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Formulation and *in vitro/in vivo* evaluation of combining DNA repair and immune enhancing nutritional supplements

R.W. Pero[a,*], A. Amiri[a], Y. Sheng[a], M. Welther[b], M. Rich[c]

[a]*Department of Cell and Molecular Biology, Section for Tumor Immunology, University of Lund, Lund, Sweden*
[b]*Arlington Family Practice, Arlington, VT 05250, USA*
[c]*Phoenix Laboratories Inc., Hicksville, New York, USA*

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Abstract

Combining nutritional supplements to achieve synergistic benefit is a common practice in the nutraceutical industry. However, establishing added health benefit from a combination of natural ingredients is often assumed, untested and without regard to the principle of metabolic competition between the active components. Here, we report on the combination of a cat's claw water extract (C-Med-100, carboxy alkyl esters = active ingredients) + medicinal mushroom extracts (*Cordyceps sinensis*, *Grifola blazei*, *Grifola frondosa*, *Trametes versicolor* and *Ganoderma lucidum*, polysaccharides = active ingredients) + nicotinamide + zinc into a formulation designed to optimize different modes of immunostimulatory action, and yet that would avoid metabolic antioxidant competition yielding less than expected efficacious effects. Isobole curve analyses of these two active classes of ingredients determined by growth inhibition of HL-60 human leukemic cells *in vitro* confirmed they were indeed synergistic when in combination, and not metabolically competitive. Furthermore, an *in vivo* study showed significant health benefit for 14 subjects treated for 4 weeks with the unique C-Med-100/mushroom extract formulation in that they had reduced pain, reduced fatigue, weight loss and a reduced presence of DNA damage in peripheral blood assessed by (8-OH) guanine DNA adducts and elevation in serum protein thiols. Because this broad-based panel of clinical parameters indicating clinical efficacy has never been demonstrated before for either of the active ingredients evaluated alone in humans, these data were taken as strong evidence that the combination of C-Med-100 + mushroom extracts + nicotinamide + zinc gave additive or synergistic effects to health benefit, and thus supported no efficacious limits from metabolic competition regarding this particular formulation.

Keywords: Chinese mushroom extracts; Cat's claw C-Med-100 extract; Nutritional additive/synergistic efficacy; Weight loss

Introduction

There are a wide variety of nutritional and dietary supplements that have been identified to possess clinically significant antiaging properties. Some of the most common preparations limit oxidative DNA damage by involving one or more metabolic pathways, and as a consequence increase cell responsiveness especially those regulating immune cellular reactions (Cross et al., 1987; Knight, 2000). For example, hot water cat's claw extracts such as C-Med-100 have been

*Corresponding author. Tel./fax: +46 46 152215.
E-mail address: rwpero@attglobal.net (R.W. Pero).

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shown to not only reduce DNA damage by scavenging free radicals (Sandoval et al., 2000) but also by preventing their formation by inhibiting their generation via proinflammatory cytokine production through NF-kB (nuclear transcription factor beta) inhibition (Sandoval-Chancon et al., 1998). Such a dramatic reduction in DNA damage by inhibiting free radical production by this mechanism has been shown to simultaneously stimulate DNA repair and immune responsiveness (Sheng et al., 2000a, b, 2001).

Parallel to this mechanism is the fact that DNA repair can also be stimulated by dietary nutritional factors such as zinc and niacin/nicotinamide. Low intracellular zinc resulting from nutritional deficiencies induce oxidative DNA damage, disrupt p53, NF-kB and AP-1 DNA binding, which in turn affect DNA repair (Ho and Ames, 2002). Likewise, niacin/nicotinamide are direct metabolic nutritional prescursors to the formation of cellular NAD (Jacob and Swendseid, 1996), which in turn is essential to energy production and it is also a co-substrate for the participation of poly (ADP-ribose) polymerase (PARP) in DNA repair (Rawling et al., 1994; Weitberg, 1989; Zhang et al., 1993). In other words, nutritional support for DNA repair enhancement is another pathway to resist DNA damage that is independent of either directly down-regulating free radical production by reducing NF-kB expression and thus the level of proinflammatory cytokines or by antioxidant free radical scavengers. In fact, nicotinamide and zinc supplements have been shown to enhance both DNA repair and immune responsiveness *in vivo*, thus confirming the health benefit of enhancing this mechanistic metabolic pathways (Sheng et al., 1998a, b).

