RESEARCH ARTICLE

Differential Behaviour of Selenium Analogs against Anticancer Drug Induced Apoptosis of Lymphocytes in Human Peripheral Blood

Sonaa Elango[1,3]*, Usha Subbiah[2], Jeong Jain[3]

Abstract

Sensitising cancer cells and at the same time desensitizing normal cells is a double task in cancer management. Agents which can combat the debilitating side effects of cancer therapeutics and simultaneously synergize with anticancer agents in specifically targeting cancer cells are needed. Selenium, a proven anticarcinogen, gains due importance in terms of its efficacy to combat the side effects of cancer therapy. This study is a comparative analysis of the chemoprotective effects of selenium compounds, methyl selenol (generated from organic selenomethionine (5mmol/L ; METase 40U/L)) and sodium selenite (inorganic form)(30μM) in peripheral blood human lymphocytes exposed to cisplatin and mitomycin. Biochemical alterations occurring in many cells during apoptosis include loss of plasma membrane phospholipid asymmetry, DNA fragmentation, and activation of caspase-3. The present study demonstrated that the selenium metabolite and selenite are efficient in protecting lymphocytes undergoing DNA damage and exerted their activity by reducing caspase 3 expression. Interestingly organic methylselenol (MeSe) was found to offer more protective effects compared to inorganic selenite (SeL), by reducing the induction of apoptosis by the cytotoxic agents. This suggests that MeSe and to a lesser extent selenite might have potential for assessment in clinical trials and could be considered as strong candidates in pharmacogenomics or in the nutriprotective arena.

Keywords: Apoptosis - anticancer drugs - lymphocytes - selenomethionine - DNA

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Introduction

'Apoptosis of lymphocytes' is a hallmark of immune suppression. This is considered as a distinct morphological type of cell death which has association with the generation of free radicals (Tynga and Abrahamse, 2015). Chemo- or Radiotherapy is also associated with enormous production of free radicals, which induce apoptosis in normal cells (Tabassum et al., 2010). Cisplatin and Mitomycin C that are often used in the treatment of cancer, display numerous physiological, toxic side effects and infact, might lead to secondary cancers. The search for methods to suppress their toxic manifestation is still enduring.

There is always a continuous search for, to analyse the capability of drugs/agents to enhance the effectiveness of chemo- or radiotherapy by sensitising cancer cells and at the same time desensitising normal cells as well. Selenium is one of the trace metal studied in this direction. Selenium is an essential trace element and is involved in antioxidant protection and the redox-regulation in humans (Castaldo et al., 2016). As an effective free radical scavenger selenium acts through the selenoenzyme-glutathione peroxidase (Battin and Brumaghim, 2009). Deficiency in selenium level results in decrease of glutathione peroxidase activity and thereby leads to susceptibility of tissues to injury by reactive oxygen species (Brozmanova 2011).

The mechanism behind selenium function is dual, either via a pro-oxidant pathway, as seen in cytotoxicity and apoptosis of cancer cells, or via an antioxidant pathway as proposed in cancer chemoprevention (Terazawa et al., 2010). This unique behavior of selenium provoked interest in us to analyse the impact of selenium compounds on chemotherapy associated side effects. There are reports that patients who previously had chemotherapy or surgery for their cancer had significantly lower blood selenium level than those who had radiotherapy ($p<0.04$) (Elango et al., 2006; Mueke et al., 2010). Selenite (SeL) or selenomethionine (SeM) are the most common selenium (Se) compounds taken as dietary antioxidants to reduce oxidative stress (Brozmanova, 2011; Shen et al., 2001).

It is quite interesting to note that cancer cells are substantially more sensitive to selenite and more prone

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to apop¬tosis induction than normal cells (Drake 2006), and this gives an opportunity to use it as therapeutic agent. However, taking into account the pro-oxidant properties of selenite (Spallholz, 1997; Drake, 2006), which potentially can damage healthy tissues, it is important to pay more attention to natural forms of selenium, in particular to SeM (Philchenkov, 2007). A recent study observed that SeM reduces apoptosis in breast cancer cells and oxidative stress (Guo et al., 2015). Methylselenol (MeSe) has been hypothesized to be a critical selenium(Se) metabolite for anticancer activity in vivo, and previous study has demonstrated that submicromolar methylselenol generated by incubating methionase with seleno-L-methionine inhibits the migration and invasive potential of HT1080 tumor cells (Zeng et al., 2009),including regulation of gene expression. However, its antiapoptotic/antioxidative property isunknown. Seleno methionine is a less-available metabolic source of selenium than selenite or selenate. So we proposed to analyse selenium metabolite methyl selenol, as elenomethionine origin and also the inorganic sodium selenite.

