MEDICAL SYMPOSIUM - RESEARCH DAY

December 4th - 2003

8:00 A.M. – 2:30 P.M. in Seminar Hall – [New Auditorium]

CHIEF GUEST

Mr. Elvis Newton
Permanent Secretary
Ministry of Health & The Environment
St. Kitts, West Indies

Coordinated by
Surendra S. Parmar
Ph.D (Lucknow), Ph.D (McGill)
Vice President (CME)

Windsor University School of Medicine
St. Kitts
West Indies

Program has been sponsored by the American Physiological Society [APS]

8:00 A.M. – 9:00 A.M.
REGISTRATION

[Coffee and Snacks Served]
9:00 A.M – 9:15 A.M.

WELCOME REMARKS

Dr. Brijinder K. Gupta
Dean
Windsor University School of Medicine

INTRODUCTORY REMARKS

Surendra S. Parmar, Ph.D
Vice President (CME)

INAUGURATION OF RESEARCH DAY

INTRODUCTION OF THE CHIEF GUEST
Dr. Brijinder K. Gupta, Dean
Windsor University School of Medicine

Mr. Elvis Newton

Permanent Secretary
Ministry of Health & The Environment
St. Kitts, West Indies

Greetings from American Physiological Society [APS]
Dr. Martin Frank Ph. D.
Executive Director [APS]

Scientific Session - I

9:30 A.M. – 10:30 A.M

CHAIRPERSON: Margaret C. Biber, Ph.D.
Professor & Chairperson, Department of Physiology
Virginia Commonwealth University School of Medicine
Richmond, Virginia.

EXERCISE TRAINING AND SYMPATHETIC REGULATION IN EXPERIMENTAL HEART FAILURE

Irving H. Zucker, Ph. D.
Theodore F. Hubbard Professor of Cardiovascular Research & CHAIRMAN
Department of Physiology and Biophysics
University of Nebraska Medical center
984575 Nebraska Medical Center
Omaha, Nebraska.

GENE EXPRESSION SIGNATURE OF FAILING AND NON-FAILING HEART

Meredith Bond, Ph D
Professor & CHAIRPERSON
Department of Physiology
University of Maryland
Baltimore
Maryland

SCIENTIFIC SESSION II

10:30 A.M. – 11:30 A.M.

CHAIRPERSON: Dr. Sundaresha T.V.

THE USE OF β – ADRENERGIC AGONISTS FOR THE TREATMENT OF SEVERE PULMONARY EDEMA: IS DESENSITIZATION OF THE β – ADRENERGIC SIGNALING PATHWAY A PROBLEM

Micheal B. Maron, Ph.D.
Professor and CHAIRMAN
Department of Physiology and Pharmacology
Northestern Ohio Universities College of Medicine
Rootstown, Ohio

GASTRIC ASTHMA: A STUDY WITH A WEST INDIAN PERSPECTIVE

Dr. Deepak Chandra Mohan, M.D.
Department of Emergency Medicine,
Windsor University School of Medicine,
West Indies.

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11:30 A.M. – 11:45 A.M.
Coffee Break

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SCIENTIFIC SESSION III

11:45 P.M. – 12:45 P.M.

CHAIRPERSON: Dr. Ravi Kumar Madhireddy

HORMONE-STIMULATED MAGNESIUM FLUXES AND REGULATION OF CELLULAR AND PLASMA MAGNESIUM HOMEOSTASIS.

Antonio Scarpa M.D., Ph.D.
The David and Inez Myers/ A Scarpa Professor & CHAIRMAN
Department Of Physiology and Biophysics
Case Western Reserve University School of Medicine
Cleveland, Ohio

CALCIUM CHANNEL GAMMA SUBUNITS: DIVERSITY IN FORM AND FUNCTION

Philip M. Best, Ph.D.
Professor & CHAIRPERSON
Department of Molecular & Integrative Physiology
University of Illinois at Urbana-Champaign
Urbana
Illinois

SCIENTIFIC SESSION IV

12:45 A.M. - 1:45 P.M.

