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Chemoprevention of Prostate Cancer With Selenium: An Update on Current Clinical Trials and Preclinical Findings

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Abstract Prostate cancer is the most common cancer diagnosed and the second leading cause of cancer-related deaths in men in the United States. The etiological factors that give rise to prostate cancer are not known. Therefore, it is not possible to develop primary intervention strategies to remove the causative agents from the environment. However, secondary intervention strategies with selenium (Se) compounds and other agents represent a viable option to reduce the morbidity and mortality of prostate cancer. In this review, we discuss ongoing clinical trials. In addition, we discuss preclinical mechanistic studies that provide insights into the biochemical and molecular basis for the anti-carcinogenic activity of both inorganic and organic forms of Se. J. Cell. Biochem. 91: 443–458, 2004.

Key words: selenium; morbidity; anti-carcinogenic activity; prostate cancer; signaling; selenomethionine

PROSTATE CANCER

Prostate cancer is the second most frequently diagnosed malignancy in men in the US and is surpassed in incidence only by non-melanoma skin cancers. In 2002, approximately 189,000 men were diagnosed with prostate cancer and the disease caused an estimated 30,000 deaths [Clegg et al., 2002]. There has been significant progress in the past decade that has improved our understanding of the disease, however, there is still much to be learned about the causes, early detection markers for diagnosis and prognosis determination, treatment and prevention of prostate cancer. Although approaches to primary prevention of prostate cancer are being tested, none have been proven to be effective yet. The most common strategy for reducing prostate cancer morbidity and mortality is periodic examination of the prostate by digital rectal exam and screening of peripheral prostate specific antigen (PSA) [Stein and Lindenmayer, 1997; Ward et al., 1997; Nivens et al., 2001]. However, the value of currently employed screening methods still remain a topic of controversy [Perkins et al., 1998; Edlefsen et al., 1999; Godley, 1999; Voss and Schectman, 2001]. Prevalence of indolent prostate cancer found during autopsies can be greater than 40% for men over the age of 60 [Gatling, 1990] emphasizing the need for development of agents

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that can prevent initiation of carcinogenesis as well as have activity in the early stages of prostate carcinogenesis.

Our current inability to differentiate between primary tumors that will result in a fatal disease from a tumor that will grow very slowly and hence be clinically insignificant in some men presents a significant clinical challenge. Several areas of research must be approached. First, it is critical that we identify genetic, physiological, and environmental factors that contribute to increased risk. Second, molecular and cellular processes contributing to development, invasion, and metastases of prostate cancer must be examined for development of improved early detection methods and targeted therapies. Third, continuous epidemiological studies must continue to recognize the relationship of incidence and mortality in different populations and within families. Finally, in vitro and in vivo models of prostate cancer must be developed to facilitate preclinical studies that will lead to effective agents for treatment and prevention of prostate cancer.

This review takes a comprehensive look at the current literature regarding selenium (Se) and prostate cancer. Ongoing clinical trials and endpoints are discussed as well as preclinical mechanistic studies that provide insights into the mechanisms of both inorganic and organic forms of Se.

EPIDEMIOLOGIC AND RETROSPECTIVE STUDIES OF Se AND CANCER

Epidemiological studies conducted over the past 40 years examining the relationship between dietary intake of Se and total cancer risk have been somewhat controversial. In addition, numerous in vivo studies have showed that dietary supplementation with Se reduces cancer incidence in a variety of animal models including a model of mouse melanoma [Hanada et al., 1986] and models of cancer of the colon [Wattenberg, 1974; Temple and Basu, 1987], breast [Schrauzer et al., 1976; Watrach et al., 1984; el-Bayoumy, 1994], liver [Yu et al., 1988; Bansal et al., 1990; Popova, 2002], esophagus [Guttenplan et al., 2002], kidney [Schroeder and Mitchener, 1972; Poirier and Milner, 1983], and lung [Liu et al., 1987; el-Bayoumy et al., 1993; Prokopczyk et al., 1997, 2000]. Table I lists four ecological studies that were conducted that showed an inverse relationship between intake of dietary Se and overall cancer risk. However, for some of these studies, statistical significance was only marginal. The studies conducted by Schrauzer et al. [1977a,b] analyzed age-corrected mortalities from cancer at 17 major body sites. Significant inverse correlations were observed between Se intake and risk for cancers of the colon, prostate, breast, ovary, and lung as well as with hematopoietic cancers, while only weak inverse correlations were observed for cancers of the pancreas, skin, and bladder. Although not all of the studies showed a protective effect of high plasma Se level, contradictory results are thought to be partially related to the variations in data collection and methods used to measure Se levels [Navarro-Alarcon and Lopez-Martinez, 2000].

Data obtained from subsequent observational and retrospective studies are also conflicting. Of the studies shown in Tables II and III, approximately half showed a statistically significant inverse correlation between Se and overall cancer risk. Studies conducted by Willett et al. [1983] measured peripheral Se levels in 111 subjects with cancer compared to 210 cancer-free, age-matched controls. Analyses of these data suggested that risk for subjects in the lowest quintile of serum Se was twice that of subjects in the highest quintile. The effect was most pronounced in gastrointestinal and prostate cancers. Salonen et al. [1984] also demonstrated an inverse association between peripheral Se level and cancer risk. In these studies, a matched-pair analysis conducted with data derived from a prospective, 6 year follow-up of a random population of over 8,000 persons in Finland in 1972. Subjects were men and women between the ages of 31–59

TABLE I. Ecological Studies Showing an Inverse Relationship Between Dietary Intake of Se and Overall Cancer Risk

Investigator	Year published	Population
Shamberger and Frost [1969]	1969	USA and Canada
Schrauzer et al. [1977a]	1977	International
Clark and Marshall [2001]	1985	USA
Yu et al. [1988]	1988	China

TABLE II. Observational Studies Examining the Effects of Se on Overall Cancer Risk