The aim of the present study is to make comparative analysis of the antiapoptotic efficacy of organic and inorganic form of selenium com¬pounds, namely methyl selenol of seleno methionine origin (an organic form) and Sodium Selenite (inorganic form) on anticancer drug cisplatin and Mitomycin C induced apoptosis in peripheral blood lymphocytes of human. The anticancer effect of selenium may have the ability to improve the immune status. With instance, to the production of anticarcinogenic Selenium metabolites, its antiapoptotic potentiality remains to be explored in depth. Likewise, the possible role of Selenium metabolite MeSe with immune response are also yet to be studied.

Materials and Methods

Compounds used

SelenoMethionine(SeM), METase enzyme (Sigma Chem. Co.,St. Louis, MO USA). Sodium Selenite (Cassel research laboratories, Chennai ,India). Cisplatin and Mitomycin C (Malar Hospital, Chennai, India). All other reagents were of analytical grade.

Treatment regimen

The experiments conducted were as follows: (Each group consist of 30 subjects) (Informed consent was obtained from each subject and all procedures were done with approval of the hospital ethics committee)

Group (I) control, lymphocytes were incubated for 24 hours. Group (II a,b) Methyl selenol(Selenomethionine 5mmol/L ; METase 40U/L) and Sodium Selenite (30μM) were added separately to the isolated lymphocytes and incubated for 24 hours. Group (III a,b) Cisplatin(10 μM) and Mitomycin C (5μM) were added individually to that of the isolated lymphocytes and incubated for 24 hours. Group (IV a,b,c,d) Cisplatin and Mitomycin C were added separately and incubated for 10 hours to the lymphocytes pre-treated with Methyl selenol and Selenite separately after incubating for 14 hours. Group (V a,b,c,d) Cisplatin and Mitomycin C were added individually to the lymphocytes and incubated for 10 hours and post-treated with Methyl selenol and Selenite separately followed with incubation for 14 hours.

Isolation of Lymphocytes

Ficoll-Hypaque (Lymphoprep, Nyegard, DenMark) lymphocyte separation medium, was used to isolate the lymphocytes from blood samples .The isolated lymphocytes were cultured in RPMI 1640 medium . For each experiment, 2 x 106 cells were grown in medium with L-glutamine supplemented with 10% heat inactivated foetal bovine serum (Sigma, USA), 100 IU/ml penicillin and 100 µg/ml streptomycin (Malar Hospital, Chennai) and incubated at 37°C for 24 hours.

Methylselenol generation: The enzyme (METase, L-methionine-g-lyase) solutions were prepared as previously described(13), divided into aliquots, and stored at -80°C. For cell treatments, the enzyme substrate seleno-L-methionine (SeM) (1.25-5 mmol/L) was added to the culture medium and was immediately followed by the addition of METase (40 U/L) for 16 hours.

Apoptosis detection

Cellular morphology analysed by the light microscope, phase contrast microscope, and interference contrast microscope (Zeng et al., 2009; Bedner et al., 1999). 2. Nuclear morphology, sister chromatid exchange analysed by light microscope and DNA quantitation by feulgen cytochemical reaction (Auer et al 1994; Kiernan et al 1981). 3. DNA damage by Comet assay (single cell gel electrophoresis (Singh et al., 1995). 4. Biochemical changes associated with lymphocyte apoptosis:a. Malondialdehyde, lipid peroxidation product(Ohkawa et al., 1979), b. Thiol protein ,c. Gpx(Rotruck et al., 1973), d. Selenium level measured by atomic absorption spectrophotometry (The detailed protocol of all of the above mentioned methods were given elsewhere, published already).

For methods to analyse cell nuclear morphology, DNA quantitation, and biochemical changes,Groups I,III,IV,V were analysed at the time zero (the time of separation of the lymphocytes),after 24 and 72 hours. Group II analysed at time zero hr, after 10 mins, 24 hr. For method to analyse sister chromatid exchange (SCE) all groups were analysed at time intervals of 0, 24, 48 hours.

SCE assay

In order to avoid possible interference between selenium analogs and serum components, we exposed the cells to serum-free medium. This permitted the lymphocytes to grow faster than in the presence of serum, due to the absence of inhibition by serum components. Preliminary experiments (unpublished observations) showed that up to 65% of the cells were in the second division at 48 h after anticancer agents stimulation, and >60% of the cells were in the third division at 72 hours. Under these conditions, we harvested cells at 66 hours. Reproducible results were obtained from subjects, and Selenium analogs appeared to be toxic at higher concentrations, >50×10-3 M (Data not shown). SCE frequencies were greatest at this higher concentration, so no further experiments were performed

with them. Nonetheless, these results suggest a possible role in protecting the target cells (lymphocytes) from the toxicity of selenium analogs. In the set of experiments conducted with lymphocyte cultures, we exposed the cells to selenium analogs for a dose of 30μM for periods of time, at 24 hours, 72 hours after initiating the cultures.