CHAIRPERSON: Dr. Deepak Chandra Mohan

CORONARY COLLATERAL GROWTH: A BALANCE BETWEEN GROWTH FACTORS AND GROWTH INHIBITORS

William M. Chilian, Ph.D.
Boyd Professor and HEAD
Department of Molecular and Cellular Physiology
Louisiana State University Health Sciences Center
New Orleans, Louisiana

ANTIOXIDANTS IN CANCER CARE: WHEN AND HOW TO USE THEM AS AN ADJUNCT TO STANDARD AND EXPERIMENTAL THERAPIES

Kedar N. Prasad, Ph.D.
Professor & DIRECTOR
Center for Vitamins and Cancer Research
Department of Radiology
University of Colorado Health Sciences Center
Denver, Colorado

VOTE OF THANKS

Synthia Marrero – Conti
4th semester Basic sciences student
Windsor University School of Medicine

[LUNCH – SERVED]
2:15 P.M.

Exercise Training and Sympathetic Regulation in Experimental Heart Failure

Irving H. Zucker, Ph.D.

Department of Physiology and Biophysics
University of Nebraska Medical Center
984575 Nebraska Medical Center
Omaha, NE 68198-4575

Significant advances in the treatment of chronic heart failure (CHF) have been made over the past 20 years. The use of angiotensin converting enzyme (ACE) inhibitors and beta adrenergic blockers has clearly prolonged life in the setting of CHF. More recently, exercise training (EX) has been used as a therapeutic modality in patients with CHF. Well controlled clinical trials have now shown that EX improved the quality of life in patients with CHF. More importantly, EX also has a significant survival benefit for these patients. While these studies have been encouraging and may reflect a new paradigm in CHF management, the mechanisms that are involved in these beneficial effects are not clear. Out laboratories have carried out several experiments designed to further understand the relationship between EX training and neuro-humoral activation in animals with experimental CHF. We evaluated the effects of EX in rabbits and rats with CHF on several cardiovascular reflexes including the arterial baroreflex, the cardiopulmonary reflex, the arterial chemoreflex and the cardiac sympathetic afferent reflex. In each case, reflex function was either normalized or significantly improved by EX. Furthermore, evaluation of resting renal sympathetic nerve activity (RSNA) in conscious rabbits indicated a reduction in sympathetic outflow. We focused on two possible mechanisms that might account for the salutary effects on RSNA and reflex function, namely angiotensin II (ANG II) and nitric oxide (NO). Plasma ANG II was reduced by EX and the effects of ANG II receptor blockade on arterial baroreflex function were abolished by EX. Furthermore, EX caused an increase in the neuronal isoform of NO synthase (nNOS) in the paraventricular nucleus of both rabbits and rats with CHF. No effects were seen in normal animals. These findings suggest that EX training causes a reduction in the sympatho-excitatory effects of central ANG II and the sympato-inhibitory effects of central NO in the setting of CHF. While EX may have direct myocardial effects that are beneficial, our data suggest that a trend towards normalization of cardiovascular reflex function and neuro-humoral activation plays an important role in the beneficial effects of EX.

Gene Expression Signature of Failing and Non-failing Heart.

Meredith Bond, PhD
Department of Physiology, University of Maryland, Baltimore

Heart failure is on the increase in the US and is now reaching epidemic proportions. The elderly, in particular, are likely to develop symptoms of heart failure. In fact, the discharge diagnosis for over half of hospital admissions for people over 65 is congestive heart failure. Despite some success with some new therapies in decreasing morbidity and mortality from heart failure, there is an enormous range of individual responses to drug therapy. For example, up to 1/3 of individuals with symptoms of heart failure do not show improvement with beta blocker treatment. In many instances, heart failure patients are taking multiple drugs in the hope that the optimal combination of drugs will be identified. Thus, our current approach to treating heart failure patients does not adequately recognize individual differences in response to medication. **It is time for a paradigm shift: we need to develop novel approaches to allow medical treatment to be tailored to the individual.**

