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CONTEMPORARY NEUROSCIENCE MEETS TRADITIONAL MEDICINE -TOWARDS UNDERSTANDING GINKGO BILOBA NEUROPROTECTION

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ABSTRACT: ***Use of alternative and complementary medical approaches, including herbal remedies, has increased substantially in the last decade, both in the United States and globally. The disease prevention theory associated with traditional Eastern medicine has the potential to both increase quality of life and reduce health care costs in Western societies. Action mechanisms of herbal remedies are, unfortunately, poorly understood. Moreover, Eastern medicine has developed within a theoretical and cultural framework that is significantly different from that of conventional Western medicine. For these reasons, understanding the mechanisms of action of herbal remedies is potentially more challenging, but not unapproachable. Application of contemporary neuroscience theories and available methodologies might help provide a better understanding of the effectiveness of traditional Eastern medicine. The purpose of the present paper is to provide an overview of how a complex herbal product, an extract from Ginkgo biloba has been studied with the tools of contemporary neuroscience***

KEY WORDS: : Chinese Herbal Medicine, Ginkgo biloba, Neuroscience

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A TREND IN THE UNITED STATES

A consumer survey in the United States indicates that at least 50% to 75% of US population has tried some form of unconventional medical therapies or alternative medicine (Neldner, 2000). Their use has undergone a dramatic increase in recent years (Kessler et al., 2001). According to a 1998 study published in the Journal of the American Medical Association JAMA (Eisenberg et al., 1998), 42% Americans were using the alternative medicine in that year, up from 34% in 1990. Between 1990 and 1997 the total number of annual visits to practitioners of alternative therapies in the US increased from 427 million to 629 million, exceeding the total annual visits to all primary care physicians (387 million in 1990 and 385 million in 1997, according to a national ambulatory medical care survey from 1996 and 1990). The therapies that have seen the greatest increase include herbal medicine, massage, megavitamin and energy healing. These are used most frequently for chronic conditions. Indeed, Americans spent more out-of-pocket for alternative therapies ($34.4 million) than they paid out-of-pocket for all hospitalization ($9.1 million) and total physician costs ($29.3 million). Herbal therapies rank second in total out-of-pocket medical expenses (Eisenberg et al., 1998).

Historically, Chinese doctors in 3000 BC believed in a strong relation between man and nature, and that every aspect of nature influences our body. Accordingly, medicinal treatments were derived from natural sources, and typically included adding therapeutic herbs to the diet (i.e., patients were advised to "eat the root" to promote physical health). Hippocrates, the father of Western medicine, also believed that man was strongly influenced by all natural elements. Hippocrates therefore took a holistic approach, believing that it was important to treat the entire man in his natural environment, rather than address the specific manifestations (symptoms) of disease. Harmony theories of illness and health held sway until the early 20th century.

The advent of Koch and Pasteur's germ theory was instrumental in changing the focus of researchers and practitioners away from harmony theory of health and illness, and toward single entity models of pathogenesis. The notion that a single microorganism could produce an acute specific disease state in a healthy organism guided the development of such treatments as antibiotics and vaccines. Clearly, the discovery of these treatments for infectious diseases was responsible for saving many lives. However, with the advent of safe and effective medicines for infectious diseases, herbal remedies fell into disfavor among Western practitioners. Physicians advise the patients to "take the antibiotics".

By the late 20th century, antibiotic-resistant pathogen strains began to emerge. Moreover, antibiotic use can be associated with significant side effects, and new infectious diseases continue to be identified that are resistant or non-responsive to antibiotic treatment. Clearly, these developments present serious challenges for modern Western medicine. Perhaps the most significant challenge comes from the various chronic medical conditions endemic among aging Americans, which seriously affect both morbidity and quality of life.

Ubiquitous environmental pollution due to the use of synthetic pesticide chemicals for over one hundred years presents anoth-

er major challenge for Western medicine. Due, perhaps, to a perceived inability of mainstream medicine to satisfy healthcare needs, especially in the elderly, or to ameliorate pollution-associated accumulation of toxins and synthetic drugs in the human body, many health-care consumers in the Western society are once again "eating the root."

To "eat roots" is a broad implication for alternative medicine, also known as complementary and alternative medicine (CAM). CAM is loosely defined as a treatment or practice not generally included in US medical school curricula, and not generally used in hospital practice or covered by third-party insurance payments. CAM is perhaps a better term than "alternative medicine" because most patients use these therapies to complement, rather than supplant, conventional medical care. CAM therapies can include herbal remedies, dietary supplements and therapies, acupuncture, acupressure, Qigong, and Yoga. Most of these therapies originate from Traditional Chinese Medicine.

TRADITIONAL CHINESE MEDICINE (TCM)

TCM has more than 4000 year history, and was first introduced to Europe over 350 years ago. TCM was not legal to practice in the US until 1975, and a shift to mainstream acceptance of TCM was strongly influenced by President Richard Nixon's China visit (Eisenberg and Wright, 1995). To achieve a better understanding of TCM, it is essential to know the underlying TCM's philosophy. There are three basic terms associated with TCM (the Qi, the Yin & Yang, and the five elements).