Investigator	Year published	Population	Sample size	Results[a]
Willett et al. [1983]	1983	USA	111	0.30 (0.1–0.7)
Salonen et al. [1984]	1984	Finland	128	0.30 (0.1–0.7)
Fex et al. [1987]	1987	Sweden	35	0.30 (P trend <0.05)
Kok et al. [1987]	1987	Netherlands	69	0.50 (0.3–1.0)
Knekt et al. [1990]	1990	Finland	597 Males	0.4 (P trend <0.001)
			499 Females	0.9 (P trend 0.6)
Virtamo et al. [1987]	1987	Finland	109	0.9 (0.5–1.5)
Ringstad et al. [1988]	1988	Norway	60	0.70 (0.3–1.7)
Peleg et al. [1985]	1985	USA	154	1.00
Coates et al. [1988]	1988	USA	154	1.00 (0.5–1.8)
Avanzini et al. [1995]	1995	Italy	58 Males	1.12 (0.85–1.46)
			37 Females	0.93 (0.66–1.29)
Garland et al. [1995]	1995	USA	934	1.2 (0.9–1.7)

[a]Relative risk in highest vs. lowest quartile.

who were initially free of cancer. One control was age-matched to each case with regards to several confounders including gender, age, smoking status, and serum cholesterol level. The mean serum Se level in this population was 128 mg/ml. Analyses revealed a relative risk of 3.1 (95% confidence interval) was associated with a peripheral Se level of <45 μg/ml [Salonen et al., 1984].

Although the chemopreventive effect of Se has been the subject of controversy, the data have been compelling and great interest was generated for testing Se as a chemopreventive agent in human clinical trials. In 1983, at the Arizona Cancer Center, the Nutritional Prevention of Cancer (NPC) was initiated to test the chemopreventive efficacy of Se for non-melanoma skin cancers in a high-risk population. Secondary endpoints included overall cancer mortality and incidence of cancers of the colon, lung, and prostate. The study was unblinded in 1996 for analyses. The study remained open for an additional 5 years in an open-label phase for which data analyses are now ongoing. One of the central findings of the initial analyses of the data collected from the NPC study was a greater than 60% reduction in the incidence of prostate cancer in participants randomized to 200 μg per day of Se compared to the placebo treated group [Clark and Marshall, 2001; Combs et al., 2001]. These findings led to the development of additional, randomized, blinded, placebo controlled clinical studies testing the effects of Se on prevention of primary and secondary prostate cancer [Nelson et al., 2002]. In additional, subset analyses of the NPC Trial conducted by Clark et al. [1996], which was designed to test Se as a chemopreventive agent for non-melanoma skin cancer, results showed that Se reduced incidence of lung cancer in persons with a relatively low baseline plasma Se level [Duffield-Lillico et al., 2002; Reid et al., 2002]. These data led to initiation of an additional randomized, placebo controlled clinical study examining the chemopreventive effect of daily Se supplementation in former smokers. This study, which was initiated in July 2002, is currently enrolling patients at the Arizona

TABLE III. Restrospective Studies Examining the Effects of Se on Cancer Risk

Investigators	Population	Sample size	Relationship between Se and cancer risk
Fernandez-Banares et al. [2002]	Patients with colon polyps compared to normal volunteers	28	Inverse
	Patients with colon cancer compared to normal volunteers	24	Inverse
Brooks et al. [2001]	Participants of the Baltimore Longitudinal study of aging registry	52 Prostate cancers 96 Age-matched controls	Inverse
Knekt [1993]	Cancer-free patients studied longitudinally	9,101 Cancer-free patients in 1960–1971 & 1973–1976	Inverse relationship for risk of lung cancer
Goodman et al. [2001]	Participants of the Carotene and Retinol Efficacy Trial	356 Lung cancers	No significance
		356 Age-matched controls	
		235 Prostate cancers 456 Age-matched controls	No significance

Cancer Center. Se is also being tested as a chemopreventive agent for recurrent colon polyps. This study, run by Alberts and colleagues, is testing selenized yeast alone or in combination with the COX-2 specific inhibitor, celecoxib.

Newer epidemiologic data support the hypothesis that there is a significant inverse correlation between total serum Se level and prostate cancer risk. Vogt et al. [2003] measured serum Se in 212 men with prostate cancer and 233 age-matched controls participating in a population-based, case-control study that included comparable numbers of Caucasian and African-American men between 40 and 79 years of age [Vogt et al., 2003]. Serum Se level was inversely associated with risk of prostate cancer, with similar patterns seen in both Caucasian and African-American men. These studies also showed that α-tocopherol (vitamin E), which is involved with cellular defense against lipid peroxidation, was also higher in men with plasma Se levels in the highest quartile.

Se AND PROSTATE CANCER PREVENTION ONGOING CLINICAL TRIALS

Phase II Chemoprevention Trial of Se and Prostate Cancer (the 'Watchful Waiting Study')

Study objectives. The 'watchful waiting study' is a randomized, double-blind, placebo controlled study sponsored by the National Cancer Institute (NCI), that was designed for men that have been diagnosed with biopsy proven prostate cancer and have elected to forgo front-line therapy including androgen ablation, radiation, or surgery. Participants are randomized to receive either 200 or 800 μg of selenized yeast or matched placebo once per day (Fig. 1). Eligibility criteria are summarized in Table IV. Serum PSA analyses are performed quarterly. A $\log + 1$ variance stabilizing transformation of PSA will be used to determine rate of rise. Both total PSA and free to total PSA will be measured. Serum alkaline phosphatase and chromagranin A will also be analyzed on a quarterly basis. Symptoms of progression of disease will be evaluated with a urological symptom questionnaire that will be distributed twice per year. This study was initiated at the University of Arizona, Arizona Cancer Center in August 1998. As of August 1st, 2003, 160 participants have been recruited. The specific aims of this study are to: (1) test the ability of Se to prevent the progression of clinical prostate cancer as determined by serial measurement of biomarkers of prostate cancer over a period of upto 5 years; (2) determine whether Se modifies the progression of prostate cancer based on analysis of initial biopsy, blood biomarkers, and urinary symptoms; and (3) further establish the safely profile of Se supplementation for a prolonged period of time. Secondary endpoints will also include immunohistochemical analyses of biopsy specimens for markers of apoptosis including TUNEL, bcl-2, p53, and ki-67.

