UNDERSTANDING THE INTRICACIES OF INTESTINAL IMMUNITY: INTEGRAL TO NUTRITIONAL MEDICINE PRACTICE

ASSESSMENT OF THE AREDS2 DOUBLE-BLIND CLINICAL TRIAL ON NUTRIENTS FOR RISK MITIGATION OF AGE-RELATED MACULAR DEGENERATION

CANCER A REDOX DISEASE
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Welcome to our first electronic version of the ACNEM Journal. We are also excited to announce the launch of the new ACNEM website which will going ‘live’ in the very near future.

The recent ACNEM training event in Brisbane was well attended, with fantastic lectures and discussions occurring over the 4 days. It is so heartening that the College provides both top class training and a forum for friendships, mentors and collegial support to flourish.

In this Spring edition of the ACNEM Journal are some great articles which I hope you enjoy.

Joanna Harnett has written a wonderful article around the intestinal epithelial interface. It is certainly intriguing to see the science bearing out for what has been clinically a core issue for many of our patients.

The assessment article by Benke et al on the AREDS2 trial is a concise summary of the type of research that is and needs to be going on in nutritional medicine.

A very thought provoking article - Cancer a Redox Disease - was supplied by Dr Mae-Wan Ho. In this article the ideas of electron imbalance/mitochondrial hyperpolarisation and other key shared electrical properties of cancer cells, is explored.

We also have a review of Ben Goldacre’s book ‘Bad Pharma’ and an article by Rahul Barmanray giving a broad overview of the field of CAM.

The College would like to pay tribute to an ACNEM member and a much-loved pioneer in the field of NEM. Mike Cushman passed away in late July; he was deeply respected and will be dearly missed. As a relatively new practitioner in this field I feel indebted to those whom have created the paths for others to follow.

Hoping you enjoy the Journal and with ‘Warmest Regards’

Dr Oscar Serrallach
The intestinal mucosa permits the absorption of vital nutrients from the gastrointestinal lumen in addition to providing a barrier against the passage of pathogenic substances into the body. The term Intestinal Permeability (IP) represents a defect in barrier function. This defect results in large molecules of undigested food, microbiota and intestinally derived endotoxins passing through or between the tight junctions of the intestine, activating inflammatory and aberrant immune responses. Defects in intestinal barrier function have been associated with ankylosing spondylitis, atopic dermatitis, Coeliac disease, Crohn’s disease, chronic heart failure, Chronic Fatigue Syndrome, cancer, diarrhoea-predominant Irritable Bowel Syndrome, depression, food allergy, inflammatory joint disease, juvenile onset arthritis, liver cirrhosis, psoriatic arthritis and mental health issues. The increase in publications dedicated to intestinal barrier defects and disease has shifted intestinal permeability away from a concept category to a recognised and well described pathophysiological state.

This technical review article is one of a two part series that aims to review the important function of the gastrointestinal associated lymphoid tissue (GALT) and dysfunctions that can lead to IP. Part Two will review the role of probiotics and nutrition in the clinical management of IP.

**INTRODUCING THE GALT**

Approximately 70% of the total immunologically active cells in the body are located in the GALT, which is the largest mass of mucosal lymphoid tissue in the human body. Whilst lymphoid tissue can be found in mucous membranes including the tonsils, adenoids and regions of the gastrointestinal tract, it is in highest numbers and most diffusely distributed in the lamina propria of the small intestine.

The GALT is made up of several types of lymphoid tissue that store T-and B-lymphocytes. Its specialised cells, tissues and signalling systems detect, protect and remember harmful substances and organisms while identifying and tolerating harmless ones and mediating inflammation. Tlaskalova et al delineate the characteristic features of mucosal immunity that distinguish it from systemic immunity, which include mechanisms of innate defence, populations of unique lymphocytes, colonisation of mucosal glands by cells originating from mucosal associated tissues, and the preferential induction of inhibitory responses to non-pathogenic antigens. Mucosal immunity may be fundamentally biased towards immune tolerance.

The gastrointestinal tract’s defence system is a dynamically complex and intricate orchestration of surveillance and responses. The intestinal epithelial cells (enterocytes) form a single-layered wall that protects the entry of harmful substances from the luminal contents entering the sub-mucosa by secreting mucins and defensins. In addition, the intestinal T-cells are capable of generating an autogenous innate immune response that triggers the production of antibodies that bind, deactivate and eliminate antigen antibodies.

One important physiological function of the intestinal epithelial cells is the capacity to communicate with commensal microflora to coordinate an immune response. Therefore the composition and quantities of commensal organisms are thought to play a critical role in the interplay between the epithelial barrier’s capacity to prevent pathogenic microorganisms from entering into systemic circulation.

Occasionally the GALT can orientate the immune system towards antigens in the luminal content, thereby provoking damage to the intestinal mucosa, as reflected in Coeliac disease and allergic gastritis. The immune mechanisms in this process are very complex and can be categorised relatively crudely into either (a) adaptive immunity, characterised by specificity and memory, or (b) innate immunity which is less specific, with no developmental memory.
THE INTESTINAL MICROFLORA AND GALT RELATIONSHIP

The gastrointestinal tract is normally colonised with trillions of commensal microorganisms that provide metabolomic and nutritional benefits to the host. While the role of pathogenic bacteria in inducing gastric inflammation is well understood, even commensal organisms under certain micro-ecological conditions have the capacity to be pathogenic, especially when factors allow them to overgrow usual population numbers. All microorganisms have the ability to elicit an immune response. The intestinal mucus and its secretion of antimicrobial agents is designed to limit or prevent direct interaction of the luminal contents with the intestinal mucosa. Given a certain microbial environment, potential pathogens may penetrate and interact with cellular elements of the mucus and induce an inflammatory response. A critical role of the commensal microflora is in forming a mucosal barrier effect that interacts with the mucosa via a lymphoepithelial and bacterial cross-talk, inducing immuno-tolerance and maintaining homeostasis. Tolerance is the process by which the immune system does not attack an antigen. Not all the molecular mechanisms underlying bacterial signalling and tolerance have been fully elucidated. The molecular mechanisms of the GALT play a critical role in allowing colonisation of the gastrointestinal tract with commensal microflora, and so become the host’s allies in warding off colonisation by transient pathogens. The development of this relationship between commensal microorganisms and the host is orchestrated by toll-like receptors (TLRs) and nucleotide-binding oligomerisation domain proteins (NODs).

COMMENSAL BACTERIA AND IMMUNE FUNCTION

The intestinal microflora initiate and regulate intestinal inflammation and tolerance. Commensal organisms induce immune tolerance by stimulating the production of lymphocytes from the Peyer’s patches. This is followed by a key feature of intestinal adaptive immunity, which is the generation of massive amounts of non-inflammatory immunoglobulin A (IgA) antibodies through multiple follicular and extra-follicular pathways. These operate in the presence or absence of cognate T-B-cell interactions. Between three to five grams of IgA is secreted into the intestinal lumen each day. This accumulates to 75% of the total immunoglobulin produced in the human body. A detailed description of the role of B- and T-lymphocytes in normal and aberrant immune responses is beyond the scope of this review. Briefly, in the absence of normal predominant enteral microflora appropriate populations of B- and T-lymphocytes do not locate in the lamina propria and IgA is not secreted. If tolerance of the normal commensal bacteria is not established in the GALT persistent inflammation may ensue.

Reduced numbers and diversity of commensal microorganisms are associated with a number of other processes that assist in the homeostasis of the GALT. Mulder et al. point out that decreases in gastrointestinal epithelial cell turnover, a reduction in smooth muscle function, a decreased vascularisation and a diminished development of GALT are all thought to be related to lack of numbers and microbial diversity. The indigenous commensal microflora may also stimulate adaptive immunity via their ability to stimulate the production of antigen-presenting and intestinal epithelial cells.

RESPONSE OF THE IMMUNE SYSTEM TO INTESTINAL MICROBIOTA

It is still not fully understood why commensal organisms do not trigger an inflammatory cascade similar to that of notorious pathogens. However, over the last decade our understanding of how the intestinal epithelial cells recognise commensal organisms and how they maintain host-bacterial symbiosis has advanced considerably. Endocrine cells, goblet cells and enterocytes of the intestinal epithelium express a range of pattern-recognition receptors to sense the presence of microbes. The toll-like receptors and nucleotide oligomerisation domain receptors are well known for their roles in pathogen recognition and the induction of innate effectors and inflammation.