Qi, is an ancient medical term. To live is to have Qi in every part of your body, to die is to be a body without Qi, for health to be maintained, there must be a balance of Qi . There are three kinds of Qi: the original Qi from parents, the nutritional Qi from food, and the air Qi from air we breathe. The theory of Yin and Yang is an ancient philosophic concept used in TCM. Yin and Yang represents the opposition and unity of the universe and human body, also known as systematic correspondence (Zhang, 1999). TCM is based on the principle that our body is in a state of harmony. This state of harmony represents a balance, or correspondence, between Yin and Yang, between the different organs of our body, and between our body and the environment in which we live. This harmony is constantly being broken due to our daily activity and due to influences from the outside environment. When this harmony is broken, we fall ill and disease will occur. Another important philosophical contribution to TCM is the five elements. In ancient China, it was though that the natural universe consisted of five elements: wood, water, earth, fire and metal. The human body is seen as a part of nature, and as one element controls another, balance is achieved.

TCM stresses the importance to disease prevention. As early as two thousand years ago, the theory of "Preventive Treatment of a Disease" was put forward in the book "Yellow Emperor's Canon of Internal Medicine". This theory includes two aspects: 1) adopting preventive measure before a disease, and 2) taking precautions against the progression of a disease (Zhang, 1999). "Adopting preventive measure" refers to taking various precautions against the possible occurrence of a disease, which starts with building up the Vital Qi's power to resist pathogenic factors. Satisfactory effects have been obtained in prevention of bacillary dysentery with garlic, of virus hepatitis with Chinese date, and of influenza with isatis leaf and the root. However, not all the disease can be prevented before the body is attacked. Thus, "Preventing the progression of a disease" states that a good doctor treats a disease caused by pathogenic factors as soon as they attack the body surface, suggesting that diseases should be cured at their initial stage in order to stop their progression. Therefore, when doctors deal with pathological changes in the liver, they usually make an additional remedy to strengthen the spleen and stomach at the same time, from which the theoretical basis of the Traditional Chinese Medicine of liver disease originates. Accordingly, the Four Basic Principles of Treatment guiding the therapeutic methods are: 1) searching for the primary cause of a disease in treatment, 2) strengthening the body's resistance and eliminating pathogenic factors, 3) regulation of Yin and Yang to restore the relative balance or a harmonious state, and 4) treatment in accordance with seasonal conditions, local conditions and the physical conditions of an individual.

Treatment approaches to illness and disease are affected by culture and sociohistorical context (Berman et al., 2002). With rapid changes in technology, there seems to be an increasing desire in the West to turn to traditional Eastern medicines for holistic cures. At the same time, modern Chinese generation has started on a Western journey. Though TCM and Western medicine have very different theoretical underpinnings, they are both medicine and thus have the common goals. A metaphor that illustrates the philosophical differences between the two medicines can be found in "Between Heaven and Earth" (Beinfield and Korngold, 1991): Western medicine views the body as a machine, and thus doctors are mechanics. TCM sees body as a garden, and thus doctors are gardeners. "What works in the garden may be inappropriate in the factory. Compost doesn't nourish a machine, and oil and gasoline do not enhance the soil. Chinese medicine readjusts balance, enhancing self-healing and helping chronic, long-term problems, whereas Western medicine affects the structure components, suppressing pathological phenomena, intervening in life-threatening crises" (Beinfield and Korngold, 1991). The best outcome of a cross-cultural encounter between Western science and TCM would be to take advantage of the best aspects of each approach, based on mutual understanding and appreciation.

DIFFERENCE BETWEEN WESTERN DRUGS AND THE HERBAL MEDICINE

Although some herbal extracts have become Western pharmaceutical drugs and are used within the paradigm of modern pharmacology, these drugs are no longer conceptualized as belonging to the herbal medicine. For example, aspirin was originally derived from the bark of trees, morphine from the seeds of poppy flowers, penicillin from a fungus and digitalis from the leaves of foxglove. The concept of modern pharmacology and the herbs accompanies the philosophies and principles of the two medicines. The difference is not the drug itself, or its source, form and components, but the mechanism of action and theory of the therapies.

Western pharmaceutical drugs are usually based on a single biologically active ingredient to produce a specific physiological effect. This accounts for their potency and also for their side effects (Beinfield and Korngold, 1991). Although drugs may control symptoms, they often do not treat the pathological process. For example, aspirin controls arthritic pain without altering the degenerative course of the disease. With herbs, the active ingredients are enfolded within the whole plant, which buffers their side effects. Because the entire molecules come from a living

thing, it increases the probability that they will exert an adaptive biological effect (Christen and Maixent, 2002). Indeed, many herbs are nutritive and cannot be distinguished from food, and are often used mainly for disease prevention and tonification, rather then symptom amelioration.