Statistical analyses. The sample size estimate for this study is based on a three-group design and uses information on PSA rate of rise velocity extrapolated from the NPC Study [Clark and Marshall, 2001]. The mean rate of rise expected is 0.25 ng/ml per year for 4 years before diagnosis whose initial PSA was less than 10 ng/ml. Taking into account the standardized Tau for a 50% difference in PSA velocities is 0.55, a sample size of 60 per treatment group was set arriving at 180 evaluable patients and a total of 220 randomized after accrual data are adjusted for an expected drop out rate. This sample size will detect a 50% treatment effect at 80% power and an alpha of 0.05.

The statistical analyses will utilize the 'intention-to-treat' paradigm. Therefore, participant data will not be censored if there is a short period of time in which the participant is off supplement. The analysis of the primary endpoint will be based on non-linear mixed effects regression model with the dependent variable being PSA velocity. Data will be adjusted for age, dose group, and Gleason score at the time of diagnosis. A Cox proportional hazards model will be used to evaluate the treatment effect for the secondary endpoint of time to initiation of therapy. Analyses for time to documented metastatic disease, the third endpoint, will also be based on a Cox proportional hazards model which will also be adjusted for age and Gleason score at time of diagnosis. Logistic regression analyses will be applied to evaluate differences between changes of other serum biomarkers including chromagranin A and alkaline phosphatase.

Phase III Trial of Se for Prostate Cancer Prevention (the 'Negative Biopsy Study')

Study objectives. Men who have had a negative biopsy and continue to have a sustained elevation in PSA are at relatively high-risk for

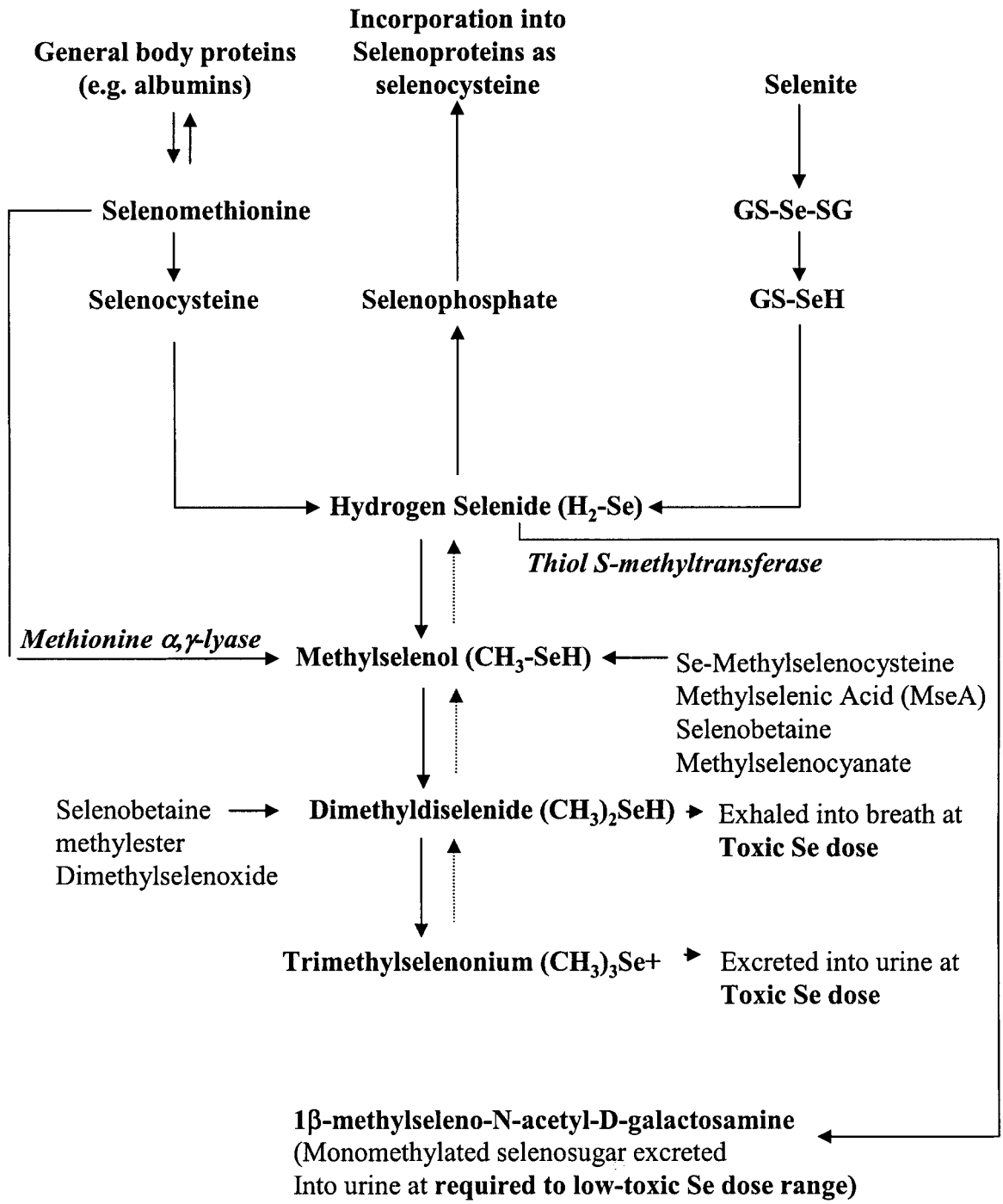

Fig. 1. Diagrammatic representation of the steps in selenium (Se) metabolism.

developing prostate cancer within 1–2 years of the initial biopsy. Smith et al. [1996] followed at cohort of 551 men who had a negative biopsy and PSA level >4.0 ng/ml. Within the 3 year follow-up, 23% of them were diagnosed with prostate cancer on subsequent biopsy. Upon further examination of the data, Roehl et al. [2002] reported that 17% of the men on this cohort were diagnosed with prostate cancer at a second biopsy performed within 1 year of the initial biopsy. Seven percent were diagnosed at the third biopsy and an additional 7% were diagnosed in the fourth biopsy [Smith et al., 1996; Roehl et al., 2002].