Still another class of nutritional supplements to be considered for developing a broad spectrum mechanistic approach for an antiaging nutritional therapy are the natural occurring polysaccharides (i.e. beta glucans). Edible mushroom extracts especially those used in Chinese and Japanese natural medicines are a rich source of beta glucans. Thus, extracts of *Cordyceps sinensis*, *Grifola blazei*, *G. frondosa*, *Trametes versicolor* and *Ganoderma lucidum* all contain high amounts of polysaccharides which can directly stimulate immune reactions by primarily modulating immune responsive cytokines such as IL-1, IL-2, IL-6 and INF-gamma

(Borchers et al., 1999; Chen et al., 1997; Ebina and Fugimiya, 1998; Hsieh and Wu, 2001; Kiho et al., 1999; Mayell, 2001; Wang et al., 2002; Wasser and Weiss, 1999).

Among the many research and development questions that still remain regarding the health benefit of nutritional supplements is the scientific logic of combining them to increase their potency and biological effectiveness. As presented above, there are a lot of natural products that differ greatly in their chemistry, but that nonetheless can have similar health benefits such as lowering DNA damage and stimulating immune function. However, there are several metabolic and mechanistic pathways by which this could be accomplished. Here, it is important to remember the role human evolution has had to play in these matters. For example, humans have had to evolve by discovering ways to obtain nutritionous food from the environment. Among the earliest ways of deciding what to eat and not eat was by a "trial and error" approach; i.e. ingest it and if it had health or nutritionous benefits it became utilized with an increased frequency over time. Having this as a background to successfully populating highly diverse environments, it would have been important for our foraging ancestors to develop metabolic controls in order to prevent over exposure to biologically active dietary nutritional factors; i.e. nutritional and health benefiting substances that we need for sustaining life but not so much of them that they could limit our survival.

One mechanism consistent with our human evolution is that if our biological responses to ingestion of food sources were diversified so that several nutrients could be utilized to accomplish the same health benefit endpoint (e.g. immune function support), then there would be less dependence on any single nutritional factor limiting survival and the chances of over exposure and toxicity would be minimized. Metabolic diversity would then encourage metabolic competition. Otherwise it would be a serious limitation on survival to be exposed to several antioxidants in the food supply that all did the same thing thus becoming additive to the final end result such as scavengering to many free radicals. Thus having different metabolic pathways to limit free radical exposure (metabolic diveristy) through nutrient ingestion would be positive to survival. Moreover, if paralleled by having metabolic competition so that when more than one type of nutrient does the same thing such as scavenge free radicals, then the presence of one food source would prevent the uptake of another food source that did the same thing. There is already strong scientific evidence that metabolic competition of nutrients in fact exists. It has been shown that vitamins E, C and carotenoids are different chemical versions of naturally occurring free radical scavengers that can strongly regulate each other's uptake (Baker et al., 1996; Niki et al., 1995).

Taken together there seems to be credible evidence that combinig nutrients having similar modes of action will not necessarily produce synergized blends of nutrients designed to improve the effectiveness of broad spectrum antiaging therapies, basically because of metabolic competition. However, taking advantage of the metabolic diversity of combining several nutrients having varied metabolic pathways to mediate desired health benefits by stimulating the natural processes of DNA repair and immune functions, seems logical to help nutritionally protect individuals against a spectrum

of diseases associated with aging such as cancer, inflammation, diabetes, autoimmune and cardiovascular disorders. Here, we report on combining a metabolically diverse nutrient spectrum containing nicotinamide, zinc, C-Med-100 (cat's claw water extract), and five mushroom extracts (*C. sinensis*, *G. blazei*, *G. frondosa*, *T. versicolor* and *G. lucidum*) into a single orally administered capsule for evaluation of clinical outcome using several subjective questionaire and biochemical endpoints.