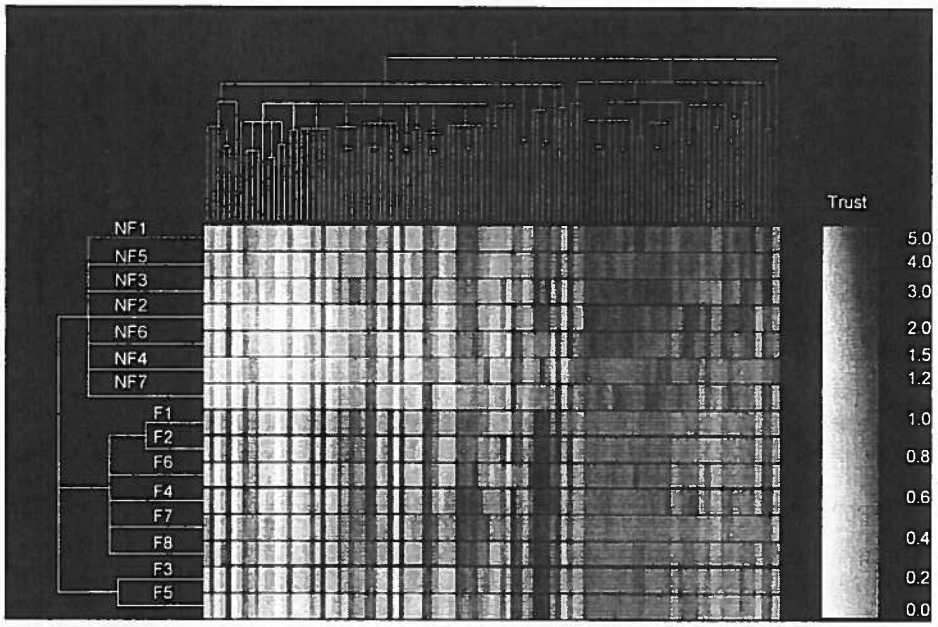

Figure 1: Gene expression fingerprint from failing (F) and non-failing (NF) human hearts. Red indicates genes with increased expression and green indicates decreased expression (compared with other hearts). Hearts are clustered according to similar gene expression patterns. From Tan et al, 2002.

Dilated cardiomyopathy (DCM) is one of the major forms of human heart failure. It is a progressive, uniform dysfunction of the entire myocardium. It can occur independently of coronary artery disease or hypertension, two common causes of heart failure. Gene expression profiling of myocardium from explanted hearts of patients diagnosed with DCM provides us with an opportunity to identify the intrinsic cardiac muscle specific genes for heart failure. Our goal is to use gene expression arrays to synthesize a composite picture of the failing heart from changes in expression of a large number of individual genes and ESTs.

In two previous studies from our laboratory (Yang et al, 2000; Tan et al, PNAS 2002), we identified, by profiling multiple human heart samples, a gene expression fingerprint for human heart failure. More interestingly, examples of failing hearts of different etiologies could be distinguished from idiophatic dilated cardiomyopathy by distinct gene expression profiles (hearts F3 and F5 in Figure 1) (Tan et al, 2002).

With the draft of the human genome now complete, focus has shifted to annotation and identification of the rest of the ~20,000 unknown genes from the human genome.

To identify genes/pathways involved in the development of human heart failure, we developed a set of statistical criteria which resulted in a comprehensive cut-off point (including parameters for evaluation for relative changes and absolute expression levels) for identifying differentially expressed genes in multiple human hearts (Tan et al. 2002). In the current study, we report the identification and characterization of a novel gene which we call HSFA (heart-specific and failure associated), expressed at a high level in the myocardium, limited in other tissues and absent in the brain. HSFA is a novel gene with a total cDNA sequence of 3171 bp encoding a protein of 157 amino acids. A cytogenetic search revealed HSFA to be located on chromosome 12 (12P13). The level of expression of HSFA in F human hearts was comparable to ANF/BNP, and, like ANF/BNP, HSFA had very low expression in NF hearts. In-situ hybridization of HSFA in F heart revealed high expression in endothelial cells of blood vessels and in cardiac myocytes.

Current directions and future opportunities will also be briefly discussed in the presentation. These include identification of novel pathways that show altered regulation in heart failure, identification of novel biomarkers of heart failure – use of EST oligonucleotide arrays and development of prognostic indicators of heart failure.

References:

Yang J, Moravec CS, Sussman MS, DiPaola NR, Fu D, Hawthorn L, Young JB, Francis GS, McCarthy PM, Bond M. Decreased expression of striated muscle LIM protein-1 (SLIM1) gene and increased expression of gelsolin in failing human hearts by high density oligonucleotide arrays. *Circulation* 102:3046–3052, 2000.

Rao JS, Bond M. Microarrays: Managing the data deluge. *Circ Res* 88:1226–1227, 2001. (editorial)

Tan FL, Moravec CS, Li J, Apperson-Hansen C, McCarthy PM, Young JB, Bond M. Gene expression fingerprint as a predictor of human heart failure and its etiology. *Proc Natl Acad Sci USA* 99:11387–11392, 2002.

