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CRYOPRESERVATION AND GENETIC DAMAGE

A thesis presented in partial fulfilment of
the requirements for the degree of
Masters of Science in Genetics
at Massey University, Palmerston North,
New Zealand

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2005

ABSTRACT

Blood analysis is time consuming and laboratories may require methods of storing samples until time permits analysis. The effects of storage on the sample are mostly unknown, yet some laboratories commonly store blood samples to allow processing of samples in batches. Cryopreservation is proposed as a convenient means of preserving blood samples, as the associated cold temperatures render cryopreservation an ideal storage method for tests requiring viable cells. In the literature, few studies have explored whether cryopreserved *whole* blood samples can be utilised effectively for cytogenetic testing.

This study extended the work on cryopreservation of blood to observe the cytogenetic effects of storing *whole* blood samples for an extended period. In this study three cytogenetic tests: Sister Chromatid Exchange (SCE), Micronucleus Assay (MN) and Fluorescence *in situ* Hybridisation (FISH) were conducted on whole blood samples from ten participants to observe whether the results from the cytogenetic tests are statistically consistent over a prolonged period of cryopreservation (fresh, one month, three months and six months). These tests were conducted on a single blood sample cryopreserved from each participant.

The results indicated that cryopreservation of whole blood is not a reliable method for storing blood samples prior to cytomolecular tests. The culturing of lymphocytes from cryopreserved blood was found to be inconsistent and the lymphocyte viability after cryopreservation reduced. When lymphocytes were successfully cultured, SCE and MN demonstrated increased genetic damage after a period of cryopreservation ($P = <0.050$ and $P = 0.016$ respectively) but FISH was not successfully performed on cryopreserved blood samples. It is unclear from the results obtained whether cryopreservation actually induces genetic damage or if the observed damage was the result of the specific storage technique.

ACKNOWLEDGEMENTS

Firstly, I would like to acknowledge and give my sincere thanks to my research supervisor, Dr Al Rowland. Over the last two years your assistance, input and moral support have been invaluable to me. Thank you.

I am indeed blessed with encouraging parents and friends. I would especially like to thank my Parents, Brad, Divya and Sarah for their full support throughout the highs and lows of this thesis and for tolerating all my 'thesis talk'. I love you all.

This research would not have been possible without the support of numerous skilled individuals. I would particularly like to thank the following people for their contributions:

Liz Nickless, Mohammed Abdul Wahab, Chad Johnson and Louise Edwards for technical advice, problem solving sessions and your company in the lab.

Vicki Scott and Chris Kendrick for the loan of equipment, endless assistance, valuable advice and for drawing numerous blood samples (even after some very difficult patients!).

The team at LA2 radiotherapy, especially Wendy for often squeezing me into your lunch break.

Clive Felix and his team at Wellington Hospital and Janine Gunderson at Palmerston North Hospital for your willing experimental assistance.

The New Zealand Nuclear Test Veterans Association and their president Roy Sefton.

Associate Professor John Podd for your assistance with experimental design.

Ted Drawneck for the statistical analysis of my results.

The William Georgetti Scholarship, New Zealand Federation of Graduate Women Scholarships, Massey Scholarship, Massey Masterate Scholarship, Goodman Family Scholarship, Molecular Genetics Research Scholarship and the Palmerston North Medical Foundation for funding.

ABBREVIATIONS

BrdU-	5-bromo-2-deoxyuridine
CB-	Cytokinesis-blocked cells
cm-	Centimeter
Cu-	Copper
dH_2O-	Distilled Water
DMSO-	Dimethyl Sulfoxide
DNA-	Deoxyribose Nucleic Acid
EBV-	Epstein Bar Virus
et al-	*Latin*, and others
EthD-1-	Ethidium Bromide Homologue
ex/em-	Excitation/Emission spectra
FISH	Fluorescence *in situ* Hybridisation
γ-	Gamma
g-	Gram
G-	Gauge
GHz-	Gigahertz
Gy-	Gray
H_2O-	Water
HCl-	Hydrogen Chloride
HCR-	Host-Cell Reactivation Assay
HIV-	Human Immunodeficiency Virus
Hrs-	Hours
KCl-	Potassium Chloride
L-	Litre
L N_2-	Liquid Nitrogen
μg-	Microgram
μL-	Microlitre
μm-	Micrometer
μM-	Micromolar concentration in moles/litre
MB-	Megabytes
M-	Molar concentration in moles/litre

M_a-	The mean number of micronucleated cytokinesis-blocked cells per 100 cytokinesis blocked cells
M_b-	The mean number of micronuclei per 100 cytokinesis-blocked cells
mg-	Milligram
mins-	Minutes
ml-	Millilitre
mm-	Millimeter
Mn-	Manganese
MN-	Micronucleus Assay
MqH_2O-	Milli-Q water
mRNA-	Messenger Ribose Nucleic Acid
N-	Sample Size
NaCl-	Sodium Chloride
NaOH-	Sodium Hydroxide
Nm-	Nanometer
No.-	Number
NZNTV-	New Zealand Nuclear Test Veterans
PBMC-	Peripheral Blood Mononucleate Cells
PHA-	Phytohaemagglutinin
PVP-	Polyvinylpyrrolidone
RAM-	Random Access Memory
rpm-	Revolutions per minute
secs-	Seconds
SCE-	Sister Chromatid Exchange
Std. Dev.-	Standard Deviation
Std. Error-	Standard Error
TM-	Thawing Media
UV-	Ultra Violet Light
W-	Watt
WBC-	White Blood Count
Zn-	Zinc

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