Herbal medicine has existed for more than 5000 years. During this long history, herbal doctors have gained rich theoretical and clinical experience. In the early Ming dynasty, more than 60,000 herbal prescriptions were recorded. A basic assumption of TCM is that nature is the source of herbal healing, thus herbs cannot be used in isolation or in a highly purified form (Zhu, 2000). Now there are more than three thousand kinds of medicine herbs. Each herb has its own characteristic, but when herbs are combined they often interact synergistically. That is, certain herbs can eliminate adverse effects or antagonize one another. It is ignorant to think that herbal medicine is safe and cannot be harmful. The appropriate use and understanding is essential (Weiss and Fintelmann, 2000).

The important principles behind modern pharmacology include: 1) the receptor theory, which states that each drug acts on a specific receptor and that drugs can function as receptor agonists or antagonists; 2) the structure-function relationship, whereby the action of a drug depends on the specific molecular structure of the compound, a higher affinity of a particular chemical structure to its receptor is often associated with a greater therapeutic efficiency; and 3) the specificity of action; most modern drugs have relatively specific sites of action, with the consequence of a specific drug being used for a specific symptom. The unity of the three principles reflects the fundamental underpinnings of modern pharmacology.

In contrast, herbal medicine relies on the following three important points: 1) Ecological regulation, recognizing that all herbs exist as part of the nature's food chain. Some of the herbs are employed in the form of simple food products, which are selected according to their modulatory role to the body's Qi. 2) The Qi properties of the herbs within. Instead of the related structure and function, herbal medicine emphasizes the balance of the Qi. The Qi properties of herbs (e.g. "cold" or "warm") are used to regulate the balance of Yin and Yang in the human body by removing the excess of either Yin or Yang, which is more complex than the structure-function relationship. 3) Comprehensive effect or systematic correspondence, modulating the body as a whole via multiple, complex polyvalent actions. In addition to the chemical transformation within the body as the Western drugs do, the herbs also undergo a biotransformation, i.e., the secondary, or even the end product often posses the efficiency. Thus, in contrast to the linear pharmacokinetics in Western pharmacology, the distribution, absorption, metabolism and excretion of the herbs are assumed to be a nonlinear process (Zhu, 2000). Often, opposite effects in human body are observed with the same herbal remedy, which is known as the "bi-directional modulation". For example, dong quai (*Angelica sinensis*), known both in China and the West for its ability to support and maintain the natural balance of female hormones, can induce both relaxation and contraction of the smooth muscles in uterus, depending on circumstances. Thus exists the herbal theory of "different remedies for the same symptom, and the same remedy for different symptom"(Gao, 2000). In fact, the therapeutic effect of herbal modulation is more than bi-directional, and should rather be called multidirectional. The ultimate goal of herbs is not only to correct the abnormality of the organ, but also to modulate the vital Qi, promoting the systematic state to balance.

The reader may note that, over the past 30 years, holistic and integrated approaches to research and treatment have attracted increasing attention in Western medicine under the rubric of "biopsychosocial" medicine (Schwartz, 1982). The term biopsychosocial represents a belief system in which human illness is thought to occur in interlocking and interdependent systems ordered in a hierarchy of increasing complexity, from micro systems (e.g., molecular) to macro systems (e.g., health delivery systems). When these factors are integrated, the effect of the system on human functioning may be greater than the individual contributions of its component parts (Engel, 1980; Reiser, 1980). Accordingly, the biopsychosocial practitioner considers the patient's psychological response to illness and treatment, and the cultural context in which the illness occurs. These factors are assumed to reciprocally affect biological functioning at the micro level, potentially altering the course of the illness. Numerous articles advocating biopsychosocial medical practice have appeared in the past three decades. Unfortunately, biopsychosocial treatments have not challenged the preeminence of conventional Western medicine (Sadler and Holgus, 1992; Gabbrd and Kay, 2001). More importantly, man's interdependence with natural world is less of a concern for Western biopsychosocial models of health than for TCM. The unity between the human body and nature has long been a major emphasis in TCM. We live in nature and are constantly influenced by the changes of environmental circumstance in nature, to make corresponding physiological and pathological responses. For example, as the climate varies with four seasons in a year, the normal pulse conditions, the rate, rhythm, volume and tension of pulse, are also varied. This provides a basis for doctors to distinguish abnormal pulse conditions from the normal ones during the diagnosis (Zhang, 1999). It is also true for changes in the color, shape and the texture of the tongue according to the season and environmental circumstances (Eisenberg and Wright, 1995). Although the biopsychosocial model is closer to TCM than the conventional biomedical model, this model may represent an intermediate product during the development of more holistic models for medicine.