The innate barrier functions of the epithelium play an important role in maintaining a harmonious relationship with the commensal community. These innate effectors are regulated by the signalling of pattern-recognition receptors, which explains why defects in NF-kappa B (a protein complex that controls the transcription of DNA) pathways of toll-like-receptor signalling are more likely to result in the development of inflammatory bowel disease. In addition, secretory immunoglobulin-A (sIgA) antibodies to the microbiota limit epithelial contact and invasion of host cells. Epithelial cells produce a B-cell-activating factor of the tumour necrosis factor family (BAFF) and a proliferation-inducing ligand, which promote B-cell recruitment in the lamina propria and class switching in response to toll-like receptors. Therefore the host’s recognition of intestinal microbes is inextricably linked to the production of sIgA and immune exclusion of microbes. Despite the existence of several mechanisms to avoid intimate contact of the epithelium with intestinal bacteria, the lamina propria has a distinctly immunosuppressive tone so as to inhibit over-reaction to innocuous luminal antigens, including the commensal microbiota. This mechanism of ‘oral tolerance’ depends largely on the development of T-regulatory cells in draining lymph nodes.

The epithelial cells produce thymic stromal lymphopoietin (TSLP). TSLP is involved in immune homeostasis or inflammation. TSLP and tumour growth factor beta (TGF-β), and possibly other factors that abolish the ability of dendritic cells to produce inflammatory cytokine responses, promote the induction of T-regulatory (T-reg) cells in the mesenteric lymph nodes. TSLP is upregulated by NF-κB-dependent pathways, suggesting that pattern-recognition receptors signalling from the luminal side of the epithelium would enhance the suppressive tone in the gastrointestinal tract, normally keeping inflammation under control. However, in the case of infection, chemokines secreted by epithelial cells would recruit unconditioned dendritic cells to mucosal sites, which changes the response to a more pro-inflammatory character.
Understanding the interactions between the microbial-intestinal epithelial cells’ interactions in immuno-regulation and inflammation is important for directing investigations concerning the gastrointestinal microbiota and disorders associated with defects in intestinal barrier function.

**INTESTINAL BARRIER FUNCTION AND TIGHT JUNCTIONS**

The epithelial tight junctions constitute the major component of the gastrointestinal tract barrier. They act as a physical functional barrier against para-cellular penetration of macromolecules from luminal contents, including microorganisms and dietary antigens. The tight junction is organised by four types of trans-membrane proteins: occludins, claudins, tricellulin and junctional adhesion molecules, which interact with scaffold proteins such as Zonulins (ZO) – ZO-1, ZO-2 and ZO-3. The role of ZO-1 is to anchor the trans-membrane protein of tight junctions to the actin cytoskeleton and it interacts with other tight junction proteins. Tight junction proteins interact with numerous signalling proteins that regulate tight junction assembly and maintenance, indicating the potential role of intra-cellular signalling pathways in the regulation of epithelial tight junction and barrier function.

The interplay of tight junctions is influenced by events in the intestinal epithelium, lamina propria and lumen and has potential to open and transfer antigenic material. Para-cellular transport of larger molecules is restricted by a normal regulatory state protecting the underlying mucosa from antigen exposure. The structural organisation of all the intestinal epithelial cell junction complexes includes both tight junctions as well as junction types found in other epithelial tissue.

The tight junctions between M-cells can be differentiated from enterocytes by the greater depth and enhanced expression of catenin actinin, polymerised actin and, in some cases, cadherin. M-cells may require a more rigid cytoskeleton and a more stable intercellular adhesion to cope with increased interactions with and transcytosis of enteral bacteria.

The term cytokine refers to the immuno-modulating agents, such as interleukins and interferons. Cytokines are small cell signalling protein molecules that are secreted by numerous cells and are used extensively in intercellular communication. Cytokines can be classified as proteins, peptides or glycoproteins. All nucleated cells have the capacity to produce cytokines but the endothelial/epithelial cells and resident macrophages near the interface of the external environment are the most potent producers of the cytokines interleukin-1, interleukin-6 and TNF-α.

Although a cellular response to cytokines show cell type specific, pleiotropic and time-dependent dose effects, common mechanisms for tight junction modulation emerge. These include cytokine induced actin remodelling and changes in tight junction structure. The effects of cytokines on tight cell junctions appear consistently to be independent of apoptosis. Epithelial and endothelial barrier continuity, when disrupted by cell apoptosis, leads to increases in tissue permeability. Cytotoxic effects due to cytokines have been described in a variety of cells lines and are dependent on dose and duration of tumour necrosis factor (TNF-α) and interferon gamma (IFN-γ) exposure. An example of this is when pharmacological inhibition of apoptosis fails to attenuate increases in monolayer permeability.

Therefore cytokines are capable of directly modifying tight junction composition and structure through signalling pathways independent of cell death.

**THE MECHANISM OF INTESTINAL INFLAMMATION**

Inflammation is essentially a protective tissue response to injury or destruction of tissues, which serves to destroy, dilute, or wall off both the injurious agent/pathogen and the injured tissues. Intestinal inflammation is a localised or general defensive response to a pathogenic organism or antigen which in turn leads to its elimination. An inflammatory response triggered by the release of mediators is essential for host defence. However, unregulated or excessive inflammation is harmful, as is demonstrated in a number of inflammatory bowel diseases. Control mechanisms directed from cells of the intestinal epithelium in response to luminal contents have the ability to maintain homeostasis, maintain inflammatory response or terminate immune responses. It is well understood that the inflammatory response orchestrates the activation of both innate and adaptive immunity through the release of cytokines.

In normal health there is usually active down-regulation of inflammatory signals to maintain a state of controlled tolerance in order to coexist with commensal bacteria. Even small changes in the heavily regulated system may lead to chronic inflammation and functional disturbances of the intestinal tract. Inflammation induces blood vessel dilation, increased expression of adhesion molecules, exudation of fluids and extravasations of
the neutrophils, macrophages and mast cells, activated Th-cells, cytotoxic T-cells, and memory T- and B-cells into tissues\textsuperscript{50}. Activation of inflammation at a molecular level is triggered by TNF-\(\alpha\), IL-6 and IL-1\(\beta\), which stimulate endothelial cells to up-regulate receptors for immune cells\textsuperscript{51}.

The innate immune response is inextricably intertwined with the mechanisms of inflammation. Inflammatory responses mediated by the innate immune system are immediate. Innate immune mechanisms that elicit an inflammatory response employ a number of transiently synthesised preformed non-specific effector molecules and phagocytes\textsuperscript{52}. In the event of innate immune overload an adaptive response is triggered\textsuperscript{53}.

An adaptive response is based on the recognition of the antigen presented by antigen presenting cells. Pattern recognition receptor signalling in response to the pathogen-associated molecular pattern molecules (PAMPs) of microorganisms activates immune mechanisms, causing a release of TLRs and nucleotide-binding oligomerisation domains (NODs). This triggers a signalling cascade which alerts and protects the host. However, an aberrant response can turn normal physiological function into a pathological situation, leading to inflammation\textsuperscript{54}. A number of groups have proposed that host-derived negative regulators cross-talk to TLR and NOD signalling cascades and shape the magnitude and duration of inflammatory processes\textsuperscript{20, 54, 55}.

In summary, the small intestine is home to a complex and intricate immune system (the GALT) that regulates both adaptive and innate responses. Normal function of the GALT is critical in differentiating between beneficial and pathogenic microorganisms. Appropriate responses by the GALT are known to play an important role in the balance between pro- and anti-inflammatory responses. Polarisation towards inflammation can lead to a number of pathologies, including autoimmunity and numerous chronic health conditions. This technical review article has provided the foundation for the next review publication that will discuss the evidence for the use of specific probiotics in the clinical management of dysfunctions of the GALT and defects in intestinal barrier function.
Assessment of the AREDS2 Double-Blind Clinical Trial on Nutrients for Risk Mitigation of Age-Related Macular Degeneration

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ABSTRACT: Results have been released from the US government funded AREDS2 study on the effect of nutrients (antioxidants, vitamins and minerals) on the progression of age-related macular degeneration. The results showed mixed outcomes on a number of issues but supported the primary AREDS formulation. The issues under scrutiny included the relative impacts of certain supplements in comparison to the previous AREDS trial, the effect of the carotenoids lutein and zeaxanthin, and the effect of omega-3 fatty acids. The trial was significant because it was a randomised double-blind clinical trial on a large scale and tested various well known supplements identified from anecdotal evidence and investigated in previous small-scale trials. The study results were also important as it was perhaps the most significant rigorous study to date on the subject. In this paper, we describe the background to the study, review the published results and discuss implications.