Caspase -3 protein level assay by ELISA Method

Lymphocytes were treated with mouse monoclonal primary antibody against human caspase-3 protein. After secondary antibody treatment the activity of bound alkaline phosphatase conjugate was monitored by the addition of 100 µl of substrate BCIP/NBT reagent. The purple color reaction product was then read at the absorbance at 590 nM in a micro plate reader and the activity of Caspase-3 protein was expressed as OD.

Statistics analysis

Statistical analysis was performed with SPSS version 15.0 for windows (SPSS, Chicago, IL, USA). All data presented as mean ±SD values, and assessed by t test. Values of $p< 0.05$ were considered significant.

Results

The results are expressed under three distinct lines: I. Cellular changes, including viability, morphology, SCE, DNA quantitation and DNA damage; II. Biochemical changes, including lipid peroxidation activity assessed by MDA, and scavenger systemassessed by thiol protein and selenium level; and III. Apoptotic cell death index

Incubation of lymphocytes with anticancer drugs

The non-viable lymphocytes obtained from 100 cells for each individual subject of the normal was 4.22±0.51, 40.72±7.11, 100±0.0, at time zero, after 24 and 72 hours incubation respectively. The cellular morphology was assessed by phase contrast microscopy, the apoptotic processes were followed as such stage I: membrane bleb formation, stage II: flattening of cells with protrusion of echinoid spikes and stage III: formation of membrane blisters and disintegration of cells into apoptotic bodies. The progress in apoptotic process was time dependent as shown in Table 1.The apoptosis of the lymphocytes after 10 minutes of incubation with cisplatin and mitomycin C, was 11.5±2.66;13.9±1.98. After 24 hours all lymphocytes were apoptotic with statistically significant difference. There was a rapid reduction in DNA contents, Figure 1c (190.76±7.38; 198.31±8.13 O.D unitx 10-3), after 24 hours. These changes are associated with a significant increase in TBE, MDA production (Figure 1a, 1e) ($p<0.05$), depletion of thiol, Gpx and selenium (Figure 1g,i,k)($p<0.05$).

Incubation of lymphocytes with antioxidants

Controlled cell viability observed in all study groups is given in Table 2. After 72 hours incubation with MeSe and SeL 57.31 ±10.11; 69.11 ± 11.3 of lymphocytes were

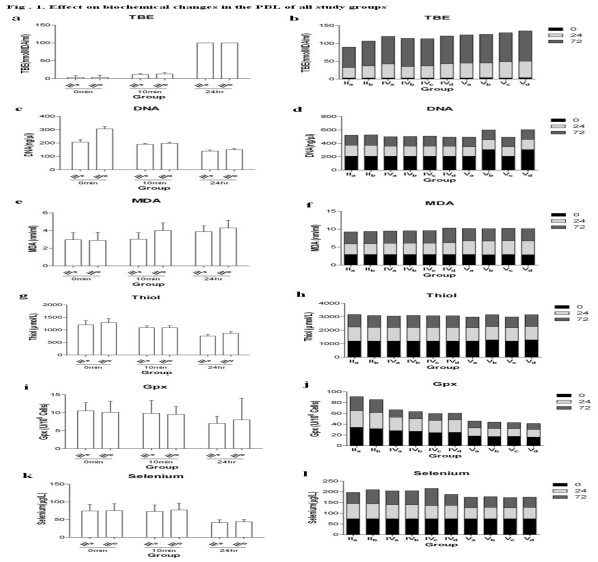

Figure 1. Effect on Biochemical Changes in the Human Peripheral Blood Lymphocytes of All Study Groups. (a) TBE level of Cisplatin , Mitomycin C treated cells ; (b) TBE level of MeSe , SeL treated cells ; (c) DNA level of Cisplatin , Mitomycin C treated cells ; (d) DNA level of MeSe , SeL treated cells ; (e) MDA level of Cisplatin , Mitomycin C treated cells ; (f) MDA level of MeSe , SeL treated cells ; (g) Thiol level of Cisplatin , Mitomycin C treated cells ; (h) Thiol level of MeSe , SeL treated cells ; (i) GPx level of Cisplatin , Mitomycin C treated cells ; (j) GPx level of MeSe , SeL treated cells ; (k) Selenium level of Cisplatin , Mitomycin C treated cells ;(l) Selenium level of MeSe , SeL treated cells

Table 1. Efffects of Cisplatin, Mitomycin C, Methylselenol and Selenite on Morphological Stages of Lymphocyte Apoptosis