With the World Health Organization's shift in focus from the treatment of manifest disease to disease prevention, the use of the herbal medicine continues to grow in the United States, as well as many other countries in the world. A consumer survey in US conducted in 1999 determined that 49% of all adult Americans had used at least one herbal medicine during the previous year, with 24% acknowledging that they use herb on a regular basis (Blumenthal, 2000). Because of the preventive properties of herbal medicine, TCM has the potential to increase the quality of life and to decrease health care costs in the United States. However, the limitation is the lack of understanding of the herbal medicine, which is associated with both the safety and effectiveness. The mechanism of action, from the point of view of modern pharmacology, is unknown.

Americans spent $27 billion on unproven remedies in 1997 (Stokstad, 2000), and most of these compounds and treatments have not been rigorously tested for either safety or efficacy. For this reason, by the year 2001, the National Center for Complementary and Alternative Medicine (NCCAM) of National Institutes of Health (NIH) budget reached $80 million for CAM research, almost a double of the year 1999 (Couzin,

1998; Stokstad, 2000). The current director of NIH/NCCAM has announced that testing the efficacy and safety of specific CAMs, and dissemination of this information to the American public, is an important part of his mission (Stokstad, 2000).

TOWARD UNDERSTANDING THE MECHANISM OF *GINKGO BILOBA* NEUROPROTECTION

The *Ginkgo biloba* leaves have been used in ancient and modern Chinese herbal pharmacopoeia as treatment for dysfunctions of heart and lung and as promoter of longevity (Del Tredici, 1991). A standardized extract of *Ginkgo biloba* leaves, EGb761, is becoming one of the most popular dietary supplements in the United States to enhance memory. In Europe it is a commonly prescribed drug for treatment of age-related deterioration, including degenerative dementias of the Alzheimer type (AD) (De Feudis, 1998).

During the past decade, *in vivo* and *in vitro* experiments in mammalian systems and clinical studies in humans demonstrated that EGb761 exhibits a range of biochemical and pharmacological effects, which include vasoregulation, cognition enhancement, and alleviating stress (De Feudis, 1998). In human studies, available data have confirmed the clinical efficacy of EGb761 in primary degenerative dementia of Alzheimer's type (Le Bars et al., 1997; Oken et al., 1998; Le Bars et al., 2000). The evidence supporting EGb761 enhancement of learning and longevity in healthy animals and humans is yet inconclusive (Winter, 1998; Curtis-Prior et al., 1999; Solomon et al., 2002). The readers may have heard a recent study (Solomon et al., 2002) indicating that EGb761-treated human subjects did not differ from placebo-treated controls in any of the cognitive tests that were administered. However, the authors did mention that the dose they used might have been too low for observing effects in healthy subjects. Also, EGb761 may have a more pronounced effect on patients who have significant cognitive impairment than on healthy subjects. From our own animal experiments, EGb761 is more effective in stress conditions than in non-stressed controls, observed in experimental mice (Ward et al., 2002), as well as in the nematode *C. elegans* (Wu et al., 2002). Although negative results are extremely important for the whole picture, all the well-controlled studies must be considered. It is noteworthy that in another independent study (Mix and Crews, 2002), eleven out of the thirteen outcome measures numerically favored EGb761. Upon considering all studies conducted to date, it appears that EGb761 has an effect, although a moderate one, on the brain function. EGb761 can be useful as symptomatic treatment for the cerebral insufficiency that occurs during normal ageing or which may be due to degenerative dementia, and vascular dementia (DeFeudis, 2002 personal communication). There are sufficient data to support the view that the extract has neuroprotective properties (Smith et al., 1996; Luo, 2001).

As summarized by Y. Christen at a recent conference on "Ginkgo biloba Extract: From Traditional Medicine To A Medicine Of The Future", EGb761 acts at all levels of life: from molecules, cells, tissues, to the entire organism (Christen and Maixent, 2002). Accumulating evidence has suggested that many of the actions of EGb761 are so-called "polyvalent" actions, i.e., the therapeutic activity of EGb761 is the net effect of interactions between the various biological activities of the individual substances of EGb761. This is in agreement with the philosophy of Chinese herbal medicine, in which systematic correspondence of a mixture of compounds from nature resource, acting simultaneously in combination and synergy, are required to balance body's Yin and Yang (Luo, 2001). It appears that it is the multiplicity of effects by EGb761, or the polyvalent action, that complicates the obtaining clinical proof of its efficacy in human studies. One of the core theories of Chinese herbal medicine, that "different remedies for the same symptom, and the same remedy for different symptom," (Gao, 2000) may provide an explanation. That is, individual differences in patients and their natural circumstance all have to be taken into account in the diagnosis and treatment with Chinese herbal medicine.

Our research involves a programmatic exploration of scientific explanations for the mechanisms of traditional herbal medicine. Using neuronal cell culture, organism nematode *Caemorhanditis* elegans and animals behavioral test in mammals, our results showed that EGb761 exhibits multiple cellular and molecular neuroprotective mechanisms, including modulating the cellular survival machinery (Smith et al., 2002), inhibition of Aβ aggregation (Luo et al., 2002), and augmentation of organism's stress-response (Ward et al., 2002; Wu et al., 2002).