KEYWORDS: AREDS2 trial, age-related macular degeneration, nutrients, antioxidants, supplements.

INTRODUCTION

The importance of vision and blindness prevention cannot be underestimated and many studies have been published on various aspects of health and safety related to environmental hazards and nutritional impacts, especially with respect to the retina1,2,3. In human vision, macular degeneration is associated with loss of central high resolution discrimination needed for various tasks involving fine detail work, such as facial recognition, reading and driving. Risk factors are related to genetics, diet, health status and environmental hazards. For example, certain ethnic groups are more prone to macular degeneration (such as European nationals). Also, poor diet may lead to lack of essential nutrients required by the retina, micro-circulation may be affected by high blood pressure and cardiovascular disease, and harmful environmental factors may include tobacco smoking, UV radiation and blue light exposure4,5,6. It has been reported that age-related macular degeneration is the leading cause of blindness in the developed world and accounts for more than 50% of cases in the United States1.

The process of diagnosis of macular degeneration involves many factors1,2,5,6. Eye tests can reveal pigmentary changes in the retina and the presence of drusen (yellow deposits of cholesterol and cellular
matter). Although the presence of small drusen is part of the aging process, increasingly larger drusen are characteristic of the progression to macular degeneration (from early to intermediate stages).

Advanced degeneration is characterised by the breakdown of the central retinal area (dry macular degeneration) or the growth of fragile abnormal blood vessels under the retina that leak blood and cause damage (wet macular degeneration). The wet form can be treated to reduce further progression by drugs that inhibit a protein, vascular endothelial growth factor (VEGF). These drugs are administered by injection and slow down the growth of the abnormal blood vessels1.

Several years ago, a large US government funded study investigated the effect of supplements on age-related macular degeneration (AMD). Now referred to as AREDS (age-related eye disease study)5,6, the study results indicated that the risk of progression to advanced AMD was reduced by about 25% over 5 years compared to placebo in those with existing moderate to severe AMD5,6. A second study referred to as AREDS2 was completed recently with the aim of testing a modified formulation incorporating added nutrients and lower doses of some of the original components1,2. The changes were in response to concerns relating to the use of beta carotene for smokers and the high dose of zinc in the original formulation (which was related to indigestion and possible anaemia in the case of long term use). The AREDS2 trial results were widely disseminated by the US researchers in May 2013 and are reviewed in this paper and some implications discussed.

**HISTORICAL BACKGROUND**

**Aims of Study**

The published aims1 of the AREDS2 study were to determine whether ‘adding lutein and zeaxanthin, together with DHA and EPA, or both, to the original AREDS formulation decreases the risk of developing advanced AMD and to evaluate the effect of eliminating beta carotene, lowering zinc dose, or both, in the AREDS formulation’. DHA and EPA are omega-3 fatty acids (see Nutrient Interventions).

**Experimental Design**

The AREDS2 randomised double-blind clinical trial was a 2 x 2 factorial design commenced in 2006 and completed in 2012. It was conducted at 82 sites in order to investigate whether the AREDS formula (see Table 1) for advanced AMD and cataract reduction may be enhanced further in performance by adding omega-3 long chain polyunsaturated fatty acids and the antioxidants lutein and zeaxanthin (see Table 2).

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<tr>
<th>Nutrient</th>
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<tr>
<td>Vitamin C</td>
<td>500</td>
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<td>Vitamin E</td>
<td>400</td>
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<td>Beta-carotene</td>
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<td>Zinc</td>
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<td>Copper</td>
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**Table 1. AREDS formulation**

The sample size for AREDS2 was 4203 (aged 50-85 years) and was limited to those with intermediate AMD (both eyes), or intermediate in one eye and advanced in the other eye5,6. Subjects were assigned randomly to treatment groups in the double-blind trial and progression to advanced AMD assessed by retinal imagery classification and treatment for neovascular AMD following enrolment.

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<th>Nutrient</th>
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<td>AREDS</td>
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<td>Lutein</td>
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<td>Zeaxanthin</td>
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<td>DHA</td>
<td>350</td>
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<td>EPA</td>
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**Table 2. AREDS2 trial components**

In Table 2, modifications with primary and secondary goals are listed in **AREDS2 Trial Modifications** below. Hazard ratios and confidence intervals were computed using SAS Ver 9.2 statistical software.

The lead author in the publication was Dr Emily Chew at the National Eye Institute, at Bethesda, Maryland, which is one of the US National Institutes of Health1.

**Nutrient Interventions**

The omega-3 long-chain polyunsaturated fatty acids used were docosahexaenoic acid (DHA) and its precursor eicosapentaenoic acid (EPA)1,2. These fatty acids may be both plant and animal in origin and are most commonly associated with fish oils. They are linked with lower rates of cardiovascular disease, and DHA is associated with the development and repair of retinal cells.

Lutein and zeaxanthin occur in the lens and retina and are antioxidants that aid in absorption of harmful UV radiation and blue light. They are major components of the macular pigment. Together with beta carotene, they belong to the family of organic pigments called carotenoids, originating from plants and green leafy vegetables in particular. Beta carotene converts to Vitamin A in the body, and is used in light detection and conversion to electrical signals for neural processing4.

**AREDS2 Trial Modifications**

The use of a double-blind clinical trial was intended to provide more rigorous statistical support following anecdotal evidence from other observations and studies8. The AREDS2 study had primary and secondary goals, with multiple treatment streams, for addressing both AMD and cataract formation1,5:

A. **Primary goal (four treatments):**

1. AREDS (Control)
2. AREDS + lutein/zeaxanthin
3. AREDS + DHA/EPA
4. AREDS + DHA/EPA + lutein/zeaxanthin

continued next page
B. Secondary goal (randomised subgroups) :

1. AREDS
2. AREDS without beta-carotene,
3. AREDS with low zinc (25 mg instead of 80 mg),
4. AREDS with low zinc and no beta-carotene.

In summary, the primary goal of randomized interventions involved participants taking one of the four variations of the AREDS formulation. The secondary goal represented further randomized sub-studies.

RESULTS AND DISCUSSION

Different combinations of lutein/zeaxanthin and DHA/EPA used in the AREDS2 formula did not lower the overall risk of progression to advanced AMD from that already achieved by the original AREDS formula\(^1,2\). It was found that the substitution of beta carotene in the original AREDS formula by the carotenoids lutein and zeaxanthin may be appropriate to reduce the risk of lung cancer in smokers or former smokers. Further analysis of the trial is still in progress and results are under continuing interpretation.

The study population was a selected group of educated participants with no dietary deficiencies who adhered strongly to experimental protocol. It may be that generalisation to a different population may have produced more positive results. This was suggested by the earlier study that malnourished patients who were deficient in essential fatty acids and carotenoids showed the most significant benefits. Moreover the efficacy of the study may also have been an issue, and sample size or duration may not have been appropriate. In general the study was a significant trial that added to the research knowledge in this area and provided material for fine-tuning the original AREDS formula.

Some further observations:

- Although this study showed no significant effect from DHA and EPA supplementation, previous studies with various dose levels have been more positive\(^3,4,5\). For example, in one study\(^6\) participants reporting the highest baseline consumption of omega-3 fatty acids were approximately 30% less likely than their peers reporting the lowest consumption to develop advanced AMD by the end of the 12-year follow-up period. Also, Christen et al\(^7\) reported a large-scale study of women health professionals, based on a detailed food-frequency questionnaire with 10-year follow-up, which suggested regular consumption of DHA and EPA resulted in decreased risk of AMD, with relative risk of AMD of 0.58 (95% CI: 0.38-0.87).

- It appears that with the omega-3 fatty acids, DHA and EPA, more research is needed on dose level, duration, and stage of disease at administration. The results from the AREDS2 trial in isolation suggest that omega-3 supplements are not as effective for treatment of medium level AMD, but perhaps may support a long term strategy for risk minimisation in healthy individuals. Further research is also necessary on the omega-3 dose, interactions and duration effects for application to existing disease as opposed to prevention.

- It was noted that previous clinical trials involving lutein and zeaxanthin, albeit with limited sample size and duration, suggested improved visual acuity\(^8\). Although this was not supported in the current study, once again the issue of stage of disease, dose and duration are raised. A subgroup analysed in the study found a further 18% reduction in risk of developing advanced AMD when lutein and zeaxanthin were used as a substitute for beta carotene in the original AREDS formula.