The use of β-adrenergic agonists for the treatment of severe pulmonary edema: Is desensitization of the β-adrenergic signaling pathway a problem?

Michael B. Maron, Ph.D.
Professor and Chairperson
Department of Physiology and Pharmacology
Northeastern Ohio Universities College of Medicine
Rootstown, Ohio

We have found that 48 hours of isoproterenol (Iso) infusion in rats impairs the ability of β-adrenoceptor (βAR) agonists to increase alveolar liquid clearance (ALC, the rate at which fluid is absorbed from the airspaces) and that this impairment is accompanied by a significant decrease in βAR numbers on alveolar epithelial type II (ATII) cells freshly isolated from rats infused with Iso (Morgan et al., *Am. J. Physiol. Lung Cell. Mol. Physiol* 282:L666-L674, 2002). In a subsequent study (Morgan et al., *Am. J. Physiol. Lung Cell. Mol. Physiol.* 285:L578-L583, 2003), we determined if post-receptor defects in βAR signaling contribute to the impaired ability of βAR agonists to increase ALC in Iso-infused rats. In these experiments, Iso was infused using subcutaneous miniosmotic pumps (4, 40, or 400 μg/kg/hr) in rats for 48 hr. At this time, the ability of forskolin to increase ALC was measured by mass-balance one hr after the instillation of a Ringers solution containing 5% albumin. Forskolin-stimulated ALC (33.4 ± 2.1 (SE)% of the instilled fluid absorbed/hr in vehicle-infused rats) was reduced by 25 and 38%, respectively, after the 40 and 400 μg/kg/hr Iso infusions. The ability of forskolin to increase cAMP was reduced by 70% in ATII cells isolated from rats infused with 400 μg/kg/hr Iso. Additionally, the ability of the stable cAMP analog and PKA activator Sp-8-Bromo-cAMPS to increase ALC (48.7 ± 3.0% in vehicle-infused rats) was reduced by 25 and 51%, respectively, after the 40 and 400 μg/kg/hr Iso infusions. Finally, the ability of cAMP to increase PKA activity was eliminated in ATII cells isolated from rats infused with Iso at 400 μg/kg/hr. These data demonstrate that prolonged βAR-agonist exposure can impair alveolar epithelial βAR signaling downstream of the βAR. They further suggest that impaired PKA function might play a critical role in producing the desensitized ALC response. To further test this hypothesis, we delivered the PKA catalytic subunit to distal lung epithelial cells of rats infused with Iso by using the protein transfection agent, Chariot™ (Maron et al., *FASEB J.*, in press). Two hr after liquid instillation, ALC in control rats was 30.1 ± 1.4% of the instilled volume. PKA catalytic subunit administration increased ALC to 54.5 ± 4.6% ($P<0.001$). After 48 h of Iso infusion (400 μg/kg/hr), ALC (27.9 ± 2.2%) was no different than that observed in control rats. PKA catalytic subunit administration to Iso-infused rats increased ALC to 45.0 ± 3.4% ($P<0.001$), a value equivalent to that produced by PKA in control rats. These data indicate that 1) protein transfection agents are capable of delivering biologically-active proteins to lung epithelial cells of intact rats, 2) PKA desensitization may be an important rate-limiting step in development of Iso-induced desensitization of the ALC response, and 3) Iso infusion does not impair the function of alveolar epithelial Na^+ transport proteins. (Supported by AHA, Ohio Valley Affiliate Grant 0051029B, a grant from the Ohio Board of Regents Research Challenge Program, and a AHA, Ohio Valley Undergraduate Student Summer Fellowship awarded to K.E. Mavrich)

Gastric Asthma: A study with a West Indian Perspective

Dr. Deepak Chandra Mohan, M.D.

Gastric Asthma is an entity characterized by respiratory symptoms of bronchospasm and increased airway reactivity with airflow obstruction triggered by gastroesophageal reflux (GERD). The prevalence of GERD in asthmatics is estimated at between 34% and 80%. Since most patients are initially seen at the primary care level, first line primary physicians must recognize the underlying cause of the disease. This review briefly discusses the pathogenesis, clinical features of the disease with a sample study in the prevalence of Gastric Asthma in the population of Trinidad, West Indies.

Hormone-Stimulated Magnesium Fluxes and Regulation of Cellular and Plasma Magnesium Homeostasis.