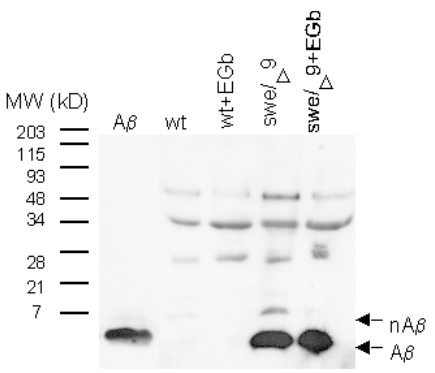

Figure 1: Effect of EGb761 on Aβ aggregation in neuroblastoma cells. Representative immunoblotting of Ab species from the culture media of an Ab-producing neuroblastoma cell line. Lane 1: purified Ab; Lane 2 and 3: wild type untreated (wt) or treated with 100 mg/ml EGb761 for 48 h (wt + EGb); Lane 4 and 5: the Ab-producing mutant cell line untreated (swe/D9) or treated with EGb761 (swe/D9 + EGb). Arrows indicate Ab monomer (Ab) or aggregated oligomers (nAb) (Luo et al., 2002).

With a neuroblastoma cell line stably expressing an Alzheimer disease-associated double mutation, we reported that EGb761 reduced the formation of amyloid-β (Ab) aggregation (Figure 1), which are the diagnostic, and possibly causative, feature of Alzheimer's disease. Ab aggregation has been postulated to link free radical production and cellular apoptosis. We observed that EGb761 significantly attenuated intracellular free radical production (Figure 2) and mitochondrion-initiated apoptosis (Figure 3), and decreased the activity of caspase 3 (Figure 4), a key enzyme in the apoptosis cell-signaling cascade. Furthermore, data from DNA microarray assay indicated that transcription of multiple apoptosis-related genes was either up-, or down-regulated in EGb761 treated PC12 cells (Table 1), a well-established cellular model for studying neuroprotection (Greene and Tischler, 1976).

However, results obtained from in vitro studies using animal cell culture may not necessarily correlate with data from in vivo studies with the experimental animals. For example, the effect on

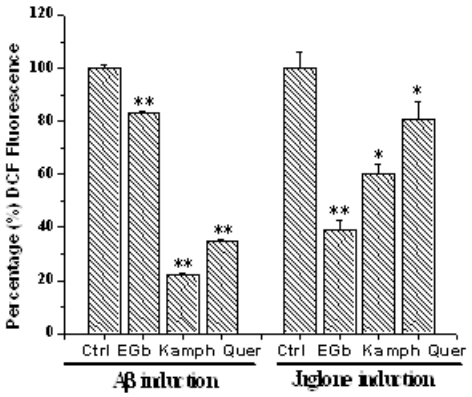

Figure 2. EGb761 effect on production of cellular reactive oxygen species (ROS). The intracellular ROS was induced either by A_ transgene expression with butyric acid (1 _M) in the mutant cells, or by exposure to an oxidative stressor Juglone (1 _M) in the wild type. Cells were pretreated with EGb761 (100 _g/ml) or its flavonoid components kamphoerol (Kamph) and quercerin (Quer) for 48 h prior to the induction. Intracellular ROS were quantitated after 50 min incubation with the cell-permeable DCF-DA (50 _M) with a fluorescence multiplate reader.

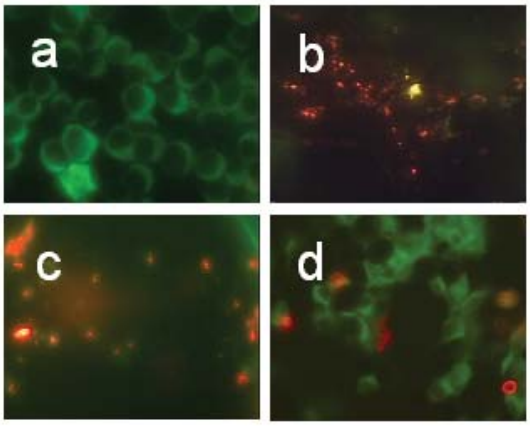

Figure 3. Representative fluorescence staining for mitochondria-sensitive A_ cytotoxicity in the neuroblastoma cells treated with or without EGb761. Mitochondrial integrity was probed using Mitosensor (Clontech). Mitochondria of healthy cells exhibit red fluorescence; cells undergoing apoptosis exhibit green fluorescence. Panel a: the A_-producing transgenic cells stimulated with 1 _M butyric acid for 12 h to express the transgene; panel b: unstimulated wild type N2a cells (wt); panel c: the A_-producing cells pretreated with EGb761 for 48 h prior to transgene expression; panel d: the A_-producing cells pretreated with vitamin E (VE) for 48 h. Similar results were obtained in 3 experiments (Luo et al., 2002).

gene expression in mice fed with EGb761 examined in two brain regions showed that only 10 out of 3,000 genes were significantly affected (Watanabe et al., 2001), whereas in a study using a cultured cell line, the expression of 400 genes out of 6,800 was modified (Gohil et al., 2000). Thus, data from in vitro studies could sometimes result in misleading conclusions (Christen et al., 2002).

