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DNA repair enhancement of aqueous extracts of *Uncaria tomentosa* in a human volunteer study

Y. Sheng, L. Li, K. Holmgren and R. W. Pero

Department of Cell and Molecular Biology, Section of Tumor and Immune Biology, University of Lund, Lund, Sweden

Summary

The *Uncaria tomentosa* water extracts (C-Med-100®) have been shown to enhance DNA repair, mitogenic response and leukocyte recovery after chemotherapy-induced DNA damage *in vivo*. In this study, the effect of C-Med-100® supplement was evaluated in a human volunteer study. Twelve apparently healthy adults working in the same environment were randomly assigned into 3 groups with age and gender matched. One group was daily supplemented with a 250 mg tablet containing an aqueous extract of *Uncaria tomentosa* of C-Med-100®, and another group with a 350 mg tablet, for 8 consecutive weeks. DNA repair after induction of DNA damage by a standard dose of hydrogen peroxide was measured 3 times before supplement and 3 times after the supplement for the last 3 weeks of the 8 week-supplement period. There were no drug-related toxic responses to C-Med-100® supplement when judged in terms of clinical symptoms, serum clinical chemistry, whole blood analysis and leukocyte differential counts. There was a statistically significant decrease of DNA damage and a concomitant increase of DNA repair in the supplement groups (250 and 350 mg/day) when compared with non-supplemented controls ($p < 0.05$). There was also an increased tendency of PHA induced lymphocyte proliferation in the treatment groups. Taken together, this trial has confirmed the earlier results obtained in the rat model when estimating DNA repair enhancement by C-Med-100®.

Key words: *Uncaria tomentosa*, human trial, leukocytes, DNA repair

Introduction

Uncaria tomentosa (Willd.) DC (Rubiaceae), also known as "una de gato" or "cat's claw", is a healing vine used in South American folk medicine to treat many human disorders including inflammations, cancer and infections (Keplinger, 1982; Keplinger et al. 1999; Wagner et al., 1985; Cerri et al., 1988, Aquino et al., 1989; 1991; Rizzi et al., 1993; Jones, 1995; Reinhard 1999). There have been several reports on its bioactive constituents. Wagner et al. (1985) using organic solvent extraction, showed that four of six alkaloids isolated from U. tomentosa (pteropodine, isopteropodine, isomitraphylline and isorynchophylline) had a pronounced enhanced effect on phagocytosis determined in two in vitro tests and the in vivo carbon clearance test while the other two, mitraphylline and rinchophylline, did not display phagocytosis effects. Other bio-active constituents have also been reported such as glycosides of quinovic acid including glycosides 1–9 (Cerri et al., 1988; Aquino et al, 1989; Aquino et al, 1991), polyhydroxylated triterpenes and others (Aquino et al, 1990). Rizzi et al. (1993) reported antimutagenic activities of chloroform/methanol extracts

Abbreviations: CAE – carboxy alkyl esters; C-Med-100® – a novel water extract from *Uncaria tomentosa*; PBL – peripheral blood lymphocyte; NF-κB – nuclear transcription factor kappa B; PHA – Phytohemagglutinin; R10 – RPMI 1640 medium supplemented with 10% fetal calf serum; SEA – staphylococcal enterotoxin A; SSB – (DNA) single strand breaks; WBC – white blood cells

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and chromatographic fractions of U. tomentosa bark. The plant extracts and fractions showed a protective antimutagenic effect in vitro against photo mutagenesis induced by 8-methoxy-psoralen plus UVA in S. typhimurium TA102. A decoction of U. tomentosa ingested daily for 15 days by a smoker also decreased the mutagenicity induced in S. typhimurium TA98 and TA100 by the subject's urine. Wurm et al. (1998) reported pentacyclic but not tetracyclic oxindole alkaloids from U. tomentosa induced EA.hy926 endothelial cells to release some yet to be determined factor(s) into the supernatant; this factor was shown to significantly enhance proliferation of normal human resting or weakly activated B and T lymphocytes. Sandoval et al (1998) showed that an aqueous extract of cat's claw elicited similar beneficial effects as an antioxidant by inhibiting indomethacin induced intestinal inflammation, consistent with previous findings using a bark methanol extract (Desmarchelier et al., 1997). More recently, Kitajima et al (2000) found a new gluco indole alkaloid, 3, 4-dehydro-5-carboxystrictosidine from Peruvian *Uncaria tomentosa*, the first example of a gluco monoterpenoid indole alkaloid having a 3, 4-dihydro-beta-carboline ring system from nature.