- The use of all three carotenoids together may have weakened the individual effects of each due to competitive absorption. This may be viewed in the context of previous trials with lutein and zeaxanthin that showed positive benefits on AMD. There is therefore a case to investigate further these carotenoids in formulations without beta-carotene being present.

- The comparison between low-dose zinc (25 mg) and high-dose zinc (80 mg) zinc showed no statistically significant difference. The lower dose was chosen in the AREDS2 trial, as it represented what was thought to be the upper limit of daily absorption. It also avoided possible side effects, such as anaemia or indigestion.
SUMMARY AND CONCLUSION

The AREDS2 study was significant because of its size and government funding, and that it also supported similar clinical trials with results that suggested common nutrients and supplements may be effective as treatments for lowering the risk of developing advanced AMD (see citations listed in the references1,2,6,9–11). The study also addressed previous criticisms originating from pharmaceutical companies that could afford the costs of large-scale randomised clinical trials, but complained about the lack of rigour of small-scale anecdotal studies or empirical observations.

The principal outcomes of the AREDS2 study were (a) it confirmed the effectiveness of the original AREDS formulation, (b) it suggested that the beta carotene in the original AREDS formulation may be replaced by lutein and zeaxanthin subject to further investigation (for the benefit of smokers and former smokers), and (c) the zinc dose may possibly be lowered from 80 mg to 25 mg (which would avoid some possible side effects).

The AREDS2 study revealed no statistically significant overall risk reduction in the development of advanced AMD using various combinations of lutein/zeaxanthin and DHA/EPA in comparison to the original AREDS formula used alone by the control group of AMD patients (25% risk reduction in progression to advanced AMD over 5 years) – but left open the issue of further improvements due to possible dependencies on dose, duration, and stage of disease at treatment. In contrast to this trial, the omega-3 fatty acids DHA/EPA were found to be significantly more effective in other reports published elsewhere9,10,12. Differences between study results also raises the issues of relative dose, adverse effects, nutrient deficiencies, and possible interactions between nutrients – which are all topics for future research. Also, use of the AREDS formula as a preventative measure versus treatment modality requires further research and clarification.

Analysis of a subgroup in the AREDS2 study suggested replacement of beta carotene by lutein/zeaxanthin in AREDS may decrease risk of advanced AMD by a further 18%. Further support of lutein/zeaxanthin for risk reduction was advanced in a different study with results that suggested common nutrients and supplements may be effective as treatments for lowering the risk of developing advanced AMD (see NIH3). The AREDS2 results will be used to fine-tune the AREDS formula and provide a reference level for further exploration of nutrients for risk reduction.

References


CANCER a Redox Disease

CANCER cells are universally disturbed in their electronic energy balance, an understanding that potentially revolutionises cancer therapy and prevention

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TWO OPPOSING APPROACHES TO CANCER THERAPY

By all accounts we are losing the war on cancer. The long-tried practice of targeting specific cancer gene mutations does not work, and for good reasons. Not only are the mutations remarkably diverse, differing between individuals and between parts within a single tumour, cancer cells soon become resistant to new drugs.

There is growing realization that cancer is not primarily a genetic disease, but an epigenetic response to chronic stress. Redundancy in diverse signalling pathways means that many different 'adaptive' mutations can enable cells to survive and multiply, predisposing them to malignant transformation.

One approach to cancer therapy is the much touted 'personalized medicine' that tailors the cure to key genes that have gone awry, but genetic heterogeneity poses a considerable, if not insurmountable hurdle.

The other approach is to target the most general characteristic of cancer cells and tumours distinct from normal cells, and that is becoming popular. Cancer cells typically have an abnormal energy metabolism, prompting some researchers to suggest that cancer is a metabolic disease.

I prefer to call cancer a redox disease, as explained later, to distinguish it from the usual 'inborn errors of metabolism' that underpinned the hypothesis of 'one gene one enzyme' of biochemical genetics.

CANCER A MITOCHONDRIAL DISEASE

The abnormal energy metabolism of cancer cells was discovered by German physiologist Otto Heinrich Warburg in the 1920s. Normal cells obtain energy by breaking down the 6-carbon molecule glucose into two 3-carbon pyruvate molecules in a series of reactions – glycolysis – that does not require oxygen, followed by oxidation reactions in the mitochondria in which oxygen is needed.

Cancer cells, however, depend heavily on glycolysis to obtain energy, even though plenty of oxygen is present. This phenomenon – aerobic glycolysis subsequently known as the Warburg effect – prompted Warburg to propose that mitochondrial dysfunction was the primary cause of cancer.

As glycolysis is much less efficient in extracting energy from glucose, cancer cells are voracious for glucose, and that is how tumours are detected by positron emission tomography (PET) imaging in which glucose uptake is measured by means of a radioactive analogue, flourodeoxyglucose.

Aerobic glycolysis is a robust hallmark of most tumours; it involves a high uptake of glucose with lactate production in the presence of oxygen, lactate being the by-product of pyruvate, even in those cancer cells that appear to have working mitochondria. The reason seems to be that cancer cells need glycolysis to generate carbon skeletons for the synthesis of proteins and nucleic acids to support rapid cell proliferation; and blocking glycolysis does appear to inhibit cancer cells (though it would also affect normal cells).

Warburg's idea fell into disfavour as the view of cancer as a metabolic disease was gradually displaced with one of cancer as a genetic disease caused by mutations in specific cancer related genes, or oncogenes.

In recent years, the idea that cancer is a metabolic disease has become fashionable again. Some commentators remark that 'molecular biology is re-discovering biochemistry'; it is more important than that.

Cancer is a disease of electronic energy imbalance, and electronic energy is the life-wire that animates cells and organisms, as the father of biochemistry Albert Szent-Györgyi had discovered three quarters of a century ago.
LIFE IS AN ELECTRONIC CURRENT

In my book, The Rainbow and the Worm, The Physics of Organisms, first published in 1993 and now in its 3rd edition\textsuperscript{11}, I presented theoretical and empirical evidence for the quantum electrodynamic nature of organisms. An organism is energized by electrons (and protons) flowing through a liquid crystalline matrix that extends into the interior of every single cell. The movement of electrons between chemical species is reduction (for the electron acceptor) and oxidation (for the electron donor). Reduction and oxidation always go together, hence ‘redox’ reactions. Redox reactions are the heart of energy transduction in living organisms. Electrons move according to the reduction potential (also referred to as reduction-oxidation potential or redox potential), the affinity of a substance for electrons. The redox potential for each substance is compared to that of hydrogen, which is set arbitrarily to zero at standard conditions of 25 °C, 1 atmosphere, and 1 M concentration.

Substances that have positive redox potentials accept electrons from hydrogen, becoming reduced, while substances that have negative redox potentials donate electrons to hydrogen, becoming oxidized.

In order to appreciate the redox theory of cancer, we need to understand the core metabolic reactions common to organisms. For a more thorough description of energy metabolism see Living Rainbow H₂O\textsuperscript{12}, a sequel to the Rainbow Worm\textsuperscript{11} and a unique synthesis of the quantum physics and chemistry of water as the ‘means, medium and message’ of life.

ENERGY METABOLISM IN ANIMAL CELLS

All air-breathing animals, human beings included, depend on oxygen to extract energy from their food in a universal set of core metabolic reactions (Figure 1). The 6-carbon molecule glucose is activated by ATP and the enzyme hexokinase, and split through a series of glycolytic reactions each catalysed by a specific enzyme into two 3-carbon pyruvate that take place in the cytoplasm, and do not require oxygen. Further metabolism of pyruvate normally takes place in the mitochondria, in which pyruvate is first oxidized by the enzyme complex pyruvate dehydrogenase and converted into a two-carbon fragment joined to co-enzyme A (acetyl-CoA) with the release of one CO₂ and water. Acetyl-CoA enters the citric acid cycle, where it is eventually fully oxidized into further molecules of CO₂ and water, generating reduced electron carriers. The reduced electron carriers shuttle electrons down the oxidative electron transport chain (ETC), and the energy released goes to make ATP (adenosine triphosphate), the universal energy intermediate in living cells.