Materials and methods

Nutraceutical product

The trade name of the nutraceutical product designed to combine metabolically diverse natural ingredients that can enhance DNA repair and immune function was manufactured by Phoenix Laboratories, Inc (Hicksville, NY) and originally called Agerasers, but recently altered and sold as Xenodrine 40+ after addition of norambrolide (Cytodyne). It contained the following active ingredients per capsule (total = 560 mg): C-Med-100 = 200 mg, nicotinamide = 100 mg, Opti-zinc (20%-international) = 10 mg, and KMA complex (*C. sinensis*, *G. blazei*, *G. frondosa*, *T. versicolor* and *G. lucidum*) = 250 mg.There were two main classes of active ingredients which were based on their well-known modes of action, i.e. KMA complex the combined 5-mushroom extracts containing beta glucans as immunostimulants, and Nicomed whose composition has the only known DNA repair enhancers C-Med-100, nicotinamide and zinc present in the formula. Nicomed and C-Med-100 are protected by US patents 6,021,351, 6,039,949, 6,238,675 B1, and 6,361,805 B2. The logic behind combining KMA complex with Nicomed was to ascertain if even greater levels of immune stimulatory efficacy could be achieved by including the beta glucan-containing mushroom extracts with Nicomed both of which are well known to stimulate immune responsiveness albeit by different metabolic pathways.

In vitro synergism studies

Both C-Med-100 and traditional mushroom extracts have been reported to modulate immune responsiveness because they contain different active ingredients that modulate immunity by varied antioxidant mechanisms (see review in introduction section above). In an effort to synergize immune responsiveness of natural products we have combined the diverse modes of action of C-Med-100 and mushroom extracts (i.e. KMA complex) into one formulation. Synergism was evaluated by calculating IC_{50} values of growth inhibition of human leukemic HL-60 cells of the following combinations of active ingredients: C-Med-100 formula (62.5% C-Med-100 + 31.5% nicotinamide + 3.1% opt-zinc) combined with KMA complex (20% *C. sinensis,* 20% *G. blazei*, 20% *G. frondosa*, 20% *T. versicolor* and 20% *G. lucidum*) in ratios of 1:0, 10:1, 5:1, 3:1, 2:1, 1:2, 1:3 1:10, and 0:1, and then subsequently evaluated by isobole curve analyses (Berenbaum, 1989) of the active ingredient concentrations present in the various assayed mixtures (Williamson, 2001). The bioassay procedures for evaluating growth inhibition of HL-60 cells *in vitro* has been presented in detail elsewhere (Sheng et al., 1998a, b).

Subjects

Fourteen subjects represented by seven females and seven males aged 52–64 years living within Bennington County in Southern Vermont were recruited into the study. Each subject was asked to fill out a case report form covering their medical history, current disease status and concomitant medications including non-prescription supplements. In addition, the clinician in charge of the study (Dr. Michael Welther, Arlington Family Practice, Arlington, VT) conducted clinical examinations via individual interviews before supplemention (baseline status) and 4 weeks after supplementation had begun using the case report form as a guideline (Table 1). Compliance records were maintained on a daily basis by the individual subjects and verified at the end of the study by the study director. All subjects were initiated on May 14, 2002 and terminated on June 14, 2002. They were all instructed not to change their eating habits or their pattern of intake of medications/supplements at initiation, so that during the supplement period with C-Med-100/mushroom extract formula there would be no added variables to before and after extract comparisons. Each subject was instructed to ingest a C-Med-100/mushroom extract formula capsule orally in the mourning and evening; i.e. 2X per day. Heparinized blood and serum samples were collected at initiation (baseline) and at termination 4 weeks after daily supplementation for biochemical endpoint analyses. Informed consent was obtained from all subjects and the study was conducted in accordance with the recommendations for guiding physicians in pharamceutical research involving human subjects decided by the Declaration of Helsinki.

Blood sampling

Peripheral blood was collected by venal puncture into 10 ml EDTA (yellow top) and serum (red top) test tubes from each subject on May 14, 2002 and June 14, 2002. The yellow top tubes were sent by overnight express courier delivery to Dr. AristoVojandi, Immunosciences

Table 1. Summary data sheet for all physician-assisted and self-reported clinical examinations used for final analysis