Although there were more than 50 dietary supplement manufacturers in the United States in 1997, human data based on scientific research are still scarce. Most of *Uncaria tomentosa* studies are based on organic solvent extraction or are attributed to the effects of indole alkaloids present in the plant parts. However, our laboratory has shown that C-Med-100®, the proprietary aqueous extract from Uncaria, has only very low concentration of alkaloids (< 0.05%) as evidenced by HPLC analysis (Sheng et al, 2000b). Previous studies have shown this hot water extract to be effective in induction of late apoptosis in vitro in human tumor cell lines (Sheng et al. 1998) and in the enhancement of DNA repair and immune response in vivo (Sheng et al. 2000a, 2000b). This study was designed to directly evaluate C-Med-100® on DNA repair and immune modulation in a human volunteer trial.

Materials and Methods

Nutraceutical Product

C-Med-100® is a patented extract (U.S. patent 6,039,949) from Cat's Claw, *Uncaria tomentosa*, manufactured by Laboratório Centroflora (Sao Paulo, Brazil) and distributed by AF Nutraceutical (Morristown, NJ) in North America. It is formulated and based on the historical medicinal use of *Uncaria tomentosa*. Basically it is a water soluble extract ultrafiltrated to remove high molecular weight toxic conjugates (> 10, 000 MW), and it contains 8–10% carboxy alkyl esters (CAE) as active ingredients, which is also essentially free of oxindole alkaloids (< 0.05 %). The active ingredients of C-Med-100® (85% of them) absorb onto charcoal, have a UV absorption maximum of 200 nm, and react with hydroxylamine and ferric chloride after conversion to hydroxamic acids (Bartos procedure), thus characterizing them as esters (e.g. CAE). The natural product extract used in the human trial was provided as 250 mg or 350 mg tablets.

Subjects

Twelve apparently healthy volunteers working in the same environment, were randomly assigned into 3 groups with 4 persons (2 males and 2 females) in each group according to sex and age. Group I was the controls, group II was the "low dose" supplement of C-Med-100® at 250 mg/day while group III was the "high dose" supplement of C-Med-100® at 350 mg/day. The volunteers were base-lined for 3 consecutive weeks using standard differential blood cell counts and DNA repair analyses (i.e. by alkaline elution). After establishing baseline, subjects in the supplemented groups took one 250 mg tablet daily (group II) or 350 mg tablet daily (group III) C-MED-100® for 8 additional consecutive weeks. No changes in food intake pattern, life style, disease or medication had occurred during the supplementation. The side effects were judged by hematological analysis, body weight change, work attendance and symptoms including diarrhea/constipation, headache, nausea/vomiting, rash/edema and pain. Total blood cell counts were also used to monitor the efficacy and toxicity. Informed consent was obtained from each participant and the study was conducted in accordance with the recommendations for guiding physicians in pharmaceutical research involving human subjects decided by the Declaration of Helsinki.

Blood sampling

About 20 ml of venous blood was collected into 3 × 5 ml heparinized vacutainer tubes for peripheral blood lymphocyte (PBL) separation (Pero et al, 1993) and 1 × 5 ml EDTA tube for whole blood analysis. The heparinized blood sample was spun at 100 g for 10 minutes to collect a plasma sample, and the original blood volume was then adjusted by addition of saline to the volume of the removed plasma. The plasma sample was spun a second time at 2000 g for 10 minutes and then stored at –80 °C until analysis. The blood cell sample was laid on top of 15 ml Lymphoprep™ and spun at 1350 g for 12 minutes. Then the lymphocyte layer was removed and washed with saline at 600 g for 10 min, washed another time with RPMI1640 medium supplemented with

10% fetal calf serum (R10) and then re-suspended in R10. The cell suspension was then counted and the cell density was adjusted to 2×10^6/ml. This cell suspension was immediately used for DNA repair assay and lymphocyte proliferation assay.

