik asai disentrik. Dalam kajian ini, kami menggunakan protokol standard untuk teknik asai disentrik. Walaubagaimanapun, untuk rawatan hipotonik limfosit, jangkamasa inkubasi telah dipelbagaikan bermula dari 10, 15, 20, 25 dan 30 minit. Seterusnya, limfosit di'tetapkan' dan diwarnakan menggunakan Giemsa. Sel kemudiannya dianalisa untuk mendapatkan latarbelakang yang jelas, yang menandakan metafasa yang baik. Kami mendapati bahawa, jangkamasa inkubasi selama 30 minit memberikan imej metafasa yang terbaik. Jangkamasa ini adalah lebih lama daripada apa yang dicadangkan oleh pihak IAEA. Ini dapat dijelaskan dengan fakta bahawa cuaca negara kita mempunyai kelembapan yang lebih tinggi jika dibandingkan dengan negara-negara Eropah.

Kata kunci : teknik asai disentrik, rawatan hipotonik.

INTRODUCTION

Dicentric assay technique using metaphase spread is the "gold standard" for biodosimetry method (IAEA, 2001). However, many factors can influence the analysis of dicentric such as duration of hypotonic treatment, preparation of slides, etc. (Henegariu *et. al.* 2001). Proper technique for preparing slides is considered essential for both classical cytogenetics (e.g. dicentric) and molecular cytogenetic procedures (e.g. fluorescence in situ hybridization "FISH"). Both cytogenetic procedures are widely used in biodosimetry field.

Each cytogenetic steps starting from cell culture, harvesting, slide preparation, aging and staining are very crucial and definitely will influence the end result. During harvesting, mitotic spindle formation is blocked by adding colcemide and cell division will stopped at metaphase level. Cells are subjected to hypotonic treatment, which will increases their volume, and disrupt the cell membrane of the red blood cells allowing it to be removed from the cell suspension. A fixative solution is added to the cell suspension to preserve the cells in their "swollen" state and to remove the water, thus "hardening" the biologic material. Fixative will also removes lipids and denatures the protein, making the cell membrane very fragile, which is important for subsequent chromosome spreading.

The International Atomic Energy Agency (IAEA) has recommended that incubation time for the hypotonic treatment of lymphocytes in dicentric assay technique to be between 12 to 20 minutes. The purpose of this study is to examine the effect of varying incubation times for hypotonic treatment of lymphocytes in dicentric assay technique. This will give us the optimum duration for hypotonic treatment, and later on will help us greatly in slide preparation and slide analysis.

METHODOLOGY

Culturing

Aseptically, five blood cultures were set up using 1ml of whole blood in each of 10ml culture media. Cultures were incubated for 48 hours at 37°C in 5% CO_2 incubator. After 45 hours of incubation, colcemide were added to stop the mitosis process, and samples were returned to the CO_2 incubator for another 3 hours.

Harvesting

After the incubation period, cells were treated with pre-warmed (37°C) 0.075M potassium chloride (KCl) as a hypotonic treatment. The incubation period were varied from 10, 15, 20, 25 and 30 minutes respectively. After the hypotonic treatment, the cells were centrifuged and the supernatant were removed. Then, the cell suspension was mixed and fixed three times with 3:1 methanol-acetic acid. During the first time of fixation, the fixer were added slowly and carefully to avoid the cells from clumping together. After that, slides were prepared by dropping 15-20ul of cell suspension onto a clean glass slide, dried and stained with 3% Giemsa. Lastly, the slides were mounted with mounting media before observation.

Slide examination

Slides were examined using a light microscope (Leica DMRBE, Germany) at 20x and 40x objectives before being examine using 100x (oil) objective. A random examination will include observation of cell spreading and the "cleanliness" of cell from debris around the chromosomes. Ten images from each incubation time were captured to compare the results.

RESULTS AND DISCUSSIONS

Results of images were shown in Fig.1 to Fig.5. From our observations, it is clearly shown that longer hypotonic treatment will result in better metaphase spreading and clearer background.