Patient name, observations	Birth date		Treatment
	Baseline day 1	Second visit day 30	Comments
Body weight (kg)			
Work attendance (%)			
How many flu incidences for the past month			
How many times sore throat for the past month?			
How often headache for the past month?			
How often diarrhoea/constipation for the past month?			
Your appetite for the past week (good, normal, bad)			
Rash for the past week (Y, N)			
Pain for the past week (Y, some, N)			
Have you fatigue (Y, some, N)			
Have you fatigue (Y, some, N)			
Concentration ability (poor, good, mod.)			
Self-assessment of wellness: very sat., mod. sat., equal sat.-dissat., mod. dissat., Very dissat., very			
Energy (poor, good, mod.)			
Doctor signed			

Lab, Inc. (Beverly Hills, CA) for the isolation of mononuclear leucocytes and the determination of 8-OH guanine DNA adducts. Serum samples were prepared from the red top tubes by separating the clotted blood from the serum by centrifugation at 300*g*. The serum was then sent by express courier to Dr. Amir Amiri at the University of Lund (Lund, Sweden) after precipitation with 80% saturated ammonium sulfate for the quantitative analysis of serum protein thiols.

(8-OH) guanine adducts

This biomarker was determined in DNA isolated from peripheral leucocytes by addition to the cell pellet of 250 µl of homogenizing buffer (pH 7.3) containing 0.3 M sucrose, 0.025 M Tris, and 0.002 M EDTA. The rest of the procedure is described in detail elsewhere (Park et al., 1989). The (8-OH) guanine adduct levels are expressed as number/10^9 nucleotide bases present in the DNA of leucocyte samples. The normal range of values for this test is between 0 and 10 (8-OH) guanine adducts/10^9 nucleotides in DNA (Immunosciences, Inc., Beverly Hills, CA). For the purpose of statistical analyses a 0 value (i.e. for adduct level) was treated as an analytical failure, and the mean for the whole group was used instead.

Serum protein thiols

The thiols present in serum proteins precipitated with 80% saturated ammonium sulfate were determined as previously described with only minor modifications to accommodate a micro scale analysis (Pero et al., 2000). Briefly, 50 µl aliquots of serum were precipitated with 100 µl saturated ammonium sulfate. The resulting suspensions were vortexed, incubated for 15 min at room temperature, and the precipitated protein harvested by centrifugation at 1000*g*. The supernatant was discarded and the pellet was redissolved in 150 µl physiologic saline for 30 min. Three 50 µl aliquots of the dissolved protein pellet were prepared as follows: 50 µl sample + 200 µl physiologic saline, 50 µl sample + 200 µl DTNB reagent (5,5′-dithio-bis(2-nitrobenzoic acid), 50 µl physiologic saline + 200 µl DTNB reagent. The DTNB reagent contains 9.5 mg/ml in 0.1 M K_2HPO_4, 17.5 mM EDTA adjusted to pH 7.5 and diluted 1:50 with water before use. The assay was carried out in 96-well microtitre plates and the absorbance read at 412 nm. Substract saline blank absorbance and DTNB blank absorbance from the absorbance of saline + DTNB + sample. The 80% ammonium sulfate precipitated serum thiol samples were estimated as nmoles/l cysteine per 200 µl aliquot of serum using a 0–100 nM cysteine standard curve. The serum samples were assayed in duplicate.

Statistics

Group means of numerical values at baseline (before supplement) and after 4 weeks C-Med-100/mushroom extract formula were compared for significant differences by paired *t*-test while rates of category data were analysed by the Chi-square test. Isobole curve was fitted with non-linear regression.

Results

In vitro synergy of C-Med-100

Before proceeding with the *in vivo* evaluation of the C-Med-100/mushroom extract formulation, *in vitro* analyses that the combination of C-Med-100 with mushroom extracts would provide data that the active ingredients combined in the C-Med-100/mushroom extract formulation were in fact synergistic to each other. Table 2 presents the data for such an anlaysis. It seems apparent that C-Med-100 formula (i.e. Nicomed) combined with KMA complex gave lower than expected IC_{50} values, clearly establishing an enhancement of HL-60 toxicity (*in vitro* efficacy) as evidenced by the lower IC_{50} values than when they were compared to the theoretical values calculated from KMA mix and Nicomed assayed alone. This interpretation of the data was confirmed by the isobole curve plot and statistical analysis presented in Fig. 1 (Williamson, 2001). Here it was quite evident that a non-linear regression analysis yielded a significant concave curve plot ($r = 0.86, p < 0.01, n = 9$), demonstrating synergistic interaction. These data were interpreted to provide strong support that the C-Med-100/mushroom extract formula should be further evaluated *in vivo* as a potential synergized immune enhancer.