Alkaline elution

Freshly prepared peripheral blood lymphocytes with the cell density of 2×10^6/ml from each individual were allocated into 1 ml each for control (add the same volume of saline as treatment control), DNA damage induction (100 µM H_2O_2 for 30 min on ice) and DNA repair (H_2O_2 treatment + 30 min repair incubation at 37 °C water bath). After that, 1.5×10^6 cells (0.75 ml of the cell suspension) were layered directly onto polycarbonate filters with 25 mm diameter and 2 µm pore size (Millipore). DNA single strand breaks (SSB) were measured by alkaline elution as described by Kohn et al. (1981) with modifications to measure the unlabeled DNA by microfluorometry (Cesarone et al., 1979; Olsson et al., 1996).

Phytohemagglutinin (PHA) and staphylococcal enterotoxin A (SEA) induced lymphocyte mitogenic response

Freshly prepared peripheral blood lymphocytes from the above blood samples were cultured in microtiter plates at 25 000 cells/well in 200 µl R10 medium + 10 µl PHA or SEA 0.001 ng/ml or SEA 0.01 ng/ml at 37 °C and 5% CO_2 for 5 days then pulsed for 6 hours with [^{3}H]-thymidine (2 Ci/mmole, final concentration 0.5 µCi/ml). Labeled nuclear material was collected on glass fiber filters in a microtiter plate cell harvester, dried and counted in scintillation fluid.

Hematologic parameter

The blood samples were collected into K_3-EDTA tubes and then analyzed within one hour by an automated hematology analyzer (Sysmex, K-1000). A control blood sample (Sysmex™) was routinely checked as a quality control.

Clinical chemistry

The plasma samples collected as described in the section of blood sampling were stored at -80 °C until analysis. The samples were sent to and analyzed by The Department of Clinical Chemistry, MEDILAB Inc. (Täby, Sweden) for total protein, albumin, glucose protein, iron, sodium, potassium, calcium, magnesium, triglycerides and cholesterol concentration.

Statistics

Comparison of mean differences among groups was made by analysis of variance with further analysis between two groups by Duncan test at a significance level of $p \leq 0.05$. The comparison between the time points (before and after supplement with 3 times repeats) of the same group was done by repeated measurement of MANOVA and calculated by SPSS software package (SPSS Inc.).

Results

The averages of age for Group I were 44 ± 11 years (mean ± SD), Group II 43 ± 11 years, and Group III 44 ± 7 years (Table 1). There were no statistically significant differences in means of age and distribution of sex among the groups (t-test and x^2 test, $p > 0.05$). There were no reports on symptoms and signs connected with C-Med-100® supplementation, nor were there any body weight and work attendance (%) changes. Complete blood counts and differential white blood cell counts before and after C-Med-100® supplement are shown in Table 2. There were no statistically significant differences among the groups in white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), platelet (PLT), and numbers/percentages of monocytes (MONO), eosinophils (EOS), basophils (BASO) and lymphocytes. The only difference found among the groups were mean corpuscular hemoglobin concentration (MCHC). However, considering only the low dose C-Med-100® group increased the value both before and after the supplement, this effect is rather induced by grouping due to the small group number than by the drug effect, as confirmed by no significant difference comparing the same low group before and after supplement ($p > 0.05$ by paired t-test).

The results of clinical chemistry analysis before and after C-Med-100® supplement are presented in Table 3. There were no statistically significant differences in plasma total protein, albumin, glucose protein, iron, sodium, potassium, calcium, magnesium, triglycerides and cholesterol concentration among the groups of control, C-Med-100® 250 mg/day and C-Med-100® 350 mg/day both before and after supplement. Neither were there any changes in these parameters after supplement by both statistical and biological significance.