TABLE IV. Eligibility Criteria

Watchful waiting study
<85 Years of age at time of study entry
Biopsy proven prostate cancer within 48 months
PSA <50 ng/ml
Have not received any therapy for prostate cancer including surgery, radiation, hormone, or chemotherapy
Have not been diagnosed with metastatic disease
At least 3 year life expectancy
No history of any type of malignancy within the past 5 years with the exception of non-melanoma skin cancer
Liver and kidney function within 1.5 times upper range of normal
Are not taking more than 50 μg of Se per day as a supplement
Gleason score <8
No participation in any interventional study within 30 days of enrollment
Negative biopsy study
<80 Years of age at time of study entry
Negative prostate biopsy within 12 months of enrollment
High grade prostatic intraepithelial neoplasia must not be present (Grade 1 PIN is acceptable)
Liver and kidney function within two times upper range of normal
Have not been diagnosed with metastatic disease
At least 3 year life expectancy
No history of any type of malignancy within the past 5 years with the exception of non-melanoma skin cancer
Liver and kidney function within normal limits
No history of any type of malignancy within the past 5 years with the exception of non-melanoma skin cancer
Are not taking more than 50 μg of Se per day as a supplement
Gleason score <8
No participation in any interventional study within 30 days of enrollment
Pre-prostatectomy study
<80 Years of age at time of study entry
Biopsy proven prostate cancer
Have not received any therapy for prostate cancer including surgery, radiation, hormone, or chemotherapy
PSA <50 ng/ml
Liver and kidney function within two times upper range of normal
Have not been diagnosed with metastatic disease
Have not received any therapy for prostate cancer including surgery, radiation, hormone or chemotherapy
At least 3 year life expectancy
No history of any type of malignancy within the past 5 years with the exception of non-melanoma skin cancer
Liver and kidney function within normal limits
Are not taking more than 50 μg of Se per day as a supplement
No participation in any interventional study within 30 days of enrollment

The 'negative biopsy study' is a randomized, double-blind, placebo controlled, NCI-sponsored intervention study designed to determine whether daily supplementation with selenized yeast would be effective in primary prevention of prostate cancer in high-risk men. Participants enrolled in the negative biopsy study have undergone a negative prostate biopsy within 1 year of study entry. Eligibility criteria are summarized in Table IV. These subjects are randomized to receive daily supplementation with either 200 or 400 μg per day of high Se yeast or matched placebo. PSA analyses are performed at 6 month intervals. Endpoints for this study include incidence of prostate cancer and PSA velocity. Other serum biomarkers, including chromagranin A and alkaline phosphatase, will also be analyzed. This study was began enrollment in August 1999. As of August 1, 2003, 440 participants have been enrolled. The goal for recruitment is 700.

Statistical analyses. The sample size for this trial is based on a three-group design and has been based on expected incidence of prostate cancer in this high-risk cohort (approximately 25%) to estimate the percent reduction in median time to diagnosis. The sample size of 700 will allow detection of a 50% treatment effect with 90% power, alpha of 0.05, and a participant drop out rate of 5% per year [Roehl et al., 2002].

Statistical analyses will employ the 'intention-to-treat' paradigm. Standard techniques for survival analyses will be used for incidence of prostate cancer. The analysis of the primary endpoint will be based on non-linear mixed effects regression model with the dependent variable being PSA velocity. Results will be adjusted for age at enrollment and Se dose. Logistic regression analyses will be used to evaluate secondary serum biomarkers including alkaline phosphatase and chromagranin A.

Chemoprevention Trial of Se and Prostate Cancer (the 'Preprostatectomy Study')

Study objectives. Several challenges have been encountered in the study of Se as a chemopreventive agent. Accurate assessment of dietary intake of Se is difficult due to the variability of the Se content in foods due to

growth conditions, crops, and local soil [Jackson, 1988]. Furthermore, regardless of Se intake, measurement of Se in target tissue is a challenge. It is not currently known whether plasma Se level reflects the presence of Se in the prostate. The purpose of the 'preprostatectomy study' is to examine the effects of Se supplementation on prostate tissue. This is a Department of Defense funded trial that began enrollment in July 1998. The primary hypothesis for this study is that supplementation with daily Se in the form of selenized yeast will modulate biomarkers present in prostate tissue that may be indicative of prostate cancer susceptibility. Eligibility criteria are summarized in Table IV. The study is designed to study the effects of Se supplementation in the brief period of time between diagnosis and radical prostatectomy (3–6 weeks). Participants enrolled in this study have had been diagnosed with prostate cancer and have elected to undergo radical prostatectomy. Subjects are randomized to receive either 200 or 400 μg of Se per day or matched placebo. Tissue biomarkers will be analyzed in participant tissue samples collected from the time of biopsy and post-surgery (after 3–6 weeks of intervention). Tissue specimens collected at the time of biopsy and prostatectomy will be analyzed for indicators of apoptotic index including bcl-2 and p53 as well as for thioredoxin (Trx), TR, and glutathione peroxidase (GPX) by immunohistochemistry. Tissue Se levels will also be evaluated.

Statistical analyses. The sample size estimate is based on a three-group design for comparison between two experimental groups and a control. Thirty-seven participants per group give 80% power to detect a chance of 0.66 standard deviation for each biomarker. These include mean Se level for each zone of the prostate, GPX, Trx, TR, markers of apoptosis, and proliferation.

SELECT

The Se and vitamin E cancer prevention trial (SELECT) is an NCI-sponsored randomized, prospective, double-blind study designed to determine whether Se and/or vitamin E supplementation can decrease the risk of prostate cancer in healthy men. SELECT is being coordinated by the Southwest Oncology Group (SWOG) and plans to enroll a total of 32,400 normal, healthy men at over 400 clinical study sites in the United States, Puerto Rico, and Canada [Klein et al., 2000; Tom, 2002]. Preclinical, epidemiological and phase III data imply that Se and vitamin E have potential efficacy for prostate cancer prevention [Drago et al., 1988; Fleshner, 2002; Yu et al., 2002; Ni et al., 2003]. The four arms of this study include: (1) Se + vitamin E, (2) Se + placebo, (3) placebo + vitamin E, and (4) placebo + placebo. Enrollment began in July 2001. The trial will be completed in 2013.