Evaluation of subjective clinical parameters after treatment with C-Med-100/mushroom extract formula

There were seven males and seven females who were all non-smokers between the ages of 52–64 years (Avg. 59.7 years) enrolled and evaluated in this study. Over the 4 weeks intervention period with C-Med-100/mushroom extract formula the compliance with daily doses of $\times 2$ capsules (morning and evening) was 95.5%. Most of the subjects were still taking prescription medications (78.6%) for the treatment of past health disorders such as cardiac disease, hypertension, diabetes, hysterectomy, thyroid disease, allergy, arthritis, and polio. In addition, 71.4% of the subjects also regularly added nutritional supplements to their diet.

The case report forms also permitted the collection of information regarding the self-reporting of the following symptoms and signs indicating general health status: work attendance, flu, sore throat, appetite, concentration, self-assessment of health, energy, stress level, headache, diarrhoea, rash, pain, and fatigue. Table 3 reports the change in incidence of symptoms before and after 4 weeks supplement with C-Med-100/mushroom

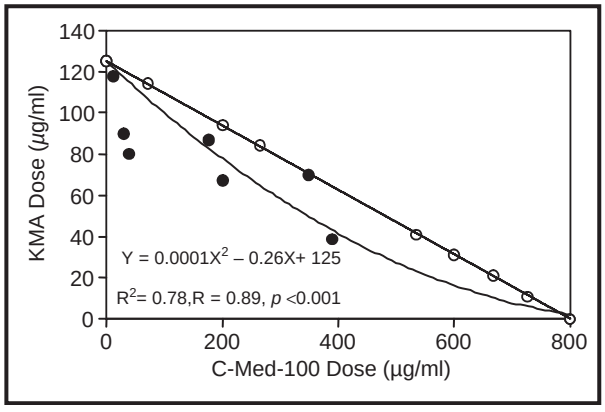

Fig. 1. The data collected and reported in Table 1 showing a synergistic *in vitro* interaction between C-Med-100 formula and KMA complex (i.e. a traditional Chinese mushroom extract of five species) presented in isobole plot format (Williamson, 2001).

Table 2. Isobole curve data for the *in vitro* evaluation of synergism between C-Med-100 formula and KMA mushroom complex in various mixtures using the IC_{50} value data of growth inhibition curves of HL-60 human leukemic cells

Mixtures (C-Med: KMA):		Component quantity (μg/ml)				C-Med, Synergism, expt. < calc. (—)[a]
		Expt.		Calc. (no interaction)		
Ratio	IC^{50} (μg/ml)	C-Med	KMA	C-Med	KMA	
1:0	800	800	0	800	0	0
10:1	430	390	39	727	11	(—)
5:1	420	349	70	667	21	(—)
3:1	268	200	67	600	31	(—)
2:1	265	177	87	536	41	(—)
1:2	120	40	80	264	84	(—)
1:3	120	30	90	200	94	(—)
1:10	130	12	118	72	114	(—)
0:1	125	0	125	0	125	0

[a]Isobole curves comparing no interaction with various ratios of two active ingredients mixed together, and their biological activity (IC_{50} values) determined experimentally, establish synergism when the mixture values are less than those predicted from the IC_{50} values determined as single active ingredients (i.e. no interaction possible, Fig. 1).

Table 3. Summary of the subjective clinical data collected from physician interviews and self-reported case report forms that were recorded in this study

Clinical indications	Incidence		% Change	*p*-Value
	Before	After		
Work attendence	14/14 = 100%	14/14 = 100%	0	n.s.
Flu symptoms	0/14	0/14	0	n.s.
Sore throat	0/14	0/14	0	n.s.
Appetite	0/14 good	0/14 good	0	n.s.
Concentration	0/14 good	0/14 good	0	n.s.
Self assessment	0/14 satisfied	0/14 satisfied	0	n.s.
Energy	0/14 good	0/14 good	0	n.s.
Stress test level	4.7	5.0	+4.8	n.s.
Headache	3/14	5/14	+14.2	n.s.
Diarrhoea	5/14	6/14	+7.1	n.s.
Rash	1/14	0/14	−7.1	n.s.
Fatigue	6/14	3/14	−21.4	0.08
Pain	9/14	4/14	−35.7	0.04

