RELATIONSHIP BETWEEN CELLULAR RADIO AND CHEMOSENSITIVITY AND ENDOGENOUS GLUTATHIONE IN MAMMALIAN CELLS WITH RESPECT TO CYTOGENETICAL END POINTS

By

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I, Ansuman Chattopdhyay, hereby declare that the subject matter of thesis is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University / Institute.

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CONTENTS

ABBREVIATIONS

Ab. M.	Aberrant metaphase
AGT	Average generation time
AET	β-aminoethyl isothiouronium
BLM	Bleomycin
BMC	Bone marrow cells
BSO	Buthionine Sulfoximine
BUdR	5-Bromo 2-deoxyurudine
CAs	Chromosome aberrations
Chd. bk.	Chromatid breaks
DEM	Diethyl malate
Dicen.	Dicentric
DTNB	5-5' dithiobis 2 nitro benjoic acid
DTT	Dithio threitol
EDTA	Ethyl diamine tetraacetic acid
Exch	Exchange
FPG	Fluroscence plus Giemsa
GSH	Reduced-Glutathione
GSH-ester	Glutathione ethyl ester
Gy	Gray
HCT	Homocysteine thiolacetone
HPBL	Human peripheral blood lymphocytes
Isochd. bk.	Iso-chromatid break
KCl	Potassium Chloride
M1	Metaphase 1
M2	Metaphase 2
MISO	Misonidazole
MPA	Metaphosphoric acid
MPG	Monopropionyl glicine
NaCl	Sodium Chloride
Na_2HPO_4	di-Sodium Hydrogen Phosphate
Na_2CO_3	Sodium Carbonate
$NaHCO_3$	Sodium Bicarbonate

OER	Oxygen enhancement ratio
SCE	Sister Chromatid exchange
SCU	Sister Chromatid Union
SSb	Single strand break
TCA	Trichloro Acetic acid
TM	Total metaphase
WR-1065	Aminopropyl amino ethanethiol
WR-2721	Amino-propylamino ethylphodphorothioic acid

INTRODUCTION

The journey of the discovery of chemical radioprotectors in living systems started almost about 50 years ago. In early 1940's, the role of sulphur compounds in protection against radiation damage in complex chemical systems was recognised. Dale in 1942, first reported that colloidal sulphuur and thiourea can protect some enzymes against inactivation by X-rays. In 1948, Laterjit and Epharti reported that bacteriophages were effectively protected against radiation damage by thioglycolic acid, glutathione, cysteine and cysteine. In 1949, Barron et al. provided evidence that radiation induced inactivation of some SH-containing enzymes could be inhibited by glutathione(GSH). However, the first in vivo study of radioprotection was reported by Patt et al. (1949), that cysteine, a naturally occurring amino acid can increase the survivality of mice.

From the earliest days of research in this area, it was also proposed that radiotherapeutic procedures could be improved by the use of radioprotectors to protect normal tiissues, but not tumors, from radiation damage. As research developed, it was clear that studies with radioprotectors could also provide important information in the mechanisms of interaction of radiation with biomolecules. Ideas on the use of protective agents in combination with chemotherapeutic agents as well as in combination with radiotherapy, were merging because of the possibilities of common mechanisms of damage by the two trearment modalities. In addition to this, studies on endogenous protective systems that are important in protection against radiation and chemical induced damages were increasing dramatically.

Glutathione (γ- Glutamyl-cystenyl-glycine, GSH), is present almost universally in animal cells, most plant cells and bacteria. It is the dominant low molecular weight nonprotein thiol (NPSH) in mammalian cells and its level is inversely correlated to the cellular radiosensitivity (Revesz et al. 1963). It has been demonstrated that GSH plays an important role in cellular detoxification processes (Revesz and Modig 1965, see Revesz et al. 1984), regulates various enzymatic pathways by acting as a cofactor (see Meister and Anderson 1983) and it is involved in cell growth and replication process (Mazia 1961; Holmgreen 1979). Several reports suggest that increased radiation sensitivity is associated with defective GSH-metabolism. Various diseases like cataract of lens (Beutler and Srivastava 1974), 5- oxoprolinuria (Larson 1081), leukemia and anaemia (Sabine 1964; Macdougall 1968) have been reported to be associated with defective GSH metabolism.