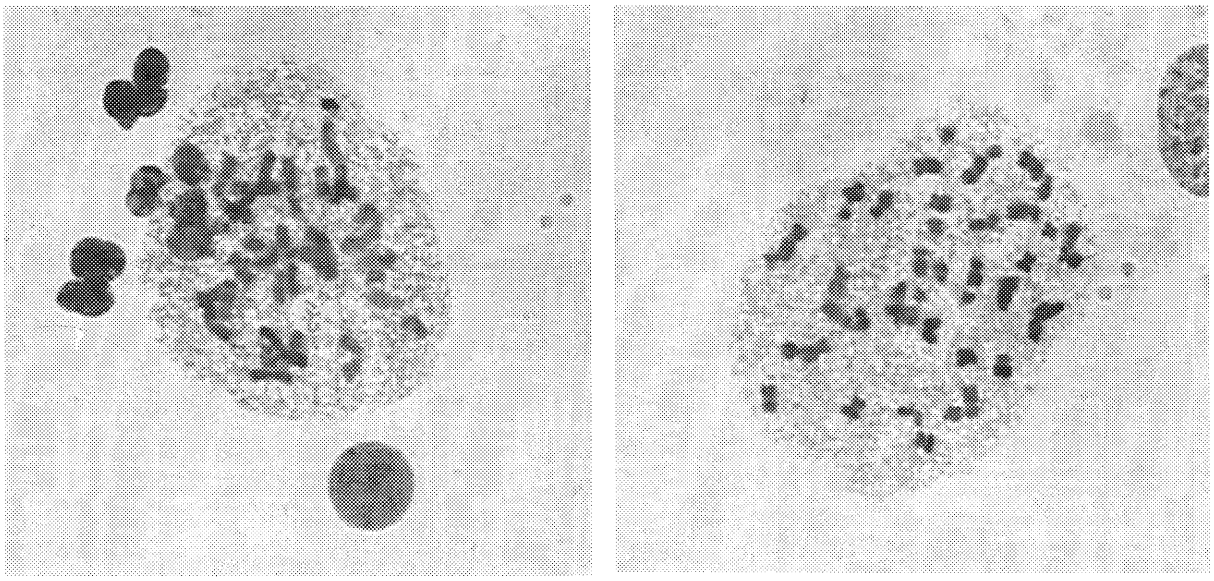

Fig.1. Images of metaphase spreading with 1000x magnification at 10 minutes of hypotonic treatment.

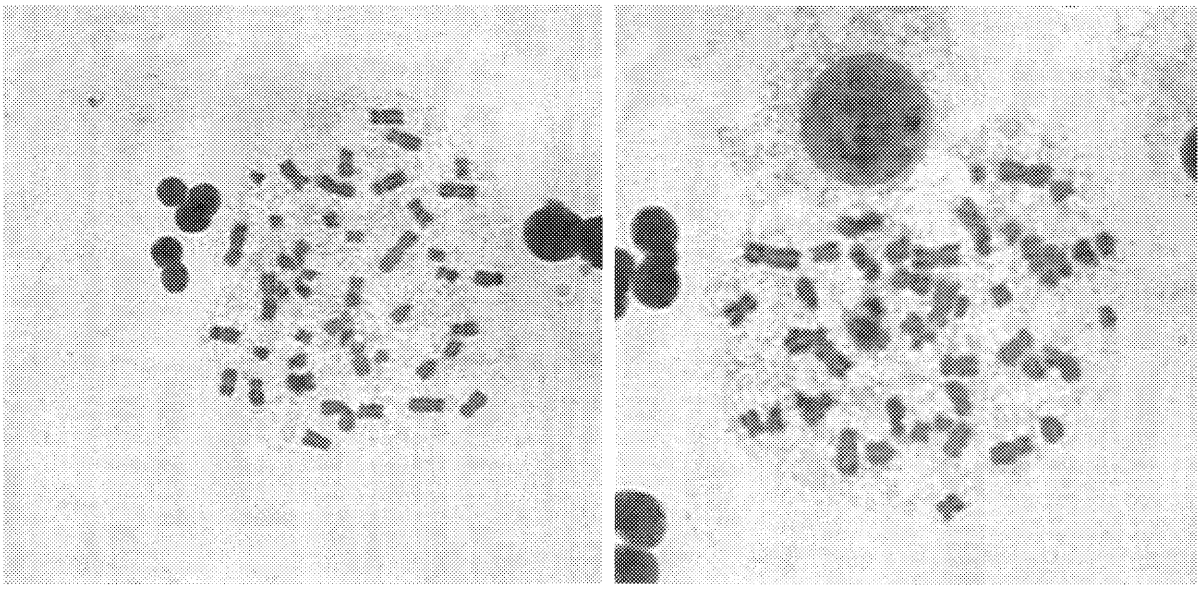

Fig.2. Images of metaphase spreading with 1000x magnification at 15 minutes of hypotonic treatment.