Se METABOLISM—ACTIVE Se METABOLITES WITH ANTI-CARCINOGENIC ACTIVITY

Next, we discuss metabolism of Se and the importance of Se biotransformation to the anticancer effects associated with this element. Se exists in both organic and inorganic forms in natural diet and has been implicated as an essential micronutrient for human health. Organic Se is present mainly in the form of selenomethionine, selenocysteine, and Se-methylselenocysteine, whereas inorganic Se occurs either as selenite or selenate. Among organic forms, selenomethionine is the predominant form of Se in most Se rich diets [Yang et al., 1997]. Both chemical forms of Se have been under intense study as a promising chemopreventive agent for different types of cancer. The varying degree of anti-carcinogenic activity of different chemical forms of Se may be related to their metabolism in vivo. Therefore, information regarding Se metabolism and active Se metabolites with anti-carcinogenic activity would equip the researchers in designing more efficient therapeutic strategies in Se chemoprevention of cancer. A flowchart describing the events in Se metabolism is presented in Figure 1.

Both organic and inorganic Se appears to be utilized with similar efficiency in the body [Shiobara et al., 1998] and enter at different points in the metabolic pathway. Metabolism of selenite is tightly regulated and is reduced by glutathione to form hydrogen selenide (H_2Se) [Hsieh and Ganther, 1975]. This selenide either serves as a precursor for the synthesis of selenoproteins such as GPXs, TR, iodothyronine deiodinases, and selenoprotein P or undergo stepwise methylation with the enzymatic reaction of thiol *S*-methyltransferases to generate mono-, di-, and tri-methylated forms of Se such as methylselenol, dimethyldiselenide, and trimethylselenonium ions, respectively. This methylation is reversible in vivo [Ip et al., 1991].

Selenomethionine, however, shows both regulated as well as non-regulated metabolism and is subjected to different metabolic fates: (a) since cell machinery does not distinguish between methionine and selenomethionine, this natural selenoaminoacid gets incorporated into the general body proteins such as albumins in place of methionine when methionine is a limiting factor. This non-specific incorporation of Se into proteins could account for the observed dose-dependent increase of tissue Se levels with selenomethionine supplemented diets compared with the diets supplemented with other chemical forms of Se [Shiobara et al., 1998], (b) it gets converted into selenocysteine by transsulfuration pathway, which subsequently convert into H_2Se [Esaki et al., 1981] and follow the similar route as mentioned in the case of selenite, and (c) it could generate methylselenol with the enzymatic reaction of methionine α,γ-lyase (also known as methioninase).

Selenocysteine, another form of organic Se, derived either from diet or from selenomethionine gets converted into selenide. It can be seen that inorganic Se and some forms of organic Se enter the Se metabolic pathway through a common intermediate, i.e., H_2Se. Se-methylselenocysteine, a predominant form of organic Se in brazil nuts, is converted to methylselenol directly by β-lyase [Foster et al., 1986]. Like Se-methylselenocysteine, synthetic Se compounds such as selenobetaine, methylseleninic acid (MseA), and methylselenocyanate readily generate monomethylated Se (MMSe), and thus, can be useful in studies as good precursors for generating methylselenol.

H_2Se after converted to selenophosphate by selenophosphate synthetase gets incorporated into selenoproteins as selenocysteine by the TGA codon [Combs and Gray, 1998]. This selenocysteine residue forms an active catalytic site in selenopreoteins and is essential for the activity of selenoproteins. In type III iodothyronine deiodinase, replacement of the SeCys residue with alanine resulted in inactivation of the enzyme, whereas SeCys $\rightarrow$ cysteine mutagenesis showed reduced turnover of the enzyme and also altered substrate specificity [Kuiper et al., 2003]. Reduced or complete elimination of catalytic activity of TRs in case of mutations in SeCys residue was also reported [Lee et al., 2000; Zhong and Holmgren, 2000; Bar-Noy et al., 2001].

Se compounds that enter either H_2Se pool or methylselenol pool undergo methylation by thiol *S*-methyltransferases and generate different methylated forms sequentially that are excreted either into urine or exhaled in breath, which might lead to maintenance of Se homeostasis in the body. MMSe is the predominant urinary Se form at low doses of Se. Analysis of Se forms in urine from rats supplemented with increasing dose of selenite (0.1–1.0 mg Se/kg body weight) clearly demonstrated excretion of monomethylated forms at low doses of Se, while trimethylated forms are being the predominant at high doses [Itoh and Suzuki, 1997]. When the trimethylselenonium ion levels reach plateau, dimethyldiselenide (DMSe) is exhaled into breath. Recently, Kobayashi et al. [2002] identified a selenosugar, 1β-methylseleno-N-acetyl-D-galactosamine as the major monomethylated form of urinary Se metabolite in deficient to low toxic Se range. Other minor excretory forms are yet to be identified. Therefore, the order in which different methylated forms of Se appear in urine depends on the initial dose of Se supplied, and less toxic forms of Se are being excreted with increasing doses of Se. Excretion of different Se species in urine at different doses thus may be useful as an indicator of the healthy and toxic doses of Se [Kobayashi et al., 2002].