To establish an in vivo model, Caenorhabditis elegans was introduced to our laboratory for pharmacological and genetic evaluation of EGb761 effects on stress-response and aging (Wu et al., 2002). The C. elegans is a small free-living soil nematode, which feeds on bacteria E.coli. Its short life span and ability to self-fertilize permit the production of large numbers of genetical-

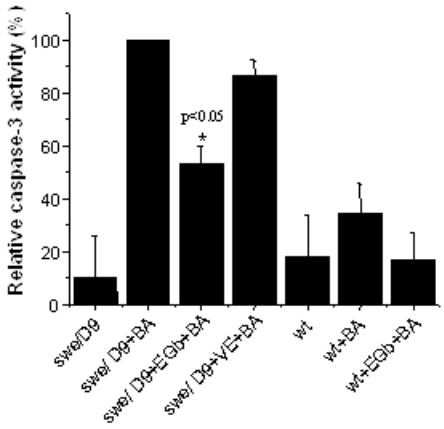

Figure 4. Effect of EGb761 on caspase-3 activity in neuroblastoma cells. Caspase-3 enzymatic activity assay in the N2a cells (wt), and the A_-producing cells (swe/_9) cells alone, or treated with EGb761 for 48 h prior to transgene expression with butyric acid (BA). Data are expressed as percentage of the maximum caspase-3 activity in the mutant cells, which, in average, was equivalent to1.6 pmole/mg protein/min. * statistical significance ($p<0.05$, n= 4) by unpaired t test (Luo et al., 2002).

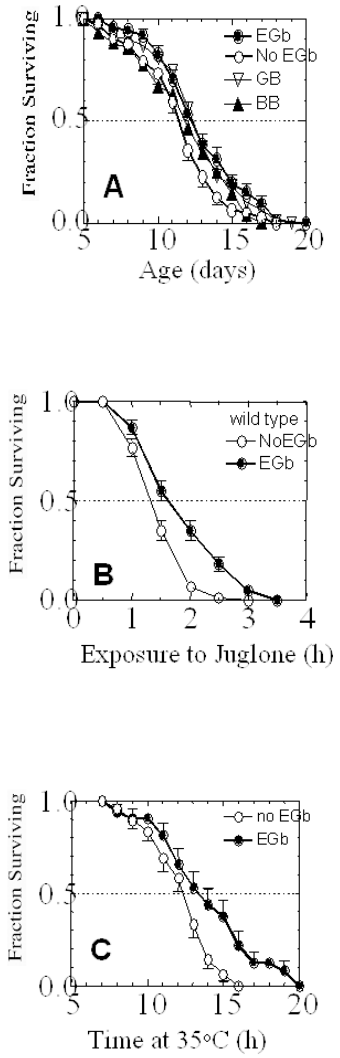

Figure 5. EGb761 and its constituents effect on life span, and stress response of C. elegans. A. Life span of the adult worms fed with E. coli OP50 supplemented with either 100 _g/ml EGb761, or 50 _g/ml ginkgo terpenoids ginkgolide B (GB) or bilobalide (BB). The control group's food was supplemented with the vehicles (ethanol and Tween-80) at appropriate concentrations. Standard errors for each data point were calculated using the GraphPad Prism software. B. The survival assay in C.elegans under an acute, lethal dose of oxidative stress. The worms were pretreated with EGb761 for 48 h, transferred onto the medium containing 472 _M juglone and survival was monitored at regular intervals. Total number of worms was 100 in each experimental group. C. The survival assay in C.elegans under heat stress. Four-day old worms, raised at 23∞C and either untreated or treated with EGb761, were transferred to 35∞C and survival was measured at regular intervals. Total number of worms in the experimental groups was 189 (control) and 179 (EGb761) (Wu et al., 2002).