The oxidation of glucose into carbon dioxide and water is respiration, the reverse of photosynthesis in green plants, algae and blue green bacteria. Photosynthesis captures energy from...
sunlight to ‘fix’ or reduce carbon dioxide from the atmosphere into carbohydrates (glucose) using electrons (and protons) obtained by splitting water, releasing oxygen back into the atmosphere in the process. The regeneration of oxygen is just as important as sequestering carbon dioxide, if not more so as far as air-breathing organisms are concerned, though it has been generally overlooked by climate change scientific community.

Water splitting and reforming is the redox dynamo, the magic roundabout that creates practically all life out of inanimate substances.

Mitochondria are special membrane-bound organelles that serve as ‘powerhouses’ in the cell (Figure 2). A mitochondrion has an outer membrane enclosing the entire structure, and a much-folded inner membrane that encloses a matrix, projecting numerous thin plate-like folds or cristae into it. Between the two membranes is a labyrinthine intermembrane space. Each mitochondrion also has 5 to 10 circular molecules of mitochondrial DNA that are replicated and inherited independently of the cell’s genome.
ATP synthase, resulting in the synthesis of ATP from ADP (adenosine diphosphate) and Pi (inorganic phosphate). This oxidative phosphorylation is absolutely essential for the life of all air-breathing animals. Most of the ATP is produced by oxidative phosphorylation in the mitochondria. The complete oxidation of glucose generates 36 molecules of ATP, of which 32 are produced within the mitochondria, and only 4 by glycolysis in the cytoplasm.

However, glycolytic reactions are much faster. It is estimated that in the time it takes for the mitochondria to produce 36 molecules from one glucose, another ten glucose molecules are turned into lactate with the generation of 20 additional ATP molecules in the cancer cell, making a total of 56 ATP molecules compared to the 36 in a normal cell.

ABNORMAL MITOCHONDRIA IN CANCER CELLS

Cancer cells not only exhibit aerobic glycolysis, they resist apoptosis (cell suicide), a fate that would normally befall cells with dysfunctional mitochondria. It thus appears that aerobic glycolysis and apoptosis are linked.

Evangelos Michelakis and his team at University of Alberta in Canada were among the first to note that aerobic glycolysis and apoptosis meet up in the mitochondria. They demonstrated the remarkable therapeutic potential of a cheap, readily available chemical dichloroacetate (DCA) that reactivated the gate-keeper enzyme for oxidation in the mitochondria, pyruvate dehydrogenase, and as a result the cancer cells committed suicide and the human tumour grown in cancer-prone rats shrank. We shall look at his results in some detail, as they are relevant to our understanding of cancer as a redox disease.

The link between glycolysis and apoptosis is apparent, as many glycolytic enzymes also regulate apoptosis, while several oncoproteins induce the expression of glycolytic enzymes. This web of circular causation is what one has come to expect as a consequence of the fluid genome, which also makes therapeutic interventions based on single molecular targets often ineffective, if not also fraught with side-effects.

The protein Akt, for example, which stimulates glycolysis and induces resistance to apoptosis, also activates hexokinase, an enzyme catalysing the first and irreversible step in glycolysis (see Figure 1) in which glucose is phosphorylated by ATP to glucose-6-phosphate. Akt induces the translocation of hexokinase - normally residing in the cytoplasm - to the mitochondrial membrane via its downstream mediator, glycogen synthase kinase 3 (GSK3). In the mitochondrial membrane, hexokinase binds to the voltage-dependent anion channel (VDAC), an important part of the mitochondrial transit pore that controls the permeability of the mitochondria to small hydrophilic molecules. This suppresses apoptosis, presumably by making the mitochondrial membrane impermeable. Inhibiting GSK3 in cancer cells presumably causes hexokinase to unbind from the VDAC, making the mitochondria permeable to small molecules, thereby inducing apoptosis and increasing sensitivity to chemotherapy.

This suggested to Michelakis’ team that perhaps the metabolic phenotype in cancer is due to a remodelling of the mitochondria that suppresses (or disturbs) oxidative phosphorylation, enhances glycolysis and stops apoptosis.

In keeping with this hypothesis is the observation that cancer cell lines have more hyperpolarized mitochondria membrane potential (more negative compared to the outside) (see Box 1). Cancer cells are also relatively deficient in the cell membrane voltage-gated K+ (Kv) channels (channels for K+ that open only if the electrical potential is beyond a threshold value). K+ channel deficiency is known to suppress apoptosis in several cell types including cancer cells.

DOWNSTREAM EFFECTS OF DCA

Treatment with DCA decreased the hyperpolarized mitochondrial potential to normal levels, accompanied by a decrease in tumour cell growth in vitro and in vivo, as reported.

The mitochondrial potentials in three human cancer cell lines: A549 (non-small-cell lung cancer), M059K (glioblastoma), and MCF-7 (breast cancer), were compared with healthy, noncancerous human cell lines: small airway epithelial cells (SAEC), fibroblasts and pulmonary artery smooth muscle cells (PASMC). All cancer cell lines had significantly more hyperpolarized mitochondrial potential compared to normal cells, as measured by increased fluorescent of the potential sensitive dye tetramethyl rhodamine methyl ester TMRM. Incubation of all three types of cancer cells with DCA reversed the hyperpolarization and returned it to the level of normal cells after

**BOX 1: CANCER CELLS HAVE HYPERPOLARIZED MITOCHONDRIA**

Hyperpolarized (more negative than normal) mitochondrial electric potential (Δψm) has been linked to malignant transformations since the 1980s. Tumour cells are typically highly heterogeneous, and within a population of tumour cells, there are minor subpopulations with stable differences in their Δψm that survive cell cloning. Cells with high Δψm typically have decreased sensitivity to chemoprotective agents and increased secretion of VEGF (vascular endothelial growth factor, promoting growth of blood vessels), and in metastatic tumours, but not in non-metastatic tumours, correlated with invasive potential.

However, mechanisms involved in generating and maintaining difference in Δψm are unclear; they may reflect alterations in the composition of the mitochondrial membranes, modulations in expression of mitochondrial targeted nuclear genes, or enrichment in a particular mitochondrial population.
48 h. But normal cells were unaffected. The DCA effects on mitochondrial electric potential occurred as quickly as 5-10 min and were dose dependent.

The DCA-induced decrease in electrical potential of the mitochondria was limited by an inhibitor of the VDAC; indicating that transport out of the mitochondria is important for the DCA response. As consistent with this hypothesis, DCA caused the efflux of pro-apoptotic factors from the mitochondria, as well as increased reactive oxygen species production (see below). In untreated A549 cells, cytochrome c and the proapoptosis inducing factor (AIF) were restricted to the mitochondria. But in DCA treated cells, cytochrome c was diffusely present in the cytoplasm and AIF was translocated to the nucleus, both indicative of apoptosis.

Moreover, DCA increased glucose oxidation by 23% and concomitantly suppressed glycolysis and fatty acid oxidation in A549 cells. After 48 h of DCA treatment, the extracellular lactate level was decreased, while pH increased in A549 cells compared with untreated cells. There is disagreement as to whether normally functional mitochondria actually export ROS, I believe it is entirely possible that ROS is only produced as the result of diminished coherence in electron transport, resulting in partially oxidized intermediates, because that is what ROS consist of. DCA increased the production of the ROS hydrogen peroxide (H2O2) in a dose-dependent manner from 25% at 0.05 mM DCA to 35% at 0.5 mM DCA. This increase was inhibited by rotenone, suggesting the involvement of complex I of the electron transport chain, presumably in a reversed electron transport due to a build-up of NADH, another sign that the mitochondrial ETC is not functioning normally in cancer cells. Consistent with this hypothesis is the observation that isolated mitochondria exposed to DCA showed an increase in NADH levels within the mitochondria.

One complication is that ROS at lower levels, characteristic of chronic stress and inflammation, are a ‘second messenger’ for cell proliferation - a predisposition to malignant transformation - supporting the idea that cancer is an epigenetic disease. However, the evidence linking mitochondrial ROS, presumably at higher concentrations, to apoptosis is equally strong.

The main ROS produced in mitochondria is H2O2 (see Box 2). If it is not eliminated by the cell’s antioxidant system, it can be further transformed to hydroxyl radical (·OH) in the presence of metal ions. ·OH is highly reactive, and damaging.

A wide range of mitochondrial ROS-induced damages has been described, to proteins, lipids and mitochondrial DNA. These damages can result in an energetic catastrophe.