The resistance of in vivo treated C-Med-100® human mononuclear leucocytes (HML) to cellular DNA damage induced by a standard dose of hydrogen peroxide (100 µM), measured by alkaline elution before and after C-Med-100® supplement is presented in Panel A of Figure 1. DNA single strand breaks, as measured by alkaline elution and expressed as DNA filtered (100% minus % of DNA retained on filter), did not change significantly before C-Med-100® supplementation (3 estimates) or after supplementation (2 estimates). Only the last blood sampling point (week 8 after supplement) showed a significant difference in DNA damage between control and the group supplemented with C-Med-100® at 350 mg/day.

Table 1. General information of the test groups.

Group	Treatment		Age Mean ± SD (range)	Number (male/female)
I	C-Med-100®	0 mg/d	44.0 ± 10.5 (29–53)	4 (2/2)
II	C-Med-100®	250 mg/d	43.3 ± 11.0 (28–54)	4 (2/2)
III	C-Med-100®	350 mg/d	43.8 ± 6.70 (37–53)	4 (2/2)

Table 2. Complete blood count and differential white blood cell count before and after C-Med-100® supplement.

Index	Group	Control	C-Med-100® (250 mg/day)	C-Med-100® (350 mg/day)	p-value**
WBC (10^3/mm^3)	Before	6.1 ± 0.6	5.4 ± 1.1	5.7 ± 0.4	0.475
	After	5.5 ± 1.0	5.5 ± 1.5	5.7 ± 0.8	0.941
RBC (10^6/mm^3)	Before	4.4 ± 0.4	4.7 ± 0.5	4.6 ± 0.4	0.573
	After	4.5 ± 0.4	4.8 ± 0.5	4.6 ± 0.6	0.770
HGB (g/dl)	Before	138 ± 9	149 ± 13	140 ± 16	0.450
	After	140 ± 11	149 ± 13	142 ± 20	0.645
HCT (%)	Before	40.8 ± 2.5	41.7 ± 3.4	40.7 ± 4.3	0.750
	After	40.7 ± 3.3	42.0 ± 3.4	41.1 ± 5.3	0.896
MCV (fl)	Before	91.4 ± 3.0	89.0 ± 2.9	89.4 ± 3.2	0.522
	After	90.6 ± 3.1	88.5 ± 2.8	89.2 ± 3.2	0.619
MCH (pg)	Before	31.7 ± 0.8	31.8 ± 0.8	30.8 ± 0.6	0.243
	After	31.1 ± 1.1	31.4 ± 0.9	30.7 ± 0.6	0.561
MCHC (%)	Before	347 ± 3	358 ± 4	345 ± 10	0.032
	After	342 ± 2	355 ± 5	344 ± 9	0.043
Platelet (10^3/mm^3)	Before	248 ± 15	245 ± 55	223 ± 50	0.680
	After	232 ± 18	240 ± 54	201 ± 53*	0.446
LYM (%)	Before	28.5 ± 3.4	36.3 ± 12.3	32.0 ± 7.6	0.467
	After	34.1 ± 11.3	38.2 ± 14.2	32.5 ± 3.7	0.745
MXD (%)	Before	10.6 ± 3.7	10.7 ± 3.8	8.9 ± 2.7	0.733
	After	10.5 ± 3.6	10.3 ± 3.9	8.1 ± 3.7	0.606
NEU (%)	Before	60.9 ± 5.5	53.0 ± 12.1	59.1 ± 8.9	0.484
	After	55.4 ± 12.4	51.5 ± 13.0	59.5 ± 4.3	0.592
LYM (10^3/mm^3)	Before	1.7 ± 0.3	1.9 ± 0.1	1.8 ± 0.4	0.693
	After	1.8 ± 0.4	2.0 ± 0.3	1.9 ± 0.3	0.789
MXD (10^3/mm^3)	Before	0.7 ± 0.3	0.6 ± 0.3	0.5 ± 0.2	0.712
	After	0.6 ± 0.2	0.6 ± 0.3	0.5 ± 0.2	0.865
NEU (10^3/mm^3)	Before	3.7 ± 0.2	3.1 ± 1.6	3.4 ± 0.6	0.751
	After	3.1 ± 1.2	2.9 ± 1.3	3.4 ± 0.6	0.835
LYM Ratio	Before	0.41 ± 0.66	0.62 ± 0.56	0.49 ± 0.44	0.444
	After	0.56 ± 0.72	0.70 ± 0.46	0.49 ± 0.51	0.643