Most functions of Se in the body are mediated by selenoproteins. Major emphasis in Se chemoprevention of cancer is on identification of active Se metabolites that exhibit anti-carcinogenic activity with less cytotoxicity [Ganther, 1999]. Using dimethylbenz(α)anthracene (DMBA) induced tumorigenesis model in rats, Ip et al. [1991] found that the four Se compounds tested for their relative efficiency in tumor growth inhibition in the range of 1–3 ppm Se were in the following order: Se-methylselenocysteine > selenite > selenocystine > dimethyl selenoxide. Further studies based on blocking the methylation of selenite with arsenite showed reduced anti-tumorigenic activity of selenite, while arsenite increased the chemopreventive activity of Se compounds that enter the metabolic pathway beyond the H_2Se [Ip et al., 1991]. It is evident from these studies that the chemical form of Se that gets metabolized directly to methylselenol has potential anti-carcinogenic activity at low doses compared to those that enter H_2Se pool. Dimethylselenoxide, which gets converted into dimethyldiselenide is low in the order, which suggests that methylselenol, a monomethylated

form of Se, could be the active Se metabolite in bringing the anti-carcinogenic effects. Methylated forms also undergo demethylation to replete the enzymatic activity of different selenoproteins [Ip et al., 1991, 2000]. More direct evidence in support of anti-carcinogenic activity of methylselenol was provided in in vitro experiments with methionine α,γ-lyase (also known as methioninase) using selenomethionine as a substrate in DU145 prostate cancer cells [Wang et al., 2002]. Methylselenol generated from the enzymatic reaction of methioninase using as low as 1 μM selenomethionine as a substrate induced apoptosis in DU145 cells, which was not apparent even when the same cells were treated with 100 μM selenomethionine alone. As selenomethionine undergo different metabloic fates, either little amount of Se may be available for generating methylselenol or the conversion of selenomethionine to methylselenol by methioninase is very slow process in vivo.

Synthetic compounds such as MseA, a precursor for methylselenol, have been used to identify the underlying molecular mechanisms that are responsible for the growth inhibition. Ip et al. [2000] have studied the in vitro and in vivo effects of MseA and Se-methylselenocysteine on cell cycle, apoptosis, and tumor inhibition. Although both these compounds generate methylselenol directly, MseA was found to be more powerful in vitro in inhibiting cell growth and inducing apoptosis in mouse mammary hyperplastic epithelial cells at one-tenth the concentration of Se-methylselenocysteine. However, the tumor inhibitory effects of both these compounds were comparable in vivo. The rapid in vitro growth inhibition of MseA may be attributable to quicker generation of methylselenol compared to that of Se-methylselenocysteine. Similar observations were also made by Gasparian et al. [2002] while evaluating the effects of MseA and selenite on their inhibition of NF-kB activity in JCA1 and DU145 prostate cancer cells. MseA showed a rapid, but transitory effect, while selenite showed much slower response but consistent effect in inhibiting NF-kB activity. These studies suggest that generation of a steady stream of monomethylated species is important for a Se compound to exhibit greater chemopreventive activity for extended period of time.

The Se compounds that enter methylselenol pool inhibit the expression of molecules involved in angiogenesis, but not those that enter H_2Se pool [Jiang et al., 2000]. Jiang et al. [2002] reported distinct effects of methylselenol pool and H_2Se pool on cell cycle, apoptosis, and the expression of different protein kinases in DU145 prostate cancer cells. Different pathways might be activated by these two metabolite pools in exerting their anti-proliferative effects. Current literature suggests that potent in vivo tumor inhibition can be achieved with low doses of Se using the compounds that enter methylselenol pool such as Se-methylselenocysteine. However, while evaluating a Se compound for their chemopreventive activity, their cytotoxicity, retention in the body, and its bioavailability for the maintenance of the activity of essential proteins should be considered, which would otherwise lead to pathogenesis of other diseases. Current understanding on various aspects of Se biochemistry supports the inclusion of selenomethionine over other forms in Se chemoprevention trials.

SODIUM SELENITE

Se is an essential dietary nutrient for all mammalian species, including humans. This essential biological trace element plays a vital part in many metabolic functions [Burk, 1983; Gladyshev et al., 1998]. Se in selenoproteins is in the form of selenocysteine, which is synthesized cotranslationally from serine and selenide as selenocysteine by a series of enzymatic reactions dictated by the UGA codon [Leinfelder et al., 1988] and SECIS sequences [Sunde, 1990; Stadtman, 1991]. Many biological functions are performed by the selenoprotein family, ranging from antioxidant protection and thyroid hormone metabolism to proper reproductive performance. Selenoproteins are crucial to many cellular functions. For example, over-expression of GPX1 [Esworthy et al., 1995; Kayanoki et al., 1996; Lu et al., 1997] and TR [Baker et al., 1997; Gladyshev et al., 1998] have been reported to inhibit apoptosis and selenoprotein P appears to be a survival factor for several cell types. Thus, the mechanism of action of Se compounds either via a pro-oxidant pathway, as seen in cytotoxicity and apoptosis, or via an antioxidant pathway as proposed in cancer chemoprevention remains to be elucidated (see below). One possible mechanism for prevention of prostate cancer is prevention of oxidative damage to prostate DNA. To this extend, Waters et al. [2003] have suggested that DNA-damage and apoptosis are Se-responsive events that may be

important regulatory points in multistep prostatic carcinogenesis.

Sodium Selenite and Cell Signaling in Prostate Cancer Cells

Inasmuch as great effort has been concentrated on the chemopreventive and/or chemotherapeutic effects of Se, the fact that Se also participates in cell signaling was not appreciated until recently. ROS such as H_2O_2, which are formed in association with a variety of oxidative stress-induced disorders, are related to cell death and may play an important role in apoptosis [Simon et al., 2000]. Selenite has been demonstrated to regulate signal molecules, especially apoptotic and anti-apoptotic signals. In fact, Se directly regulates the activity of many proteins crucial for various intracellular signaling pathways. For example, the activities of NF-κB, AP-1, p53, HIF-1 alpha, c-Jun-NH2 terminal kinase 1 (JNK1) [Park et al., 2000], and caspase-3 are inhibited through the redox regulation of their reactive cysteine residues. The key to NF-κB regulation is the inhibitory κB (IκB) proteins that in response to diverse stimuli are rapidly phosphorylated by IκB kinase complex, ubiquitinated, and undergo degradation, releasing NF-κB factor. Recently, Gasparian et al. [2002] showed that selenite inhibited IκB kinase activation and IκB-alpha phosphorylation and degradation induced by TNF-alpha and liposaccharide in DU145 prostate cancer cells. Similarly, apoptosis signal-regulating kinase1 (ASK1)–JNK1 pathway is inhibited by physiological concentrations of selenite. Selenite has been suggested to possibly inhibit JNK1 activity through interacting with redox active cysteine residue(s) on the enzyme in 293T human embryonic kidney cells [Park et al., 2000]. Selenite induced-apoptotic DNA fragmentation was shown to be associated with the phosphorylation of JNK and p38 mitogen-activated protein kinase/stress activated protein kinase 2 (MAPK/SAPK2) [Jiang et al., 2001] in DU145 prostate cancer cells.