The subjects were evaluated for each of the listed parameters before and after 4 week intervention with C-Med-100/mushroom extract formula, and the patterns of occurrence within the group tested for significant changes by Chi-square analysis ($p \leqslant 0.05$). Percent change in incidence between before and after interviews for the group of $n = 14$ are shown.

extract formula. Chi-square analyses have shown significant reductions in the incidences of pain ($p < 0.04$) and a tendency for fatigue ($p < 0.08$) while all the other parameters analysed remained significantly unchanged.

Evaluation of objective clinical parameters

The following clinical endpoints were determined experimentally before and after 4 weeks supplement with C-Med-100/mushroom extract formula:

Weight — Fig. 2 displays the individual weight changes in lbs for the 14 subjects in this trial. The weight at initiation of the trial was subtracted from the weight after 4 weeks supplementation with C-Med-100/mushroom extract formula. It can be clearly seen that only one subject (#2) had any weight gain while 2 others (#1 and #7) were unchanged in weight. All the remaining 11 subjects had weight loss up to 7 lb which was statistically significant for this sample size ($p < 0.01$).

Serum thiols — This biochemical endpoint is a direct measure of the oxidation/reduction balance (i.e. and thus free radical levels) that is occurring *in vivo*, because it measures the steady state conversion of thiols to disulfides that exist in the cysteine residues in the ammonium sulfate precipitated protein fraction of serum (Pero et al., 2000). Moreover, the redox state of serum proteins are of particular importance because these proteins are likely the signal reducers of critical biological processes in the cells. Hence, it is not surprising that both lifespan and DNA repair have been associated to redox balance and serum thiols (Pero et al., 1995). The data reported in Fig. 3 also strongly support the clinical importance of serum thiols in the *in vivo* regulation of redox balance using the C-Med-100/mushroom extract formula, because several health benefit parameters known to be involved with oxidative stress were improved in parallel (Table 3). In fact, there was only one subject (# 3) that did not increase its serum thiol level by the C-Med-100/mushroom extract formula

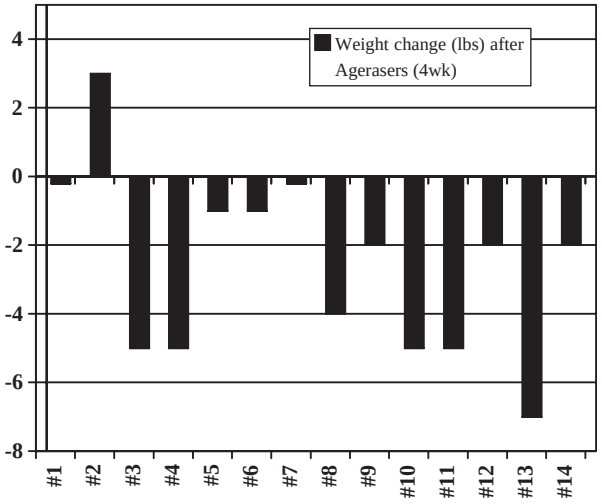

Fig. 2. Individual changes of weight in pounds (lbs) between before and after 4 weeks supplement interviews (*y*-axis). Each individual before and after weights were compared by paired *t*-test for significant weight losses in the $n = 14$ group. The group lost an average of 2.5 lbs ± 2.8 when analysed by a paired *t*-test which was significant ($p < 0.01$). Subjects #1–#14 are identified on the *x*-axis).