* $p < 0.05$ compared to before supplement by paired t-test
** $p < 0.05$ comparison among the groups by one-way ANOWA.

Panel 2 in Figure 1 shows the DNA repair 30 minutes after DNA damage at 37 °C as also illustrated in Panel 1, Figure 1. There were no statistically significant differences among the different blood sampling time points for the control groups. However, there were significant increases of DNA repair (higher DNA retained on filter) after supplement (blood sampling time points 4, 5 and 6) when the data were considered by an overall MONOVA repeated measurement analysis ($p < 0.05$) for both supplement groups (250 and 350 mg/day). This has also been confirmed by pooling different sampling time point data according to before and after sup-

Table 3. Some measurements of clinical chemistry before and after C-Med-100® supplement.

Index	Group	Control	C-Med-100® (250 mg/day)	C-Med-100® (350 mg/day)	p-value**
Total Protein (g/L)	Before	75.3 ± 2.1	72.4 ± 3.7	73.6 ± 2.3	0.374
	After	75.0 ± 1.9	73.3 ± 4.1	74.3 ± 2.8	0.750
Albumin (g/L)	Before	38.3 ± 1.8	42.2 ± 2.7	41.9 ± 2.9	0.105
	After	39.3 ± 1.5	43.0 ± 2.8*	42.7 ± 2.8	0.117
Glucose Protein (mmol/L)	Before	5.13 ± 1.11	5.79 ± 0.88	5.40 ± 0.94	0.650
	After	5.00 ± 0.55	5.61 ± 0.62	5.59 ± 0.32	0.216
Iron (Fe) (mmol/L)	Before	19.5 ± 3.7	20.5 ± 3.9	19.6 ± 3.5	0.923
	After	21.6 ± 5.7	23.8 ± 5.5	20.5 ± 4.6	0.680
Sodium (Na) (mmol/L)	Before	141.8 ± 1.4	140.5 ± 2.3	141.3 ± 1.0	0.570
	After	141.3 ± 1.7	139.9 ± 1.7	140.3 ± 0.9*	0.397
Potassium (K) (mmol/L)	Before	3.94 ± 0.19	3.72 ± 0.19	3.83 ± 0.12	0.232
	After	4.00 ± 0.16	3.86 ± 0.28	3.91 ± 0.22	0.783
Calcium (Ca) (mmol/L)	Before	2.33 ± 0.03	2.36 ± 0.07	2.34 ± 0.05	0.687
	After	2.33 ± 0.02	2.36 ± 0.08	2.32 ± 0.06	0.625
Magnesium (Mg) (mmol/L)	Before	0.87 ± 0.07	0.86 ± 0.09	0.86 ± 0.09	0.976
	After	0.86 ± 0.06	0.87 ± 0.08	0.85 ± 0.11	0.932
Triglycerides (mmol/L)	Before	1.28 ± 0.39	1.84 ± 0.66	1.03 ± 0.36	0.111
	After	1.35 ± 0.40	2.14 ± 0.60	1.28 ± 0.62	0.092
Cholesterol (mmol/L)	Before	4.59 ± 0.08	5.42 ± 1.15	5.30 ± 0.83	0.348
	After	5.01 ± 0.40	5.61 ± 1.03	5.51 ± 0.83	0.546

* $p < 0.05$ compared to before supplement by paired t-test
** comparison among the groups by one-way ANOVA.

plementation and then analyzing it by t-test as presented in Figure 2. Both supplement groups significantly increased the DNA repair from 72–74% before supplement to 81–85% (12–15% increase) after supplement.