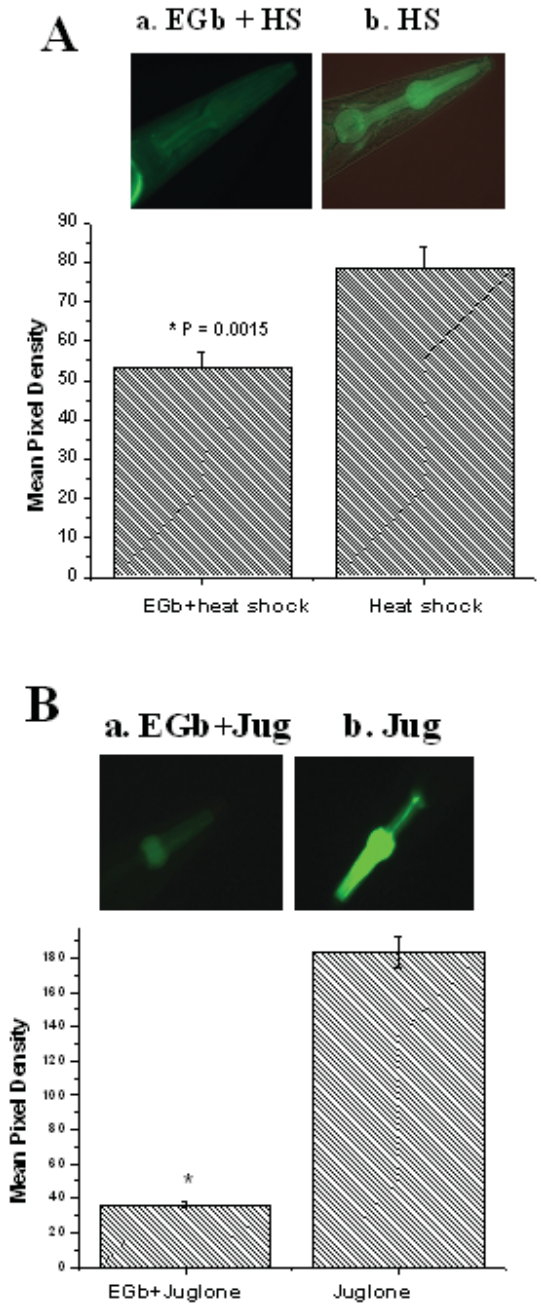

Figure 6. Regulation of hsp16 expression by EGb761 under stress conditions induced by heat (A) or Juglone (B). A. Worms were grown at 20oC, treated with or without 100 mg/ml EGb761 for 48 h at age day 2, prior to heat shock. Then the worms were exposed to 35oC for 2 h and transferred to 20oC for 4 h to recover prior to fluorescence microscopy. B. The worms were fed with EGb761 for 48 h before juglone stimulation for 24 h. The fluorescence images were obtained with an Olympus fluorescence microscope attached to a digital camera. The inset images include the entire anterior part of the pharynx displaying endogenous GFP fluorescence. For quantitate a population of reporter animals to heat shock and Juglone, each 40X image were analyzed using Imageplus software.

ly identical individuals. These traits have enabled the discovery of over forty single-gene mutations that extend life span by 10% to 400% (Jazwinski, 1996). Moreover, in such optically transparent model systems, gene expression can be directly visualized in vivo by the construction of reporter transgenes expressing jellyfish green fluorescent protein (GFP) (Link et al., 1999). Using this model, we tested the theory that EGb761 augments the natural antioxidant system of C. elegans, and thus increases stress resistance and longevity. We observed that EGb761 did not significantly extend the maximum life span of C.elegans under physiological conditions (Fig. 5A), but it restored the mean and maximum life span to the normal value in the animals exposed to a temporary, non-lethal oxidative stress (Wu et al., 2002). This supports the free-radical theory of aging (Packer and Colman, 1999). We then tested whether EGb761 can increase resistance against stress induced by heat and juglone, a redox quinone that generates superoxide anion from molecular oxygen during metabolism. We found that treatment of the nematode with EGb761 increased the worm's resistance to oxidative stress by 33%, and its thermotolerance by 25% (Fig. 5B and C), which is consistent with our previous results from an animal behavioral study with mice (Ward et al., 2002).

To visualize gene regulation in vivo in real time, the effect of EGb761 on expression of a heat shock protein gene hsp-16 was directly observed using a GFP reporter transgene (hsp::GFP) (Link et al., 1999). The small heat shock proteins (hsp16) are a collection of low molecular weight polypeptides identified in all eukaryotes examined (Fonte et al., 2002). EGb761 significantly attenuated the hsp:GFP gene expression in response to thermal and oxidative stresses (Fig.6). Post-administration effects of EGb761 (data not shown) seem to indicate that the extract functions not only as a chain-breaking antioxidant that prevented the propagation of free radical damage, but also as the enhancer of repair or turn-over of damaged macromolecules. EGb761 has been postulated to act as a biological response modifier, perhaps aiding hsp16 in its functions, one of which is to recycle proteins that have been damaged. It is speculated that in the presence of EGb761 fewer radicals are around, and fewer proteins are damaged, which makes expression of hsp16 unnecessary. Our results suggest that oxidative stress as well as other types of stress can be successfully counteracted by the Ginkgo biloba extract EGb761, probably via regulation of anti-stress mechanisms, or the endogenous "antioxidant network" (Packer and Colman, 1999).