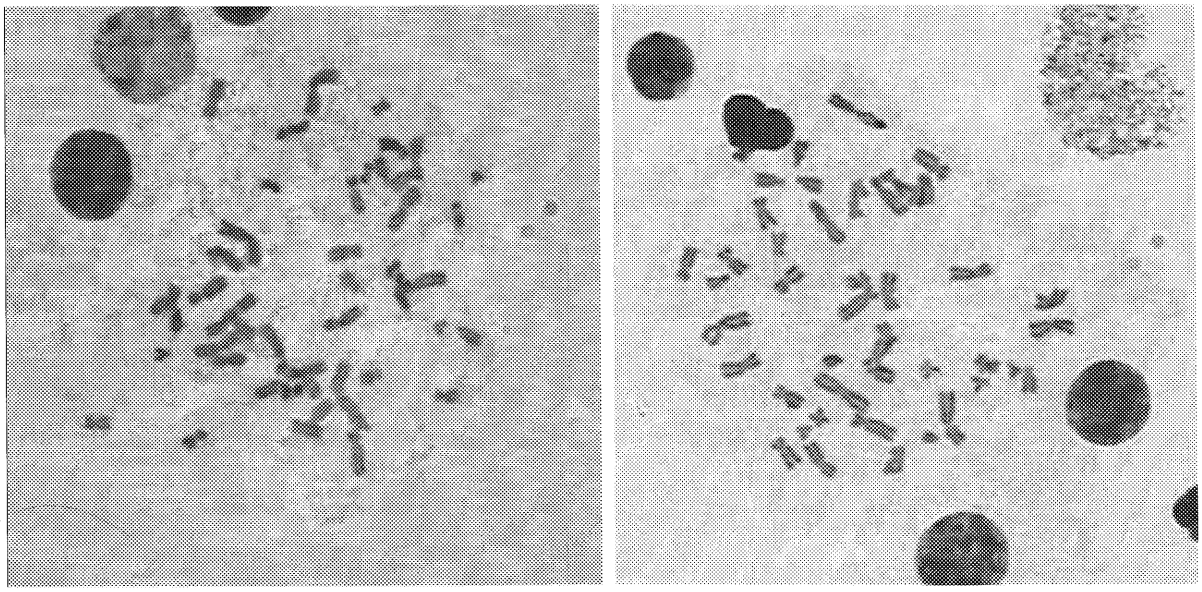

Fig.3. Images of metaphase spreading with 1000x magnification at 20 minutes of hypotonic treatment.