N=30	Time	Total non-viable cells	Stage I	Stage II	Stage III
	0 h	4.22±0.51	4.22±5.1	0	0
Blank	24 h	40.72 ±7.11	4.33±0.04	13.4±3.3	23.01± 9.88
	72 h	100± 0.0	2.11±0.31	40.91±9.99	58.21 ±13.01
Cisplatin	10 min	11.5± 2.66	8.66±0.77	3.5±0.58	0
Mitomycin C	10 min	13.9 ±1.98	10.5±0.85	5.6± 0.66	0
Cisplatin	24 h	100± 0.0	15.13±2.1	35.11±10.1	50.45±9.99
Mitomycin C	24 h	100± 0.0	16.35±2.3	39.28±9.7	52.39±10.2
Methyl Selenol	v	29.21± 5.21	5.61± 0.33	10.11±1.89	14.87±3.34
	72 h	57.31± 10.11	5.56±0.31	18.25±2.01	34.52±8.99
Selenite	24 h	35.33± 6.2	5.11±0.66	12.23±6.21	18.88±3.46
	72 h	69.11± 11.3	4.31±0.41	25.55±11.10	40.82± 5.56

Results expressed as mean ± SE of the mean

apoptoticand 99.66 cells died in the control group($p < 0.05$).The DNA contents were significantly preserved, (150.24 ± 11.3; 158.31 ± 14.61 O.D unit x 10-3) after 72 hours incubation with MeSe and SeL respectively vs. (140.62 O.D unit x 10-3) control, (Figure 1d) ($p<0.05$). The level of TBE (57.27±10.68; 69.87±10.21) (Figure 1b), MDA level (3.34 ± 0.4; 3.43 ± 0.42 μMol/ml) was observedafter 72 hours incubation with MeSe and SeL respectively vs. (5.27 μMol/ml) control (Figure 1f) ($p<0.05$). Thiol proteins were significantly preserved, which were (920.7 ± 80.72, 886.48 ± 70.43 µmol/L) after 72 hours incubation with MeSe and SeL respectively (747.45 µmol/L) compared with control(Fig 1h)($p<0.05$).Similar wise Gpx activity was found to be preserved with MeSe (26.2±9.7) and SeL (24.1±3.1) supplementation after 72hours (Figure 1j) ($p<0.05$). This is in correlation with the selenium level as Selenium was only reduced to (52.4 ± 3.2 and 66.17 ±2.4 µg/L) after 72 hours incubation with MeSe and SeL respectively vs. (42.25 µg/L) controlwhichshoweda significant statistical difference (Figure 1)($p<0.05$).In all of the above studies the pretreatment groups showed significantly better result compared with post treatment groups of MeSe and SeL with Cisplatin, Mitomycin C treated groups.

SCE determination

We determined the frequency of SCE (Figure 2), using blood samples (5ml) to establish cell cultures during 72h. Average SCEs/cell±S.E. of anticancer drugs and selenium analogs well analysed. 30 second-division metaphases

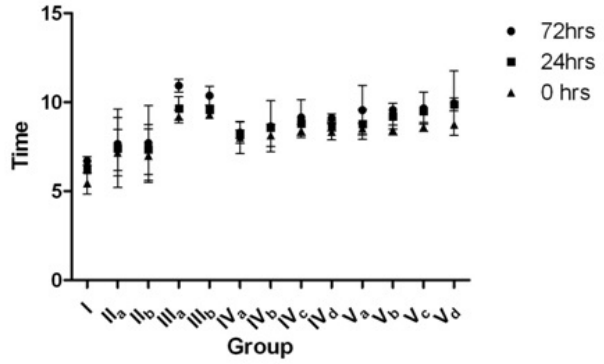

Figure 2. Sister Chromatid Exchange in Human Peripheral Blood Lymphocytes of all Study Groups

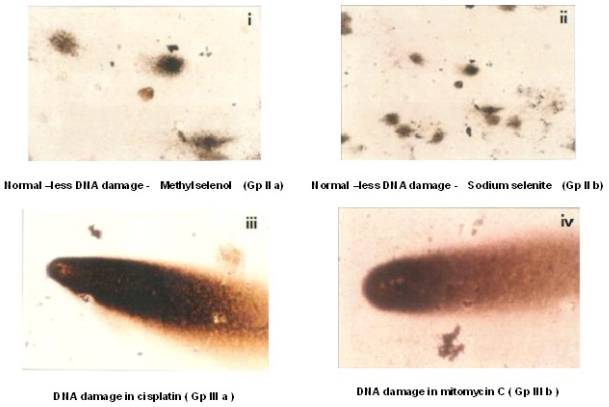

Figure 3. Comet Assay Results. i and ii denote representative photos of normal to mild damage in MeSe, SeL group, iii and iv denote extensive DNA damage by Cisplatin, Mitomycin C