A. Scarpa, Department of Physiology and Biophysics, Case School of Medicine, Cleveland, OH

Many human diseases such as cardiac arrhythmia, diabetes and chronic alcoholism are associated with a marked decrease of plasma and parenchymal magnesium. Studies conducted in our laboratory during the last ten years have focused on the hormonal regulation of Mg fluxes from the cells to the plasma and vice versa as well on the redistribution of Mg within the cytoplasm. The main conclusions of this work are:

1) Catecholamine stimulation of hearts and livers (or cardiomyocytes and hepatocytes) causes within a few min a massive Mg release through a cAMP increase and PKA dependent phosphorylation of Mg release processes
2) Carbachol or vasopressin, mostly through activation of PKC, causes an uptake of Mg in the same tissues within minutes
3) Total Mg release and uptake under these conditions is greater than 10% of total tissue or cell Mg.
4) Under these conditions of massive total Mg movement, free cytosolic Mg changes are negligible, indicating a major cellular Mg redistribution.
5) By contrast, under those conditions total plasma Mg changes within min by 20-40%, far more if measured in the interstitial extracellular space.
6) Three different Mg exchangers (two electrogenic Na-Mg exchangers and one electroneutral Ca-Mg exchanger) operate Mg release in liver and have been characterized.
7) Mg uptake is the result of an opening of a Mg channel.

The conclusion is that Mg remains constant in the cytosol but greatly oscillates in the plasma and in organelles. Hence regulation of metabolism and cell function by Mg is expected to occur in protein within organelles or facing the plasma domain of the plasma membrane. Additionally, Mg redistribution could be regarded as an additional major part of the adrenergic stimulation.

Calcium Channel Gamma Subunits: Diversity in Form and Function

Philip M. Best, Ph.D.
University of Illinois at Urbana-Champaign

Voltage-dependent calcium channels are integral membrane proteins that permit the entry of extracellular calcium into the cytoplasm and thus contribute to the regulation of a number of cellular processes including membrane depolarization, hormone secretion, neurotransmitter release, muscle contraction and gene expression. Structurally, calcium channels consist of a pore containing α_1 subunit that binds with auxiliary β, $\alpha_2\delta$, and γ subunits to form hetero-oligomers. The biophysical and pharmacological diversity of native calcium currents is thought to be a consequence of variation in the molecular identity of the pore forming and auxiliary subunits each of which is encoded by multiple genes and their splice variants. Extensive experimental work has characterized the functions of the α_1, β and $\alpha_2\delta$ subunits. However the modulatory effects of the γ subunits on calcium currents are less well understood.

Eight γ subunit genes have been identified to date including three that are expressed in cardiac myocytes. While sequence homology between the three subunits expressed in the heart is low, they are all predicted to contain four transmembrane segments with intracellular N- and C-termini. The γ_6 subunit is unique in that it contains a long, positively charged N-terminal sequence that is not found in other γ subunits. It also lacks the C-terminal PDZ-binding motif present in γ_4 and γ_7. These structural differences may indicate that γ_6 has functions that are distinct from those of other γ subunits.

To test this idea, we have used a heterologous expression system to study the effects of the γ_4, γ_6, and γ_7 subunits on calcium current produced by the α1G (also known as Cav3.1) pore forming subunit. The α1G subunit is expressed in cardiac myocytes and produces the low voltage activated (LVA) calcium current that is robustly expressed in embryonic ventricular myocytes, adult atrial myocytes and cells in the SA node and conduction system. Expression plasmids containing γ_4, γ_6 or γ_7 subunit DNA along with a GFP marker were individually introduced into a HEK cell line that had been stably transformed to express the α1G pore forming subunit. Analysis of LVA calcium current in control and transfected cells revealed that the γ_6 subunit had a unique and dramatic inhibitory effect (>50%) on current expression that was not seen with the other γ subunits. Quantitative RT-PCR and Western blot analysis showed that the decrease in calcium current did not result from a γ subunit dependent inhibition of transcription or translation of the α1G subunit. Nor did the γ subunit disrupt trafficking of the α1G subunit to the surface membrane. Thus it appears that the γ6 subunit, but not γ4 or γ7, can act as a negative regulator of the α1G calcium channel.