Data available on the protective effect of GSH on radiation induced chromosome aberrations (CAs) are limited to deletion in *Tradescantia* root tips (Mikaelsen1952), anaphase bridges in grasshopper (Chaudhuri 1968) and *Hordeum* root tips (Reddy 1971) and sex linked lethals in *Drosophila* (Jacob and Roychoudhuri 1973). Only one study was made using Fluorescence plus Giemsa (FPG) staining to restrict scoring in the first cycle metaphases (Chatterjee and Jacob-Raman 1986). They reported that reduced GSH minimized X-ray induced CAs and cell cycle delay in muntjac lymphocytes.

In contrast to radiation, very few informations are available on the role of endogenous GSH, on chemosensitivity. Studies have shown an increased level of GSH and its related enzymes in tumour tissues, indicating a correlation to drug resistance (Carmichael et al. 1988; Cook et al. 1991; Di Ilio et al. 1988; Volm et al. 1991). GSH was also found to provide resistance to several anticancer drugs. These include alkylating agents (nitrogen mustards, nitrosourea-s), redox-cycling agents (adriamycin) and also to vinca alkaloids and epipodophilotoxins (Black and Wolf 1991). Mytomicin C (MMC) toxicity was found to be inversely proportional to cellular GSH-level (Kennedy et al. 1985).

Therefore, it will be interesting to see the influence of endogenous GSH-level on cellular damages induced by radiomimetic chemical bleomycin (BLM), an important anticancer drug.

Accumulated evidences clearly indicate that endogenous GSH is involved in many radiation induced chemical processes in cells and thus represents an important factor in determining internal cellular radiosensitivity. The role of endogenous GSH on radiosensitivity have been observed with both GSH-deficient and proficient cell lines, however, its potential contribution to the *in vivo* response has not been quantified,

So far, the yield of DNA single strand breaks (SSbs), and clonogenic survivality have been used as a criteria for the radiation response and cytogenetical parameters have not yet been considered as end points. Survivality may not be an appropriate criteria due to probable interference of biochemical processes and the damages on DNA analyzed by SSbs also do not reflect the true picture at chromosomal level (Hittelman and Pollard 1982). Therefore, in the present investigation, analysis of CAs, Sister chromatid exchanges (SCEs) and cell cycle kinetics are considered as the end points to asses the radio and chemosensitivity in normal and GSH-depleted cells.

The bone marrow cells of mouse (BMCs) and human peripheral blood lymphocytes (HPBLs) is considered to be an well established *in vivo* and *in vitro* experimental system respectively. This two systems were selected in the present investigation with the following objectives:

- Evaluation of the influence of endogenous GSH on CAs induced by Y-irradiation in both in vivo and in vitro systems
- Analysis of the involvement of GSH in repair/misrepair type of phenomenon after induction of damages on DNA by radiation.
- Analysis of the influence of endogenous GSH on radiomimetic chemical induced CAs, with a view to draw a comparison between the mechanism of action of the two clastogens and the influence of endogenous GSH on them.
- Determination of the role of GSH alone on cell cycle delay and SCEs.

The present investigation is presented on the basis of each objective. Therefore, total five separate chapters are formed. Each chapter comprises more background literature survey along with materials and methods, results and discussion. However, the present study is mainly inclined to establish a relationship between cellular radio and chemosensitivity and endogenous GSH-level. Therefore, the first chapter is mainly concerned about the measurement of endogenous GSH both in mouse bone marrow cells and human peripheral blood lymphocytes.