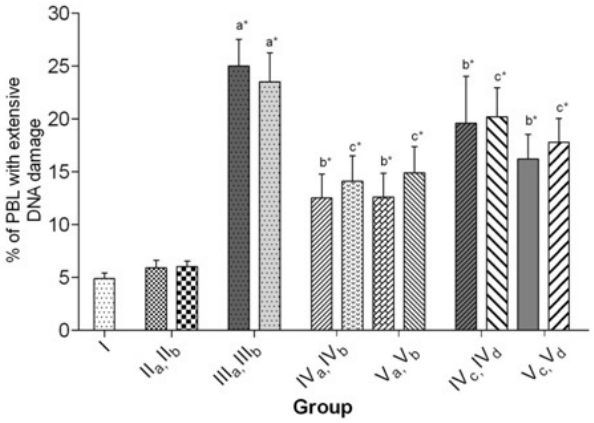

Figure 4. Percentages of Peripheral Human Blood Lymphocytes with DNA Damage by Comet Assay. a - group IIIa,b compared with group IIa,b ; b - group IVa,c, Va,c compared with group IIIa ; c - group IVb,d, Vb,d compared with group IIIb. * - denote statistically significant value $p<0.05$

Table 2. Effects of Methylselenol and Selenite on Morphological Staging of Apoptotic Lymphocytes Stimulated by Cisplatin and Mitomycin C

M=30	Time	Total non viable cells	Stage I	Stage II	Stage III
IVa	24h	49.11 ±10.1	4.33± 0.31	12.23± 6.21	14.88± 3.46
	72h	60.91± 7.01	5.56± 0.66	12.56± 9.01	14.98± 2.31
IVb	24h	50.1 ±1.21	4.31± 0.51	12.91±2.66	15.01± 2.32
	72h	74.01 ±2.31	4.41± 0.55	13.52± 1.31	14.91± 7.01
IVc	24h	49.98± 2.31	4.23± 6.34	12.11± 1.66	31.88± 3.46
	72h	62.31± 1.11	5.11± 0.31	12.25± 1.89	34.52± 8.99
IVd	24h	51.9± 1.39	4.91± 1.89	12.55± 2.01	35.18± 7.05
	72h	76.98± 1.85	5.99± 9.88	13.28± 6.21	35.97± 4.31
Va	24h	59.99± 1.98	4.11± 0.31	25.13± 9.99	39.91± 0.31
	72h	86.89± 2.66	14.99 ±0.55	30.82± 5.56	38.23± 6.21
Vb	24h	63.21± 7.10	1.35± 2.01	29.55± 11.10	45.11± 0.66
	72h	81.51± 1.89	13.91± 0.33	31.23± 6.11	46.31± 0.41
Vc	24h	64.31± 6.2	14.9± 0.41	12.23± 0.58	40.11± 5.66
	72h	88.33± 10.1	13.31± 0.31	25.55± 2.01	41.23± 0.39
Vd	24h	61.12± 6.2	13.93± 6.34	26.11± 1.89	40.77± 6.21
	72h	87.22± 1.98	13.51± 7.41	25.51± 9.71	42.59± 7.15

Results expressed as mean ± SE of the mean

were examined from each subject. The results showed a significantly higher SCE frequency in Cisplatin (9.2 ±0.35),Mitomycin C (9.29 ± 0.14) ,than in MeSe (7.16 ± 1.3) and SeL(6.99 ± 1.5)($p<0.01$) treated groups. A significant difference in SCE frequency was also shown when comparing the IV a,b,c,d groups of pretreatment set ($p<0.05$) with post treatment of antioxidants V a,b,c,d. Interestingly, not much significant difference was found when comparing the groups within pre and post sets ($p>0.05$).

DNA damage extent by comet assay

The extent of DNA damage in peripheral blood lymphocytes of all groups was measured by single-cell gel electrophoresis (comet assay) (Figure 3). Results of visually scoring in 100 randomly selected cells from each sample in all study groups are shown in Figure 4. Each cell was visually scored as previously described according to the criteria as no damage, mild to moderate damage, and extensive DNA damage. Extent of DNA damage within peripheral blood lymphocytes was expressed as the percentage of cells with extensive DNA damage.

Caspase-3 protein analysis by ELISA

Both unstimulated and antioxidants stimulated lymphocytes expressed a low level of caspase-3. As shown in Figure 5, lymphocytes when exposed to cisplatin showed significant increase in the level of caspase-3 ($p<0.05$) when compared to untreated and in cells treated with MeSe and SeL. Addition of MeSe and SeL to the cells exposed to cisplatin showed statistically significant decrease in caspase-3 level ($p<0.05$) when compared to cells exposed to cisplatin. Lymphocytes treated with SeL expressed normal levels of caspase-3. SeL treated cells exposed with cisplatin showed a significant reduction ($p< 0.05$) in caspase-3, when compared with lymphocytes exposed to cisplatin alone. Similar results ($p< 0.05$) were observed with Mitomycin C groups. Similarwise, a significant decrease in caspase -3 level of pretreated antioxidants ($p<0.05$) was observed when compared to post treated antioxidants added to cells exposed to anticancer drugs.