To identify critical regions of γ6 that are required for its functional effects on α1G current, chimeric constructs were created in which portions of the γ6 subunit were replaced by the same region of γ4. These chimeras were then tested to see if the inhibitory effect of γ6 was reversed when a portion of the γ4 subunit was inserted into the molecule. Preliminary analysis indicates that the N-terminal region, including the first transmembrane domain, may be critical for the inhibitory effect of γ6 on α1G dependent calcium current.

These results suggest that structural differences within the calcium channel γ subunit family may lead to differences in their functional effects.

CORONARY COLLATERAL GROWTH: A BALANCE BETWEEN GROWTH FACTORS AND GROWTH INHIBITORS

William M. Chilian, Department of Physiology, Louisiana State University Health Sciences Center, New Orleans, LA

The mechanisms underlying coronary collateral growth and angiogenesis in response to ischemia are undefined. In order to elucidate the underlying mechanisms, we developed a canine model of collateral development that enabled sampling of myocardial interstitial fluid (MIF) to determine the expression of growth factors and growth inhibitors during the complete time course (21 days) of growth, along with simultaneous measurements of collateral flow. We have observed that the expression of vascular endothelial growth factor (VEGF) and its corroborator, angiopoietin-2 (Ang-2), are expressed during the early stages of growth. The expression of these factors peaks at Day 3, but wanes thereafter and by the end of two weeks, their expression has returned to baseline. Both VEGF and Ang-2 require normal endothelial function, including endothelial production of NO for signaling. We reasoned that in a model of vascular disease characterized by endothelial dysfunction, collateral growth would be impaired. Measurements of growth factors, growth inhibitors, and metalloproteinase activities were made in myocardial tissue in MIF from dogs during stimulation of collateral growth under control conditions or during antagonism of NO synthase (*N*G-nitro-L-arginine methyl ester [L-NAME]) to mimic endothelial dysfunction. L-NAME increased the expression of VEGF. Despite this large increase in VEGF, collateral growth and angiogenesis were impaired. MIF from control dogs induced in vitro endothelial tube formation and cell proliferation, and this response was blunted in response to MIF from the L-NAME group. Anti-angiostatin restored these in vitro responses in the L-NAME group to control levels. Angiostatin expression in MIF was increased in the L-NAME group compared with controls and shams. The activities of tissue matrix metalloproteinases (MMPs) MMP-2 and MMP-9, which generate angiostatin, were increased in the L-NAME group. Our findings indicate that angiostatin inhibits coronary collateralization during endothelial dysfunction, opposing the growth stimulatory actions of VEGF and Ang-2. Thus, coronary collateral growth is controlled by a balance between growth factors and growth inhibitors, and therapeutic strategies aimed at stimulating collateral growth should invoke means of both augmenting production or activity of growth factors, as well as neutralizing growth inhibitors.

Antioxidants in Cancer Care: When and How to Use Them as an Adjunct to Standard and Experimental Therapies. **Kedar N. Prasad, Center for Vitamins and Cancer Research , Department of Radiology, University of Colorado Health Sciences Center, Denver, CO 80262, USA.** Cancer patients can be divided into two groups: those schedule to receive standard therapy and those in remission carrying the risk of recurrence and development of a second new cancer. Except for reducing the risk of recurrence of breast cancer with tamoxifen, there is no effective strategy to reduce the risk of recurrence of other cancer or development of a second cancer. While impressive progress in radiation therapy and chemotherapy has been made, the value of these modalities in tumor control may have reached a plateau. Although additional drugs are being developed to improve the treatment outcomes of cancer, we have paid relatively less attention to other approaches that might improve the efficacy of standard therapy. Recent laboratory experiments and limited clinical studies showed that micronutrients including dietary antioxidants and their derivatives (vitamin A, retinoids, d-α-tocopheryl succinate and natural β-carotene) at appropriate doses, dose schedule and treatment period may improve outcomes in all three groups of cancer patients. Endogenously made antioxidants such as glutathione- or antioxidant enzyme-elevating agents are not recommended during standard therapy, because they may protect cancer cells against cytotoxic agents. However, the use of antioxidants has become a controversial issue, and most oncologists do not recommend antioxidants under any condition, especially during therapy. This review has identified the experimental data that are used for and against the use of antioxidant in cancer therapy. It has also provided a scientific rationale for the use of an active micronutrient treatment protocol as an adjunct to standard therapy, and to improve the quality of life and maintenance protocol together with modification in diet and lifestyle to reduce the risk of recurrence of tumor and development of a new cancer among survivors.