As described by Michalakis’ team, the major ROS target inside the mitochondria is the permeability transition pore, which becomes highly conductive in the presence of ROS, allowing small molecules to pass in both directions. Small solutes flood into the mitochondrial matrix along their electrochemical gradients (from high concentrations outside to low concentrations), dissipating the electrochemical potential and inducing swelling of the mitochondrial matrix, eventually rupturing the outer membrane, releasing cytochrome c and proapoptosis inducing factor (AIF) into the cytoplasm, resulting in apoptosis. Cells use a special form of autophagy - mitophagy to selectively eliminate defective mitochondria. Increases in cellular ROS leads to loss of mitochondrial membrane potential, which is a trigger for mitophagy. When many mitochondrial are eliminated by mitophagy, apoptosis follows.

**MITOCHONDRIA REACTIVE OXYGEN SPECIES & DCA**

Reactive oxygen species (ROS) are small molecules containing oxygen that are more reactive than ordinary molecular oxygen. ROS are produced in mitochondria as intermediates of electron transport (see Box 2).

The process of oxidative phosphorylation, oxygen is reduced one electron at a time in a sequence; oxygen to superoxide to hydrogen peroxide to hydroxyl radical, and finally water:

\[ \text{O}_2 \rightarrow \text{O}_2^- \rightarrow \text{H}_2\text{O}_2 \rightarrow \cdot\text{OH} \rightarrow \text{H}_2\text{O} \]

All except the first and last have an unpaired electron, and are very reactive, hence referred to as reactive oxygen species (ROS). Thus, oxidative phosphorylation inevitably generates ROS as intermediates, and the mitochondria are considered the major source of ROS; the primary ROS being superoxide anion, O2·-. It is the precursor of all ROS species, and in vivo it is produced both enzymatically by NADPH oxidase, and xanthine oxidase, and non-enzymatically, when a single electron is directly transferred to O2. The superoxide anion acquires a proton to become a hydroperoxyl radical (H2O2·), followed by a fast rearrangement (dismutation) either spontaneously or through a reaction catalysed by superoxide dismutases (SODs) to produce hydrogen peroxide H2O2.

H2O2 is relatively stable and membrane permeable; and can diffuse within the cell to be eliminated by antioxidant systems in the cell or mitochondria, such as catalase, glutathione peroxidase, and thioredoxin peroxidase.

**BOX 2: MITOCHONDRIA IS THE MAIN SOURCE OF ROS**

H2O2 is relatively stable and membrane permeable; and can diffuse within the cell to be eliminated by antioxidant systems in the cell or mitochondria, such as catalase, glutathione peroxidase, and thioredoxin peroxidase.
the K⁺ outward current significantly in all cancer cells but not in normal cells\textsuperscript{15}. This increase in outward K⁺ current, accompanied by an increased expression of the K⁺ channel Kv1.5, leads to hyperpolarization of the plasma membrane (becoming more negative); and is blocked by intracellular catalase, which breaks down H₂O₂ and by rotenone which inhibits complex I produced H₂O₂.

At the same time, DCA decreased intracellular Ca²⁺ by inhibiting voltage-gated Ca²⁺ channels, so DCA treated cells had lower intracellular Ca²⁺ compared with untreated cells, the decrease occurring within 5 mins and sustained after 48 hours of DCA exposure. The effects on Ca²⁺ were inhibited by rotenone and mimicked by H₂O₂, among other things.

DCA is thought to decrease intracellular Ca²⁺ and increase Kv1.5 expression by inhibiting NFAT (nuclear factor of activated T lymphocytes). NFAT is known to inhibit both apoptosis and the expression of Kv1.5 in myocardial cells, and the team found that this was also true in cancer cells. Increase in intracellular Ca²⁺ activates calcinerin, which dephosphorylates NFAT, allowing it to be translocated to the nucleus where it regulates gene transcription. DCA-induced activation of Kv1.5 leads to hyperpolarization of the cell membrane, inhibiting voltage gated Ca²⁺ channels, hence blocking the increase in intracellular Ca²⁺ and inhibiting NFAT.

**DCA & APOPTOSIS**

Michelakis and colleagues found that DCA increases annexin expression, caused a ~6-fold increase in TUNEL-positive nuclei and activates both caspase 3 and 9 in A549 cells. Terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) is a method for detecting DNA fragmentation by labelling the terminal end of nucleic acids.

DCA appears to eliminate highly proliferative cells by inducing apoptosis and by decreasing intracellular Ca²⁺ levels. It also decreases cell proliferation, as measured by BrdU (bromodeoxyuridine) incorporation, and the expression of proliferating cell nuclear antigen (PCNA). In addition, DCA decreased the expression of survivin, a mitotic indicator.

DCA induces apoptosis of cancer cells by two pathways, one in the mitochondria, where depolarization activates mitochondria-dependent apoptosis, and the other at the cell membrane, where upregulation of Kv1.5 channels decreases K⁺, activating caspasas. The mitochondrial component is thought to be more important, as other factors and manipulations to deliver the cytoplasmic component of apoptosis did not result in the degree of apoptosis induced by DCA.

These findings, in addition to the demonstration of the ability of DCA to shrink xenograft human tumours in rats, and glioblastomas in humans\textsuperscript{16} do support Warburg's hypothesis that cancer is a disease involving mitochondrial malfunction; but perhaps not in its original form, as Warburg thought mitochondrial were totally inactive.

**REDOX IMBALANCE IN CANCER CELLS**

Not much attention has been paid to the electronic state of the cell or its organelles until quite recently when voltage sensitive dyes became available. This made it much easier to measure the electric potential of cells and organelles. As a result, researchers discovered that the cell's electric potential determines its vital states, from cell division and pattern formation to differentiation, regeneration and cancer\textsuperscript{21}. This is fully in accord with the quantum electro-dynamic nature of life\textsuperscript{11,12}.

Actually, it has been known since the 1950's that the cell membrane potential, measured with microelectrodes, varies throughout the cell cycle\textsuperscript{22}. Cell types with very high resting potentials such as muscle cells and neurons show little if any tendency to divide, while a decrease in membrane potential follows malignant transformation. In the 1970s, Clarence D. Cone Jr. induced DNA synthesis and mitosis in fully differentiated neurons from the central nervous system using a variety of agents that depolarized the cell membrane (made it less negative)\textsuperscript{23}.

In the 1990s, electric potential measurements of skin sites over malignant tumours of the breast gave electropositive readings that were correlated with increased depolarization in membrane potential of cancerous cells and tissues compared with normal cells or non-cancerous cells\textsuperscript{24}.

The other well-known sign of redox imbalance in cancer cells is the hyperpolarized mitochondria (see Box 1).

Additional evidence is now coming from direct measurements of redox states. The redox pairs of the cell are NADH/NAD⁺ (nicotinamide adenine dinucleotide), NADPH/NADP⁺ (nicotinamide adenine dinucleotide phosphate) and GSH/GSSG (glutathione). The ratio of reduced to oxidized forms reflect the redox state of the cell. For example, under unstressed conditions in cultured astrocytes (brain cells that control blood flow to neurons), the NADH/NAD⁺ pair is predominantly in the oxidized state to accept electrons produced during glycolysis in the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) reaction (see Figure 1). In contrast, the redox pair NADPH/ NADP⁺ are kept in a more reduced state to provide electrons for reductive biosynthesis, while the concentration of GSH strongly exceeds that of GSSG to support efficient antioxidant defence. These ratios of the redox pairs in cultured astrocytes are similar to those reported for brain\textsuperscript{25}, and are intimately linked to cellular metabolism and function.

Thioredoxin - a class of small redox proteins present in all organisms that act as antioxidants with redox signalling functions - is believed to integrate the overall redox state of the cell, and are essential for life in mammals\textsuperscript{26}. Researchers at University of Wisconsin School of Medicine and Public Health, Madison, in the United States, examined protein levels and redox changes of thioredoxin 1 (Trx1) in human prostate tissues and culture cells\textsuperscript{27}. They found more than 4-fold increase in Trx1 protein in the nucleus of high-grade cancer cells compared with normal controls, and the increase correlated with cancer progression. The protein was also increased in the cytoplasm by about 2-fold. Despite increased protein levels, the oxidized forms of nuclear Trx1 were higher in prostate cancer cell lines compared to their benign
counterparts, suggesting that nuclear redox imbalance occurred selectively in cancer cells.