Discussion

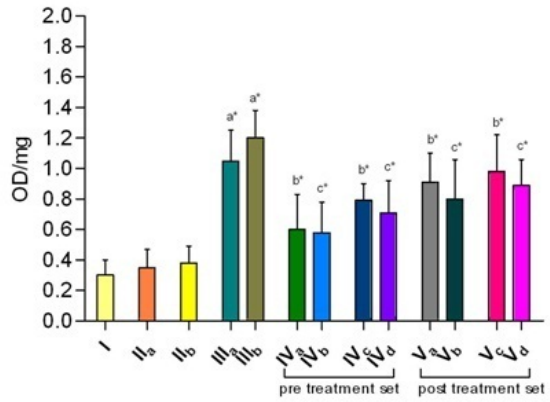

Figure 4. Caspase-3 Protein Levels in Human Peripheral Blood Lymphocytes of all Study Groups. a - group III a,b compared with group II a,b ; b - group IVa,c, Va,c compared with group IIIa ; c - group IVb,d, Vb,d compared with group IIIb. * - denote statistically significant value $p<0.05$

Selenium is a toxic mineral with a fairly small therapeutic window. Selenoproteins, shows the importance of activating T-cell function and control of the immune response. In the present study, where lymphocytes were pretreated with MeSe and exposed to cisplatin, the percentage of viable cells increased. Likewise, in MeSe pretreated cells exposed to mitomycin C, the percentage of viable cells increased with the corresponding decrease in early and late apoptosis. The lymphocytes in control as well as those treated with natural compounds maintained their viability. Cisplatin can bind to phospholipids and phosphatidyl serine in the cell membrane before it enters into the cell (Dasari and Tchounwou, 2014) and mitomycin C can also expose phosphatidyl serine on the outer membrane. Further it can enter into the cell, induce nuclear damage leading to necrotic stage. The decrease in the exposure of phosphatidyl serine due to MeSe might be that MeSe prevents the breakdown of fatty acids and inhibits the release of phosphatidyl choline and the serine content of the cell membrane (Zeng et al., 2009).

Selenium has dual nature, as it undergoes anaerobic glycolysis with the excess of glutathione present inside the cancer cells in a hypoxic environment producing anticarcinogenic metabolites like seleno diglutathione. While in the aerobic condition prevailing in the normal cells selenium acts as antioxidant, scavenging the excess deleterious free radicals. This concept is evidenced in our study results that both MeSe and SeL decrease MDA level and preserve the thiol proteins, DNA content and improve the selenium level, compared with anti cancer drugs stimulated lymphocytes. Selenium, as part of the enzyme Gpx was found to stabilize SH-group of protein and acts as a free radical scavenger (Brozmanova et al., 2010). GPx is an antioxidant enzyme which protects membrane lipids and macromolecules against the oxidative damage generated by peroxides (Jayaprakash et al., 2015) Hsu et al., 1997). As most of the seleno proteins have ROS scavenging activity, selenium has been known as an important antioxidant system in cell survival. Present study resulted in increase inthe selenium level corresponding to Selenium supplementation, directly correlates with an increased Gpx activity, which denotes the higher incorporation of selenium into Gpx.

There are reports stating the protective role of antioxidants like vitamins A ,C and E against the induced genotoxic damage in cultured human lymphocytes (Alpsoy et al., 2009). This goes in accord with our current examination of the cytogenetic changes, decreased by selenium analogs in a dose-dependent manner (i.e., as the doses increased, their protective effects also increased, data not shown). However, MeSe could effectively inhibit anticancer agent induced SCE compared to SeL. Selenium exhibits pro- as well as anti-apoptotic effects Weber et al., 2008). On one hand it is discussed as an anticancer agent, since it induces apoptosis in tumor cells via DNA damage (Ghosh et al., 2015). On the other hand it protects neuronal cells against hypoxia induced apoptosis (El-Mazary et al., 2015), inhibits reactive oxygen species-mediated apoptotic cell death *in vitro* and *in vivo*. The anticancer effect of SeM may relate more closely to its ability to

enhance the immune response or, more likely, to its ability to produce anti-tumorigenic metabolites (eg, MeSe or its precursors) that can perturb tumour-cell metabolism, inhibit angiogenesis, and induce apoptosis of cancer cells.

As a reflection of the above reports in our current study the reduction in the ability to induce apoptosis upon addition of MeSe and SeL might restabilize the membrane potential and stacking of the DNA bases; thus, reducing the damage induced by mutagens cisplatin and mitomycin, in normal cells. The cytotoxicity of cisplatin and mitomycin C are by induction of DNA damage by altering the phosphate backbone, sugar or base by modifications such as alkylation,cross-links or formation of bulky DNA adducts that are substrates for DNA repair mechanisms. The lesions produced by these agents could trigger apoptosis. A study on sepsis condition denoted that the depletion of serum selenium may contribute to accelerated apoptosis in severe sepsis. Our study also falls in acceptance to these published report that selenium analogs precisely inhibited apoptosis, which is evidenced in the decreased DNA damage in peripheral blood, shown by comet assay.