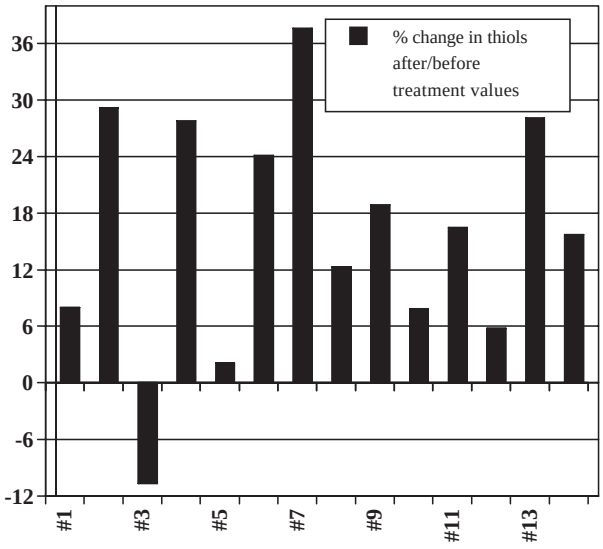

Fig. 3. Individual changes in serum thiol levels calculated as a % by subtracting the after 4 weeks C-Med-100/mushroom extract supplement thiol level from the before (initiation) thiol level and dividing by the before thiol level $\times 100$ (y-axis). Subjects #1–#14 are identified on the x-axis. The mean % change in thiols for the $n = 14$ group was: 16 ± 12.8. When the before and after serum thiol values were analysed by students paired t-test the increased level of thiols for the group ($n = 14$) was highly statistically significant, $p < 0.01$.

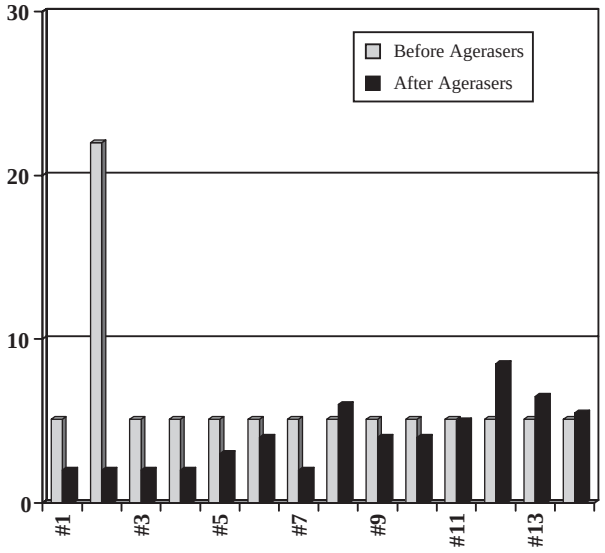

Fig. 4. Individual levels of (8-OH) guanine adducts in the DNA of peripheral blood leucocytes were expressed as number/10^9 nucleotide bases before and after 4 weeks intervention with C-Med-100/mushroom extract formula (y-axis). Subjects #1–#14 are identified on the x-axis. The range of (8-OH) guanine adducts found in leucocytes from a normal human population is Median = 5 (Range = 1–10) (Immunosciences, Inc. accumulated data). The data shown are the result of duplicate determinations for each data point.

when administered for 4 weeks. The increase of serum thiols in 13 of the 14 subjects was statistically significant when the group ($n = 14$) was analysed by paired t-test ($p < 0.01$).

DNA adducts — One of the most common oxidative damages to DNA coming from exposures to free radicals is the formation of (8-OH) guanine adducts. Hence, the levels of this adduct in DNA are a direct measure of previous oxidative stress exposures that may have occurred *in vivo* minus any removal of these lesions by DNA repair. The data reported in Fig. 4 show the levels of (8-OH) guanine adducts per 10^9 nucleotides in DNA before and after 4 weeks supplementation with C-Med-100/mushroom extract formula. In order to assess DNA damage *in vivo* from genotoxic exposures following antioxidant therapies using DNA adducts, it would have been necessary for the subjects to start out the study with elevated levels so that their removal via antioxidant treatment could be quantified above background. Unfortunately, only one subject (#3) had DNA adducts above the background levels, which are known to be between 1 and 10 (8-OH) guanine adducts/10^9 nucleotides. Hence, the effectiveness of C-Med-100/mushroom extract formula could only be meaningfully evaluated in subject #3 which was reduced from 22 to 2 DNA adducts after 4 weeks of intervention. The other 13 subjects had before and after C-Med-100/mushroom extract formula levels of DNA adducts within the normal range of a healthy population, and thus comparison of the before and after DNA adduct levels were to insignificant to detect successful antioxidant intervention above this background noise level. Nonetheless, the results with subject #3 were consistent with the thiol test endpoint analysis designed to indicate an induced DNA repair with a subsequent reduction in DNA damage.