Se, acting as an insulin-mimetic agent, indirectly stimulates tyrosine phosphorylation and activates MAP kinase in primary rat hepatocytes and 3T3 L1 adipocytes [Stapleton et al., 1997]. Finally, He et al. [2002] showed that the expression of the membrane death receptor DR5 in prostate cancer cells is up-regulated by Se and is coupled with caspase-8 activation as well as Bid cleavage.

Sodium Selenite and the PI3K/Akt/PTEN Survival Pathway

It has been recently shown that sodium selenite induced a dose dependent growth inhibition of prostate cancer cells such as DU-145, PC-3, and LNCaP [Webber et al., 1985; Menter et al., 2000]. After 72 h, the growth of the cell lines tested was decreased at $IC_{50}S$ ranging from 0.2 μM (for the LNCaP) to 3.7 and 3.9 μM (for PC-3 and DU-145, respectively). In the same study, the authors demonstrated an increase in apoptosis in selenite-treated prostate cancer cells. Our observations are in agreement with these studies and confirm the effects of selenite on Akt phosphorylation as the Ser/Thr kinase has been shown to protect from apoptosis and induce cell proliferation when activated (Meuillet et al., personal communication).

Moreover, we have recently reported that the activity of the tumor suppressor PTEN is modulated by Trx, a small redox protein, in a redox dependent manner (Meuillet et al. article submitted). The oxidized form (Trx-S2) contains a disulfide bridge in the active site that is reduced to a dithiol by NADPH and the flavoprotein TR. Thus, the TRX system is composed of Trx-1, TR, and NADPH in vivo [Buchanan et al., 1994; Holmgren, 1995]. TR is a known selenoprotein, which activity can be regulated by selenocompounds (Fig. 2) [Berggren et al., 1999].

One possible mechanism of action for sodium selenite resides in the fact that upon stress, Trx inhibit PTEN activity. The presence of Se, which increases TR activity, allows Trx to be reduced and relieve PTEN from Trx inhibition (Fig. 2). P53 has been suggested to be increased upon PTEN expression. Because TR is one transcriptional target of p53, these results support the idea that in p53 wild-type cells, TR may be increased upon PTEN expression. Ongoing experiments in our laboratories are directed towards the demonstration of the involvement of p53 in PTEN and TR in a selenite-induced process.

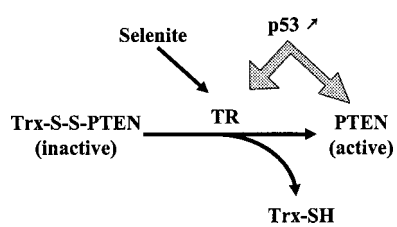

Fig. 2. Model for Selenite effects on TR-Trx system and PTEN.

ORGANIC Se FORMS AND PROSTATE CANCER

Epidemiological studies have identified low Se levels as one of the many factors that lead to the incidence of prostate cancer. Human clinical trials with organic Se as a dietary supplement have shown promise of using Se as an effective chemopreventive agent for treating prostate cancer [Clark et al., 1996]. Molecular mechanisms through which different Se forms exert their growth inhibitory effects in cancer cells are just beginning to emerge.

Cell culture studies demonstrated that at micromolar concentrations selenomethionine (SeMet) inhibits selectively the growth (1–90) and induces apoptosis in dose-dependent manner in prostate cancer cells such as LNCaP, PC-3, and DU145, but not normal diploid fibroblasts and primary prostate cultures [Redman et al., 1998; Menter et al., 2000]. MseA, an immediate precursor of methylselenol, at a concentration of 5–10 μM inhibits the growth of PC-3 cells and also induces apoptosis more potently than SeMet and selenite [Jiang et al., 2000; Dong et al., 2003]. Both selenomethionine and MSeA induce apoptosis through DNA fragmentation, and cleavage of poly (ADP-ribose) polymerase (PARP), [Jiang et al., 2000, 2001; Menter et al., 2000] which is considered as a downstream effect of caspase-3. However, the PARP cleavage products generated by these Se compounds are different in DU145 prostate cancer cells. This caspase mediated PARP cleavage is more prominent in MSeA treated cells compared to that of selenite [Jiang et al., 2001, 2002]. MSeA- induced apoptosis is accompanied by activation of multiple caspases-8, -9, -7, and -3 with caspase-8 at upstream in the pathway as demonstrated by using different caspase inhibitors, cleavage of PARP, and mitochondrial cytochrome C release. Furthermore, MSeA also reduced the expression of phospho-AKT and phospho-ERK1/2 [Jiang et al., 2001, 2002]. Activation of these two kinases is associated mainly with cell survival [Marte and Downward, 1997; Uzgare et al., 2003] and, therefore, suppression of these two kinases, in part, might account for MSeA mediated growth inhibition and apoptosis of DU145 prostate cancer cells. Jiang et al. [2002] showed that inhibition of AKT is not sufficient for inducing apoptosis in DU145 prostate cancer, which suggests the execution of apoptotic process through multiple pathways in MSeA treated cells. In contrast, selenite treated cells showed activation of phospho and total AKT, phospho-JNK, and phospho-p38 MAPK. It did not affect ERK2, but slightly inhibited the phosphorylation of ERK1. Since MSeA and selenite represent two different Se metabolite pools, they might target distinct pathways in exerting their growth inhibitory effects.