Selenium treated lymphocytes showed a normal level of caspase-3, whose activation is involved in executing apoptosis with resultant increase in DNA damage. This is well supported by earlier studies on Selenium with Doxorubicin had minimal cytotoxicity against quiescent and mitogenically stimulated human peripheral blood lymphocytes (Santos and Takahashi 2008). These results supported our data that Selenium did not induce apoptosis in control unstimulated and stimulated lymphocytes.CD95 receptor causes mitochondrial release of cytochrome C and caspase activation (Tatsukawa et al., 2011). Reports indicate that caspase-3 is not only involved in executing the apoptosis phenotype but is also essential for the processing of upstream procaspase-9 in cisplatin induced apoptosis (Elango et al., 2006). The significant decrease in the level of caspase-3 in MeSe treated cells exposed to cisplatin and mitomycin C, point out that selenium reduced the caspase-3 levels in cisplatin induced lymphocytes. The correlation of Selenium with DNA damage and caspase can be described as the decrease in caspase level by selenium analogs inhibited apoptosis with a sequential decrease in DNA damage.

SeL suppresses hydrogen peroxide induced apoptosis through inhibition of ASK1/JNK and activation of the PI3-K/Akt pathways (Bargonetti et al., 2010). Our result well supported the above reports that Selenite protected normal cells and induced apoptosis in cancer cells. With a high Se intake, the extra Se can enrich the MeSe pool by the methylation pathway (Ganther, 1999).

In conclusion, selenium is very toxic to kill cancer cells. Current findings supports the characteristics of inorganic Selenium compounds, having high redox potency, which represents a promising option in cancer therapy and organic form of Selenium good for normal cells. In this aspect of differential behavior of selenium analogs, it maybe appreciable if a combined supplementation study of these two drugs is done further, as it would render their synergistic action in killing cancer cells and at the same time protecting normal cells. Moreover, in certain studies Selenium compounds pharmacologically, i.e. in doses well above physiological level were given, while in certain studies the doses given were very high. So these aspects of dosage level and forms given for patients need to be addressed in depth to clearly understand the exact way to combat the side effects of anticancer drugs. Still more randomised controlled trials over the effect of selenium supplementation on other therapy-associated toxicities; quality of life; performance status in cancer patients are necessitated.

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References

Auer G, Eriksson E, Azaved E et al (1984). Prognostic significance of nuclear DNA content in Mammary adenocarcinomas in humans. *Cancer Res*, **44**, 394-6.

Alpsoy L, Agar G, Ikbal M (2009). Protective role of vitamins A, C, and E against the genotoxic damage induced by aflatoxin B1 in cultured human lymphocytes. *Toxicol Health*, **25**, 183-8.

Andea L, Isgro G, Pleguezueli M et al (2009). Surveillance and diagnosis of hepatocellular carcinoma in patients with cirrhosis. *World J Hepatol*, **1**, 48-61.

Alcindor T, Beauger N (2011). Oxaliplatin: a review in the era of molecularly targeted therapy. *Curr Oncol*, **18**, 18-25.

Abbas T, Olivier M, Lopez J, Houser S, Xiao G, Suresh kumar G, et al (2002). Differential activation of p53 by the various adducts of mitomycin C. *J BiolChem*, **277**, 40513-9.

Bedner E, Li X, GorczycaW, Melamed MR, Darzynkiewicz Z (1999). Analysis of apoptosis by laser scanning cytometry. *Cytometry*, **35**, 181-95.

Battin EE, Brumaghim JL (2009). Antioxidant activity of sulfur and selenium: a review of reactive oxygen species scavenging, glutathione peroxidase, and metal-binding antioxidant mechanisms. *Cell Biochem*, **55**, 1-23.

Bargonetti J, Champeil E, Tomasz M (2010), Differential Toxicity of DNA Adducts of Mitomycin C. *J Nucleic Acids*, **4**, 1-6.

Brozmanova J, Manikova D, Vlčkova V, Chovanec M (2010). Selenium: a double-edged sword for defense and offence in cancer. *Arch Toxicol*, **84**, 919-38.

Brozmanova J (2011). Selenium and cancer: from prevention to treatment. *Klin Onko*, **24**,171-9.

Castaldo SA, Freitas JR, Conchinha NV, Madureira PA (2016). The Tumorigenic Roles of the Cellular REDOX Regulatory Systems. *Oxidative Med Cell Long*, **17.**

Drake EN (2006). Cancer chemoprevention: selenium as a prooxidant, not an antioxidant. *Med Hypotheses*, **67**, 318-22.

Dasari S, Tchounwou PB (2014). Cisplatin in cancer therapy: molecular mechanisms of action. *Eur J Pharmacol*, **0**, 364-78.