Trx1 has a specific role in the modulation of redox signaling, with distinct nuclear and cytoplasmic pools, each performing different functions. In the nucleus, Trx1 interacts with certain transcription factors to regulate their binding to DNA; these include p53 (apoptosis response), nuclear factor κB (NF-κB, involved in inflammatory response) and nuclear factor-like 2 (Nrf2, involved in antioxidant response). In the cytoplasm, Trx1 can regulate apoptotic signal-regulating kinases. Trx1 is also known to move from the cytoplasm to the nucleus in response to oxidative stress. Selective oxidation of Trx1 can occur and has been detected in both the nucleus and the cytoplasm in response to cellular redox changes. Increased Trx1 protein expression has been detected in multiple cancer tissues and cancer cell lines, and an increase in Trx1 expression was associated with higher tumor grade and has been implicated in the resistance of tumor cells to certain chemotherapies and ROS generating agents.

CONCLUSION

Emerging evidence suggests that cancer cells are more oxidized relative to normal; they do not have enough electrons. This is consistent with other indications that cancer is a redox disease, a state of electronic imbalance. Rational therapy and prevention should start from here.

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General Practitioner (GP): “What can I do for you today?”

Patient: “I had the flu two weeks ago and my joints and muscles ached the entire time. I’ve still got pain all over but I’m otherwise fine, so I wanted something done.”

GP: “Tell me a bit more about your symptoms.”

…[Ten minutes pass]…

GP: “I’m convinced there’s nothing serious going on at this stage, the pain will resolve by itself with time, and some simple paracetamol will be all you need. Are you happy to go home, take paracetamol if the pain gets too much, and come back in two weeks if it hasn’t resolved?”

Patient: “Sure, that sounds good to me.”

GP: “If you have any other problems, as always just ring my receptionist to make an appointment.”

Patient: “Thanks, see you later.”

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Complementary and Alternative Medicine (CAM) Practitioner: “What can I do for you today?”

Patient: “I had the flu two weeks ago and my joints and muscles ached the entire time. I’ve still got pain all over but I’m otherwise fine, so I wanted something done.”

CAM Practitioner: “Tell me a bit more about your problems.”

…[Twenty minutes pass]…

CAM Practitioner: “We’ve discussed your pain in the context of your life at the moment and agreed that it’s nothing serious at this stage but is causing you quite some discomfort, particularly at work. Of the options that we discussed — doing nothing, taking paracetamol when required, and acupuncture of the affected areas — which would you like to move forward with?”

Patient: “Well as you said it should just go away with time so I’m happy to just take paracetamol if I need it.”

CAM Practitioner: “Ok, would you also like to discuss ways of dealing with the stress you have at work and home at the moment?”

Patient: “Actually I’m late for work right now, but I’d love to hear more about that next time.”

CAM Practitioner: “If you have any worries in the meantime feel free to ring the practice. If I’m not free my receptionist will take a message and I’ll get back to you as soon as I can.”

Patient: “Thanks, see you later.”

SO WHAT IS CAM?

Complementary and Alternative Medicine, or CAM, is the most common name used to refer to “a group of diverse medical and health care systems, practices, and products that are not generally considered part of conventional medicine”. Common CAM therapies include acupuncture, chiropractic, homeopathy, naturopathy and traditional Chinese medicine (TCM), and can be used alongside (complementary) or instead of (alternative) conventional medicine. These and other CAM therapies have over recent decades been responsible for greater and greater health expenditure, especially in first-world countries.

continued next page
WHERE DID CAM COME FROM AND WHERE IS IT NOW?

The oldest CAM therapies — complete medical systems such as TCM and Ayurveda — have existed for millennia. Developing in isolation in China and India respectively, they are based on the concept of two (TCM) or three (Ayurveda) vital forces. They propose that illness stems from derangement of these forces, with health achieved by specific physical procedures, techniques and natural substances that return these forces to balance. Newer therapies such as homeopathy and naturopathy developed independently in Europe over the last few centuries and are based on different beliefs of illness and health. But what all these therapies have in common is that they developed independently of conventional medicine, independent of each other, and espouse very different and in general simpler conceptions of illness and the body when compared to the modern scientific paradigm.

Though these therapies enjoyed their time in the sun for centuries, with the rise of conventional medicine, particularly following the introduction of the first publicly available medication aspirin in 1899, for most of last century, CAM therapies have largely been relegated to the sidelines. Yet the hippie culture of the 1960s, with its resurgent interest in mysticism, nature and alternatives to capitalist Western society, threw CAM a lifeline and brought it back into the limelight.

WHY IS CAM SO POPULAR?

It would be easy to explain away the popularity of CAM as a passing fad associated with a resurgent interest in nature, or as a by-product of increasing dissatisfaction with overburdened conventional healthcare systems. But with expenditure on CAM therapies increasing yearly and conventional medicine dissatisfaction having been summarily disproved as the reason, as a large United States survey attests, the answer lies further afield.

Consultation time

Perhaps the greatest gulf separating the practice of conventional medicine and CAM — other than the therapies themselves — is length of consultation time. One of the major drawbacks for users of CAM is that not only do consultations by practitioners on average take longer, the actual therapies themselves take significant time to deliver, such that total practitioner contact time is far greater than with conventional health professionals. This gives patients a sense of feeling valued, that they are able to spend so long a time with someone wholly interested in their health and wellbeing. It’s not a matter of CAM practitioners having more time to spare or necessarily caring more about their patients. In truth the minimum time required for CAM consultations is chief amongst the barriers to government subsidisation in public healthcare systems around the world.

Patient empowerment

A recurring theme when patients are asked about their reasons for using CAM is that of empowerment. Theories abound as to why this is the case, the most prominent being the relative simplicity of conceptions of health and disease in alternative compared to conventional medical systems, such that patients feel they understand the rationale behind CAM therapies better. It is hypothesised that the societal perception of the intellectual superiority of doctors plays a role, where CAM practitioners are seen as ‘ordinary people’ giving the patient more power in the relationship and thus increasing the popularity of CAM. Regardless, consumers of healthcare are increasingly rejecting the archetypal paternalistic ‘Doctor-as-God’ of the late 19th and early 20th Centuries in favour of the egalitarian health professional who favours individualism and restores the balance of power in favour of the patient.

Perceived safety

Side-effects are many patients’ biggest concern when receiving healthcare but this worry appears not to extend to CAM therapies. Given the relative age of CAM, therapies are often marketed as having been used by a certain group for millennia, with the implication that if it has existed for that long it must be safe. They are also seen as being natural — a perception practitioners encourage — and thus less likely and able to cause harm, despite many pill-based therapies ironically being manufactured on the same production lines as pharmaceuticals, most of which are themselves derived directly from biological substrates.

Perceived effectiveness

Despite the relative paucity of evidence on mechanisms of CAM therapies’ actions, when used for appropriate indications, CAM is effective. Examples backed by clinical trial evidence include: acupuncture for nausea, St. John’s wort for depression, and gingko biloba for intermittent claudication. The majority of CAM therapies, however, either remain unstudied or have conflicting evidence surrounding them. Yet despite this, more and more
people are using CAM and claiming positive outcomes. It has long been suspected that the beliefs of a patient regarding the therapy they are receiving are a strong determinant of treatment outcome. If this were the case then it would explain why clinical trials, which tend to control for patient expectations, would not detect the benefit experienced by CAM users who strongly believe in the effectiveness of the therapy they are receiving. When this notion was investigated in the context of a specific acupuncture intervention, it was shown unequivocally that positive patient expectations about a specific treatment improved outcomes, independent of general optimism about treatment.

WHAT CAN CONVENTIONAL MEDICINE LEARN FROM CAM?

Consultation time

Consultation time is one of the biggest challenges facing overburdened public health systems globally. There is much evidence suggesting that consultation time is correlated with patient compliance with therapy and thus outcome, and even the likelihood of medic-co-legal action in the event of an adverse outcome. There are clear financial and human resource barriers to increased consultation time in most conventional medical contexts, but that is not to say that they cannot be overcome.

It has been suggested that the majority of benefit from extra consultation time stems from greater personal contact with someone interested in the patient and their wellbeing. Thus one novel potential solution could be to enlist trained volunteer patient advocates. These advocates would be able to emotionally support patients in the majority of healthcare settings, including before and after consultations with health professionals, in hospital, and before major procedures or investigations. They would provide even greater uninterrupted support than CAM practitioners and tie in well with existing illness-specific support groups. While social workers would be better trained to serve in this role, social work tends to suffer from the same if not worse human resource issues as the medical profession.