CONTEMPORARY NEUROSCIENCE MEETS TRADITIONAL MEDICINE

Contemporary neuroscience is probably the most interdisciplinary research. It utilizes the best molecular, cellular, physiological, behavioral and imaging techniques to address a range of concerns related to the function, and dysfunction, of the brain. For example, neuroplasticity and learning can be measured in vitro at the synaptic level electrophysiologically as long-term potentiation (LTP), at the cellular level as image of waves of molecular changes, in vivo as gene expression profile, and at a behavioral level in transgenic animal model of neurodegenerative diseases or in human brain via visualization by functional imaging of brain activity in real time. The emerging technology offers significant potential to alter our view of the brain's development, organization and function (Katz and Grinvald, 2002). Apparently, modern neuroscience has at its disposal the best tools for approaching the most challenging questions, such as the mechanism complexity of herbal medicine, which is a mixture of molecules synthesized not by a chemist but by the natural selection.

Table 1. Transcriptional effects of EGb761 on NGF differentiated PC12 cells.

Gene/function	Gene ID	Clone description	Fold change
Apoptosis			
Bcl-2 interacting protein	H3103B07	Mus musculus Bcl2/adenovirus E1B 19 kDa-interacting protein 3-like	1.95
Tumor necrosis factor	H3091D11	Mus musculus timor necrosis factor superfamily member 19	1.86
Caspase 12	H3131G02	Mus musculus caspase 12	-1.74
Apoptosis regulator	H3038E03	Homo sapiens PRKC, apoptosis, WT1, regulator	-1.84
Other MitoChip Clones			
Putative phosphatase	H3157D01		4.49
ATPase like proton channel	H3027A10	Mus musculus ATPase-like vacuolar proton channel (Atpl)	1.89
choline transporter	H3102C06	Rattus norvegicus choline transporter (CHOT1)	1.76
Brain cDNA clone MNCb-0663	H3122H03	Mus musculus brain cDNA, clone MNCb-0063, liver regeneration-like	1.71
Voltage-gated-Sodium channel	H3149E11	Homo sapiens sodium channel, voltage-gated type II, beta polypeptide	-1.71
Glutathione-S-transferase	H3111F09	Rat Y-b3 glutathione –S-transferase mRNA	-1.81
Glycogen phosphorylase	H3117G06	Rat glycogen phosphorylas brain isozyme mRNA	-1.91
Serine protease	H3045E05	Mus musculus serine protease OMI	-2.02
Glycerol-3-phosphate dehydrogenase	H3005G01	Mouse mRNA for glycerol-3-phosphate dehydrogenase	-2.09
Mitotic arrest deficient	H3124D11	Mus musculus mitotic arrest deficient 1-like	-3.16

Total mRNA was extracted from the PC12 cells treated with or without EGb761 (100_g/ml for 48 h). cDNAs for array printing were amplified by PCR. Mouse Mitochip array consists of 816 cDNA clones originated from the NIA 15k mouse cDNA library (see Methods). Positive numbers indicate up-regulation of the transcription by EGb761 treatment, i.e. a ratio greater than 1. The negative numbers indicate down-regulation of the transcription by EGb761 treatment, i.e. the ratio smaller than 1. Similar results were obtained from two different preparations (Smith et al., 2002).

It is exciting to note that some results obtained with modern neuroscience methods agree with the traditional herbal medicine theory. For example, EGb761 was found to attenuate the apoptosis, a programmed cell death, in the culture, which in effect increases the number of cells (Luo et al., 2002; Smith et al., 2002). On the other hand, the same extract also inhibits cell proliferation, slowing growth of cultured cancer cells (Gohil et al., 2000; Papadopoulos et al., 2000), apparently a surprising finding. But this dual effect is in line with the bi-directional modulator theory of herbal medicine. Such a bi- and multi-directional modulation also occurs within our body. A small molecule nitric oxide (NO) functions as a neurotransmitter in synaptic plasticity, as a modulator for relaxation of smooth muscles, and a toxic free radical. Interestingly, the effect of EGb761 on NO also illustrates its multidirectional regulatory action. EGb761 induces NO production by endothelial cells; it can also scavenge the free radical form of NO and prevents endothelial activation (Pietri et al., 1997). Thus, the regulatory and adaptive effects of EGb761 can vary neurotransmitters or neuromodulators activities according to the circumstances (Christen et al., 2002).

CONCLUSION

EGb761 research serves as an example of how the combined power of contemporary neuroscience and theory of traditional medicine can provide new clues about the complexity both in the products (drugs, nutraceuticals, herbs, etc) and in their therapeutic functions. It is predicted that the twenty-first century molecular medicine will be based on decoding complexity. Given the ubiquitous and increasing use of TCM by Western health consumers, it is imperative that we achieve a better understanding of the mechanisms of action of herbal remedies. Perhaps more important, more information about how Western and Eastern approaches can be integrated in a safe and synergistic manner would be helpful to both providers and consumers of health services. Modern neuroscientists are ideally equipped to address both of these critically important needs, and should take a proactive leadership role in these endeavors.

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