Ellman GL (1959). Tissue sulfhydryl groups. Arch Biochem Biophys, 82, 70-7.

Elango N, Samuel S, Cinnakannu P (2006). Enzymatic and Non enzymatic antioxidant status in stage (III) human oral squamous cell carcinoma and treated with radical radiotherapy: Influence of selenium supplementation. Clinica Chimica Acta, 373, 92-8.

El-Mazary AM, Aziz RA, Mahmoud RA, El-Said MA,

Mohammed NR (2015). Correlations between maternal and neonatal serum selenium levels in full term neonates with hypoxic ischemic encephalopathy. Ital J Pediatr, 41, 83.

Ganther HE (1999). Selenium metabolism, selenoproteins and mechanisms of cancer prevention: complexities with thioredoxinreductase. Carcinogenesis, 20, 1657-66.

Guo CH, Hsia S, Shih M, Hsieh FC, Chen PC (2015). Effects of Selenium Yeast on Oxidative Stress, Growth Inhibition and Apoptosis in Human Breast Cancer Cells. Int J Med Sci, 12, 748-58.

Ghosh P, Roy SS, Basu A, Bhattacharjee A, Bhattacharya S (2015). Sensitization of cisplatin therapy by a naphthalimide based organoselenium compound through modulation of antioxidant enzymes and p53 mediated apoptosis. *Free Radic Res*, **49**, 453-71.

Jayaprakash R, Ramesh V, Sridhar MP, Sasikala C (2015). Antioxidant activity of ethanolic extract of Tinospora cordifolia on N-nitrosodiethylamine (diethylnitrosamine) induced liver cancer in male Wister albino rats. *J Pharm Bioallied Sci*, **7**, S40-5.

Kiernan JA (1981). Methods for nucleic acid. In histological and histochemical methods theory and practical. 2nd ed. Pergaman Press Oxford 104-7.

Menter DG, Sabichi AL, Lippman SM (2000). Selenium effects on prostate cell growth. *Cancer Epidemiol Biomarkers Prev*, **9**, 171-1182.

Mueke R, Schomburg L, Buentzel J, et al (2010). Selenium or no selenium- that is the question in tumor patients: a new controversy. *Integr Cancer Ther*, **9**, 136-41.

Ohkawa H, Ohishi N, Yagi K (1979). Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. *Anal Biochem*, **95**, 351-8.

Rotruck JT, Pope AL, Ganther HE, et al (1973). Selenium: Biochemical role as a component of glutathione peroxidase. *Science*, **179**, 558 - 90.

Stadtman TC (1991). Biosynthesis and function of selenocysteine-containing enzymes. *J Biol Chem*, **266**, 16257-60.

Singh NP, Graham MM, Singh V, Khan A (1995). Induction of DNA single-strand breaks in human lymphocytes by low doses of Y-rays. *Int J Radiat Biol*, **68**, 563-9.

Spallholz JE (1997). Free radical generation by selenium compounds and their prooxidant toxicity. *Biomed Environ Sci*, **10**, 260-70.

Santos RA, Takahashi CS (2008). Anticlastogenic and antigenotoxic effects of selenomethionine on doxorubicin-induced damage *in vitro* in human lymphocytes. *Food ChemToxicol*, **46**, 671-7.

Terazawa R, Garud DR, Hamada N, et al (2010). Identification of organoselenium compounds that possess chemopreventive properties in human prostate cancer LNCaP cells. *Bioorg Med Chem*, **24**, 7001-8.

Tatsukawa H, Sano T, Fukaya Y et al (2011). Dual induction of caspase 3- and transglutaminase-dependent apoptosis by acyclic retinoid in hepatocellular carcinoma cells. *Molecular Cancer*, **10**, 1-11.

Tynga IM, Abrahamse H (2015). Cell death pathways and phthalocyanine as an efficient agent for photodynamic cancer therapy. *Int J Mol Sci*, **16**, 10228-41.

Weber SU, Lehmann LE, Schewe JC, et al (2008). Low serum α-tocopherol and selenium are associated with accelerated apoptosis in severe sepsis. *Bio Factor*, **33**, 107-19.

Yeo JE, Kang SK (2007). Selenium effectively inhibits ROS-mediated apoptotic neural precursor cell death *in vitro* and *in vivo* in traumatic brain injury. *Biochim Biophys Acta*, **1772**, 1199-210.

Zhou N, Xiao H, Li TK, et al (2003).DNA damage-mediated apoptosis induced by selenium compounds. *J Biol Chem*, **278**, 29532-37.

Zeng H, Wu M, Botnen JH (2009). Methylselenol, a Selenium Metabolite, Induces Cell Cycle Arrest in G1 Phase and Apoptosis via the Extracellular-Regulated Kinase 1/2 Pathway and Other Cancer Signaling Genes1-3. *J Nutr*, **139**, 1613-1618.