Patient empowerment

Empowering patients is something conventional medicine has not only ignored but actively discouraged from its beginnings centuries ago until relatively recently, when modern individualism began demanding greater patient involvement in decisions regarding their own healthcare. More recently, the information revolution, led by free electronic databases such as Wikipedia, means patients are becoming more informed than ever about their own health. But curiously, rather than patients feeling empowered by being closer in knowledge to their clinicians, they are often frustrated by either the disconnect between the often incorrect or misinterpreted information they have procured online and either the process or outcome of their treatment. In turn, medical professionals’ bewilderment at the patient’s resulting non-compliance can cause them to react with more aggressive demands for compliance, often focussing on the negative consequences of failed treatment, unwittingly further reducing the patient’s empowerment and perpetuating the problem.

In this context, conventional medical practitioners can learn much from their CAM counterparts, where the latter tend to actively respect patients’ knowledge to a much greater degree. While many patients will not comprehend the specifics to same degree as their medical professional, empowerment is not purely a function of knowledge. Instead, the conventional medical system should urge practitioners to enquire of their patients’ understanding at every opportunity, correct where necessary, and encourage patients to seek information or clarification from their treating medical professional whenever required, as CAM practitioners tend to advance as a matter of course. To cope with the growing thirst for medical knowledge of patients, there is a growing body of doctor-authored literature demystifying medicine and the medical system for patients, which clinicians can encourage their information-hungry patients to read in the place of internet sources of uncertain veracity.

Perceived safety

With the rigorous trials that medications must go through before approved, followed by ongoing monitoring until production of the medication ceases, every effort has been taken to identify dangerous side-effects so as to avoid them in the patients who could not tolerate them. In fact, far more effort is put into ensuring the safety of conventional therapies with far higher standards than those applicable to almost all CAM therapies.

Yet patients still perceive closely regulated conventional therapies as more dangerous than largely unregulated CAM.

The reason for this appears to be in approach; where CAM practitioners connect with patients primarily on an emotional level, conventional medicine engages intellectually, speaking to patients of trials, evidence and regulations, which while seemingly incontrovertible to doctors often means little to patients.

In this context too, conventional medicine can learn from the personal approach of CAM. Patients may be reluctant to talk about their concerns regarding medications or procedures. Medical professionals can pre-empt this by proactively asking patients if they have such concerns. Perhaps more effective, in that patients can relate to them more readily, are members of support groups who have experienced or are experiencing a similar condition to the patient and wish to help others in the same situation. At present such groups tend to be patient-initiated and operate outside the health system, however, integration into the system would be very likely to improve their effectiveness. The health profession would ignore the value of patients themselves at its peril.

Perceived effectiveness

While the effectiveness of appropriate conventional therapies is rarely doubted, the manner in which CAM may be more effective in a subset of the population with particular health beliefs raises possible avenues for improvement in conventional medicine. Studies of CAM therapies, such as the investigation into acupuncture, suggest that greater belief in the effectiveness produces better outcomes. Belief can be increased by conscious
practitioner-provided information, which is already done to varying degrees by most medical practitioners, or by subconscious methods e.g. hypnosis.

Hypnosis was once thought to be too ethically fraught to use in medicine, but recent studies have shown it can be used safely with informed consent in the same manner as any other medical intervention\(^6\). To date it has only been used to assist patients in thinking positively about their condition and so aid therapy as well as the patients' bodies' own adjustment and defence mechanisms against the disease. While such a practice is ethically uncontested, using hypnosis to cause a patient to think more positively on the effectiveness of their therapy raises more complex issues. Having been hypnotised into thinking the therapy will be more effective than their previous subjective feelings regarding the therapy, if adverse events occur the patient will be unable to make an unbiased decision regarding continuation or cessation of the therapy. They would thus rely upon their treating team to such a degree that exploitation would be possible, for example if their treating team had a financial interest in continuing therapy. However, the situation is less fraught than other contexts in healthcare, including the ethically accepted scenario of an anaesthetised patient being completely dependent upon their operating surgical team. If these issues can be resolved we may have a new non-invasive way to improve the effectiveness of existing conventional medical therapies.

CONCLUSION

As a rapidly growing healthcare sector, CAM has a lot to offer patients as well as the conventional medical system it operates alongside. CAM's effectiveness in putting patients at ease, empowering them and giving them a system they feel is safe and that they can trust, holds important lessons for conventional medicine. While there are numerous barriers to translating those lessons into the conventional context, with some novel solutions they can be implemented.

Patient advocates can help patients feel more supported through their healthcare journey. Doctors can build rapport and therapeutic alliances by clarifying their patients' understanding of their bodies and medical conditions. Better integration of support groups into the healthcare system can provide an empathic human face of medicine to patients. Hypnosis can be used in controlled settings to augment the effectiveness of other therapies.

Though CAM and conventional medicine have such different foundations, they share a common goal, that of helping patients to achieve health. Both systems would do well to learn from each other such that we, patients past, present and future, are the ones who will benefit.

References:


**Book Review**

**Bad Pharma**  
*By Ben Goldacre*

This is a highly readable book by the same author who wrote *Bad Science*. Ben Goldacre graduated in medicine from Magdelen College, Oxford and is a Member of the Royal College of Psychiatry in London. He has a weekly column in the Guardian newspaper.

The book explores the very intimate relationships between the pharmaceutical industry, the medical profession, academia, academic journals, and to some extent with the Government.

While the author’s main focus is on the UK and American health industries, it is clear from a recent article in The Age newspaper that much of what he has written applies equally to the Australian scene. The pharmaceutical industry is all pervasive in its largesse with money for promoting its products to doctors, supporting education in universities, funding trials, funding academic journals to such an extent that few have the courage, or alternative sources of finance, to resist its charms.

While Goldacre admits there are many excellent and well meaning academics and doctors, he queries the closeness of the relationship with the pharmaceutical industry, and their ability to take an objective look at what has been happening over the last 50 years. One hundred pages are dedicated to the marketing aspects of the pharmaceutical industry, which makes for a very interesting read, and I recommend to all in the medical and allied health industries.

He also takes the industry to task over the number of trials which show missing data and negative results, which do not appear in the academic press, thus creating a bias in favour of the reported, and mostly successful trials in the meta analyses, such as the Cochrane Review process. Consequently he argues that the unexpected/unwanted side effects tend to be reduced in importance. Goldacre also tackles bad regulators and trials, and argues in favour of larger and simpler trials. Ghost written ‘academic papers’ are also a target for his rapier.

At the end of the book he puts forward some proposals as to what can be done to diminish the pharmaceutical industry’s influence. The book is very well referenced and is recommended for reading by all those concerned about the real cost of illness. As an “insider”, his information is invaluable.

*Reviewed by Dennis Crowley, BSc (Hons).*

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ACNEM Nested Qualifications

ACNEM qualifications are made up of learning modules, assessment tasks, practice logs, case studies and NEM Credit points. Learning modules are divided into Core or Elective modules and each learning module represents approximately 15 hours of lectures.

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Topics covered in Primary Course Modules 1 & 2 include:

- Introduction to NEM
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- Cardiovascular disease
- Allergies and food sensitivities
- Dietary history & the Low Stress Diet
- Cancer
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- Chronic fatigue syndrome
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- Migraines & other headaches
- Adrenal & thyroid conditions
- Women’s health & menopause
- Men’s health
- Childhood conditions
- Heavy metal toxicity
- The sensitive patient
- Arthritis, inflammation and pain
- Metabolic syndrome
- A-Z of hormones
- Contraindications & interactions
- Case histories
- Interactive panel discussions

Learning Modules Available

Online Core Modules

- Primary Module 1
- Primary Module 2
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- Gastrointestinal Conditions
- Thyroid & Adrenal Conditions
- Epigenetics & Nutrigenomics
- Women’s Health (coming soon)
- Metabolic Conditions, Diabetes and CVD (coming soon)
- Mental Health (available in 2014)
- Allergy, Autoimmune & Dermatological Conditions (available 2014)

Online Elective Modules

- Injectable Nutrients (doctors only)
- Heavy Metal Detoxification + certification (doctors only)
- Applications of NEM (available 2014)
- Menopause, Andropause & BHRT (available 2014)
- Environmental Health (available 2014)
- Oral Health (available 2014)

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November 2013 Sydney

- Primary Course in NEM – Modules 1&2 (21-24 Nov) (2 Core Modules)
- Gastrointestinal Conditions (21-22 Nov) (Core Module)
- A to Z of NEM (23-24 Nov) (Elective Module)

March 2014 Wellington

- Primary Course in NEM – Module 1 (8-9 Mar) (Core Module)
- Mental Health (Core module)