Recent studies by our group in LNCAP and DU-145 cells demonstrates dose-dependent growth inhibition of these prostate cancer cell lines. Interestingly, we also observed concentration dependent activation of ERK1/2 with selenomethionine during the growth inhibition. Although ERK is thought to play a key role in the proliferative process, recent studies also suggest that persistent activation of ERK may mediate cell cycle arrest and differentiation [Traverse et al., 1992; Pang et al., 1995; Pumiglia and Decker, 1997; Adachi et al., 2002; Wang et al., 2003]. After activation, phospho-ERK is translocated to the nucleus, where it can phosphorylate transcription factors leading to altered gene expression (Fig. 3). The relationship between selenomethionine growth inhibition and activation of ERK is remains to be clarified.

An interesting observation is that androgen-responsive LNCaP cells were found to be highly sensitive for SeMet-mediated growth inhibition compared to that of androgen unresponsive cells such as PC-3 and DU145. This differential sensitivity of prostate cancer cells to SeMet treatment is not yet known. Abdulkadir et al. [2001] found reduced prostate tumor progression in early growth response protein 1 (EGR1) deficient mice (Egr1−/−). A recent study demonstrated interaction of EGR1 with the androgen receptor (AR), which leads to the translocation of AR to the nucleus and trigger AR-dependent

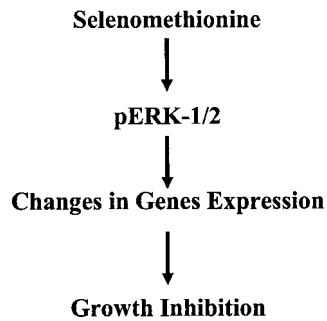

Fig. 3. Model for Selenomethionine effects on prostate cancer.

signalling pathway [Yang and Abdulkadir, 2003]. It can be speculated that blocking the interaction of the transcription factors such as EGR1 with AR by SeMet might lead to growth inhibition and increased sensitivity of the androgen responsive prostate cancer cells to SeMet treatment. After prolonged hormonal therapy, androgen dependent cells would become androgen independent. Cell culture data demonstrated that selenomethionine and MseA were able to inhibit the growth of androgen unresponsive cells, which favors the use of organic Se forms in treating prostate cancer.

Cell cycle progression is a complex event which depends on the coordinated expression of cell cycle regulatory proteins such as cyclin dependent kinases (CDKs) and their cyclin partners. SeMet treatments resulted in the growth arrest of prostate cancer cells at G2-M transition phase, which is associated with the tyrosine phosphorylation of cdc2 molecule in LNCaP, PC-3, and DU145 cells, [Menter et al., 2000] and decreased expression levels of cyclins D1 and D3 in LNCaP cells [Ni et al., 2003]. It did not affect the expression of cdk2, cdk4, and cyclin E. MseA causes growth arrest of DU145 cells at G1 phase, whereas selenite induces cells to arrest at S phase. G1 arrest of MSeA treated cells was accompanied by increased expression of CDK inhibitors such as p27kip1 and p21cip1 [Jiang et al., 2002]. It was also shown the down-regulation of cdk2 by MseA in DU145 cells [Dong et al., 2003].

Previous studies on growth inhibition of prostate cancer cells by Se have shown the requirement of high concentration of SeMet to exert its anti-proliferative effects. Wang et al. [2002] also showed that SeMet induces apoptosis at higher concentrations (>100 μM) in DU145 prostate cancer cells. However, with the exogenous addition of 0.08 U of methioninase, which generates methylselenol using selenomethionine as a substrate, as little as 1 μM selenomethionine induced morphological features of apoptosis. It shows that the methylselenol generated from 1 μM selenomethionine with the enzymatic reaction of methioninase is sufficient to exert anti-proliferative effects and in inducing apoptosis. Further the apoptotic features resembled to those exhibited by MSeA. Further, methylselenol exposure from 10 μM SeMet and 0.02 U/ml of methioninase for 20 h completely inhibited phosphorylation of AKT.

p53 is a tumor suppressor gene that protects the cells by activation of the DNA repair systems in cells that have DNA damage. Selenomethionine is non-cytotoxic and does not cause any DNA damage. In transient transfection assays of H1299 lung cancer cells with wild type p53 and 20 μM SeMet treatment, Seo et al. [2002] have shown that SeMet promotes reduction of cysteine residues 275 or 277 of p53 in a redox factor Ref-1 dependent fashion. Electrophoretic mobility shift assay and reporter gene assays showed enhanced binding of p53 to its binding site of the human *Gadd45* gene and increased p53 dependent transcription of the reporter gene. An interesting observation is that SeMet protected cells from UV-induced DNA damage in the presence of wild type p53, but not in case of null p53 allele. This study demonstrates the additional role of SeMet in protecting the cells from DNA damage through p53 dependent mechanism.

Oligonucleotide array analysis of MseA treated DU145 cells showed altered expression of numerous proteins involved in cell cycle, angiogenesis, apoptosis, cell–cell adhesion, tumor suppressors, DNA repair proteins, and a wide variety of transcription factors [Dong et al., 2003]. Further understanding of molecular targets of Se would help in designing more effective forms that target specific pathway(s). In addition to Se, other compounds such as vitamin E has been under testing in clinical trials as an effective chemopreventive agent against prostate cancer. Vitamin E succinate, a vitamin E derivative, possess antioxidant activity. Treatment of LNCaP cells with 20 μM VES or 150 μM SeMet singly inhibited the growth by 47 and 37%, respectively, but together reduced the growth by 78% [Ni et al., 2003]. Therefore, emphasis should be given in identifying compounds that exhibit synergistic effects with Se in treating prostate cancer in more effective manner.

CONCLUSION

There may not be more extensive body of evidence for a cancer prevention potential of dietary component nutrient than there is for Se. Even though there are gaps in present understanding, in regards to the exact molecular and biochemical basis for the anti-cancer effects of various forms of Se, it is clear from epidemiological evidence, preclinical studies, and recent intervention trials that it is plausible to consider Se compounds as potential cancer

chemoprevention agent against prostate cancer. Ongoing clinical trials and preclinical studies with Se alone and in combination with other compounds may lead to new effective strategies to combat prostate cancer.

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