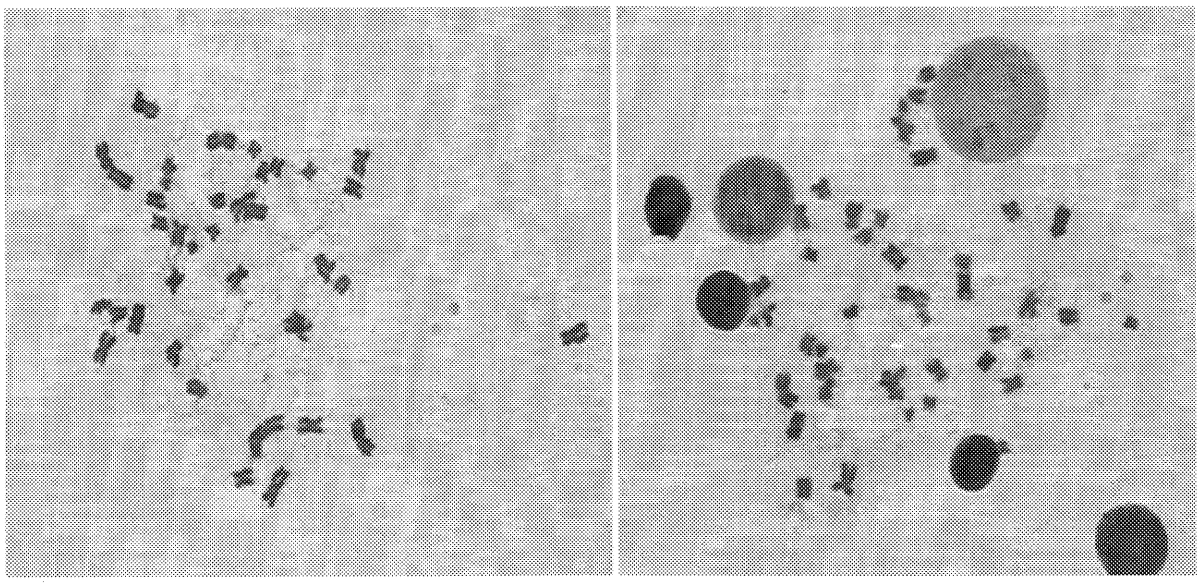

Fig.4. Images of metaphase spreading with 1000x magnification at 25 minutes of hypotonic treatment.

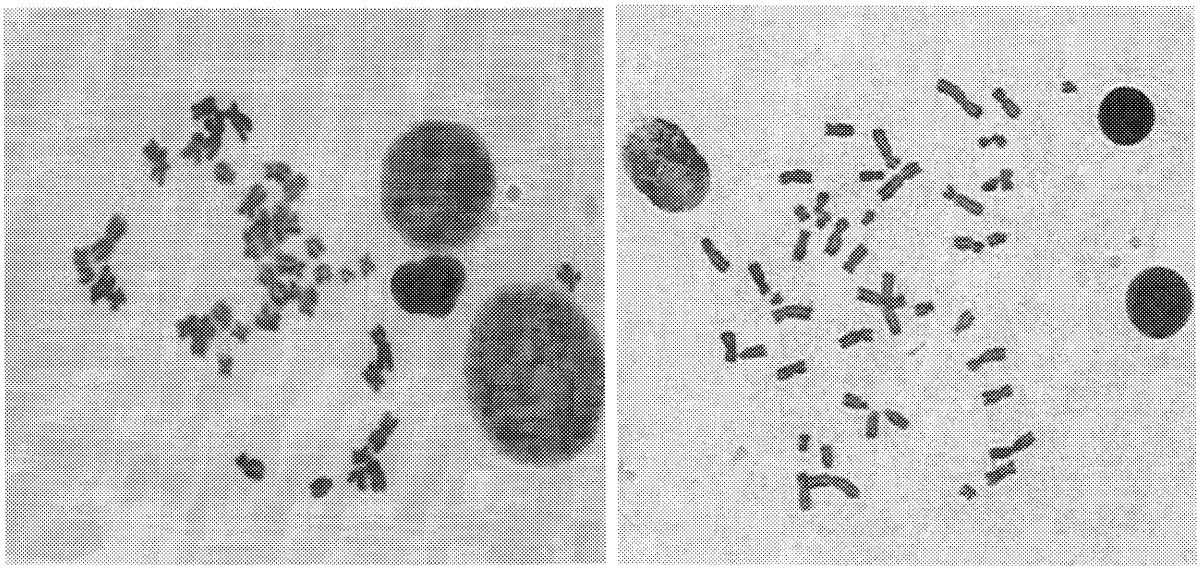

Fig.5. Images of metaphase spreading with 1000x magnification at 30 minutes of hypotonic treatment.

For metaphase spreading using lymphocytes from whole blood sample, 12 to 20 minutes incubation in 0.075M KCl at room temperature or 37°C is usually sufficient (IAEA, 2001; Henegariu *et.al.,* 2001). However, results have shown that longer incubation period during hypotonic treatment improved the metaphase images. Hypotonic treatment induces swelling of the cells and bursts open the red blood cells from peripheral blood cultures allowing their separation. Longer hypotonic incubations (45 to 60 minutes) or the use of a more hypotonic buffer resulted in longer, thicker, stickier, very difficult to spread and yielding poor banding but still good FISH signals. Too short hypotonic treatment leaves cell membranes around the chromosomes, which do not spread properly and have a poor banding and hybridization quality. We have found that hypotonic treatment of 30 minutes gives a good result in terms of chromosome spreading and clearer background. This may be explained by the fact that our country's climate is of higher humidity compared with the European countries. This study has given us the optimum duration for hypotonic treatment, and later on will help us greatly in slide preparation and slide analysis.

CONCLUSIONS

We have presented the effect of varying incubation times for hypotonic treatment of lymphocytes in dicentric assay technique. We have found that incubation time of 30 minutes gives the best metaphase images. This incubation time is longer than what has been recommended by the IAEA.

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