Discussion

The primary aim of this study was to demonstrate the added benefit of developing a nutritional supplement designed to take advantage of metabolic diverse pathways that can combat the hazardous health consequences of oxidative stress by different mechanisms. C-Med-100/mushroom extract formula is such a product whereby the health benefits of DNA repair and immune enhancements which are in turn modulated by antioxidizaton can be further enhanced by the combination of ingredients that represent non-competing metabolic pathways; C-Med-100, nicotinamide, zinc, and traditional Chinese medicinal mushroom extracts. C-Med-100 has antioxidant properties because it can inhibit NF-kB and prevent free radical formation as well as stimulate DNA repair, nicotinamide and zinc

nutritionally support the battery of enzymes involved in DNA repair, and the mushroom extracts contain beta glucans which can directly regulate lymphocyte cytokines, increase cell proliferation, repair and thus metabolic antioxidant processes. Because of the formulating design of metabolic diversity, a non-competing broader base has been potentially added to protect cells against the hazardous effects of free radicals. The data recorded in Tables 2 and 3 and Figs. 1–4 have clearly supported this scientific concept on which C-Med-100/mushroom extract formula was built on.

For example, subjects in this study had significantly reduced pain, less fatigue, weight loss, increased serum thiol status and less DNA adducts when evaluable. These data are particularly impressive when considering that 78.6% of the cohorts were taking prescription medicines, and in addition 71.4% were also already taking nutritional supplements. Therefore, any therapeutic advantages from Agerasers would have had to be realized over and above the combined benefits already being received from the self-administered medications and supplements. In other words, the subjects in this study should have been quite healthy since they were already receiving state of the art health care. However, it was obvious some symptoms still remained even after treatment with medications indicating their ineffectiveness. Equally obvious was that a variety of nutritional supplements currently available and being routinely ingested also did not relieve the symptoms for the subjects under study. Despite these treatments already being in place, C-Med-100/mushroom extract formula significantly positively benefited the health of the study subjects.

The fact that the enrolled subjects were in exceptional good condition relative to oxidative stress levels is strongly supported by the DNA adduct data presented in Fig. 4, where only one patient had adducts out of the normal range level at the start of the study. Here it is important to remember that DNA damage and DNA repair although inter-related are to separate risk factors for controlling the oxidative stress health consequences. The data presented here suggests that even if there are only marginal levels of DNA adducts present to cause harm to your health, there still can be a substantial advantage to enhance DNA repair by supplements since at least the incidence of weight gain, pain and fatigue were reduced.

There are other studies available in the scientific literature, which support the clinical outcome of the subjects enrolled in the 4 weeks C-Med-100/mushroom extract formula intervention study. First of all, water extracts of cat's claw such as C-Med-100 have already been shown clinically to reduce joint pain (Piscoya et al., 2001), enhance DNA repair (Sheng et al., 2000b), and increase antibody levels to pneumococcal vaccine (Lamm et al., 2001). In addition, nicotinamide and zinc combined with carotenoids (Sheng et al., 1998a, b), or in combination with C-Med-100 and a broad spectrum of other antioxidants called Optigene Professional stimulated immune function, enhanced DNA repair and reduced DNA adduct levels (Pero et al., 2003). These data clearly support the scientific logic and the clinical results observed for the C-Med-100/mushroom extract formula study in that joint pain was reduced and serum thiols, a surrogate assay for DNA repair enhancement, were increased (Table 3, Fig. 3). In addition, fatigue was reduced and there was also significant weight loss recorded after 4 weeks on C-Med-100/mushroom extract formula treatment (Table 3), which to our knowledge has never before been reported for the active ingredients of C-Med-100 or traditional Chinese mushroom extracts containing immune enhancing polysaccharides (beta glucans). Taken together it was concluded these data were evidence that the combination of C-Med-100/mushroom extract formula was at least additive and probably synergistic *in vivo* since neither class of active ingredients previously administered alone showed the same broad spectrum of clinical efficacy. Moreover, the added health benefit observed over earlier studies involving the two separate classes of active ingredients (i.e. DNA repair and beta glucan immune enhancement) was accomplished by utilizing the logic of combining ingredients having different antioxidant mechanisms defined by their metabolic diversity.

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