The results of in vivo treated C-Med-100® HML on proliferation after stimulation by phytohemagglutinin (PHA) are presented in Figure 3. Although there was an increased tendency of lymphocyte proliferation as expressed by increased [^{3}H]-thymidine incorporation for the supplement groups, the variation of the indicator is even greater before supplementation for the 3 groups, and that may partly explain the lack of significant differences before and after supplementation. Figure 4

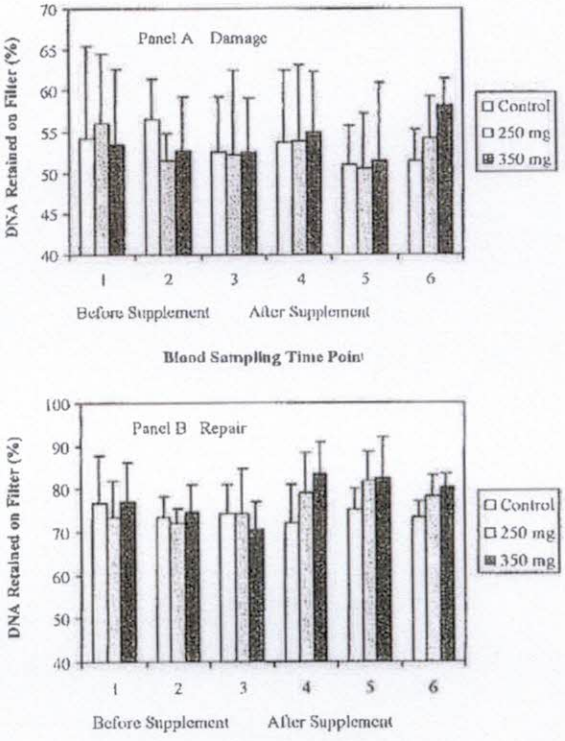

Fig. 1. DNA damage (Panel A) and DNA repair (Panel B) before and after C-Med-100® supplement at different blood sampling time points. Blood sampling time points 1–3 are before supplement while 4–6 are after supplement. Human monoclonal leukocytes (HML) taken from different time points were exposed to a standard dose of hydrogen peroxide (100 µM) on ice for 30 minutes and then the DNA single strand breaks were measured by alkaline elution. DNA repair at 37 °C was evaluated 30 minutes after the induction of DNA damage. Results are shown as mean ± SD (n = 4). * = $p < 0.05$ compared to control.

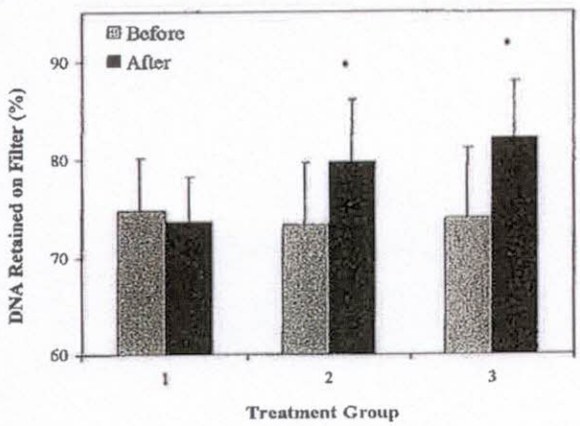

Fig. 2. DNA repair before and after C-Med-100® supplement presented in summary. DNA repair of human mononuclear leukocytes (HML) was measured by alkaline elution 30 minutes at 37 °C after DNA damage induced by a standard dose of hydrogen peroxide (100 µM). The results are pooled from 3 measurement of 4 persons from each group and shown as mean ± SD (n = 12). Group 1 is controls, group 2 is supplemented with C-Med-100® 250 mg/day and group 3 is supplemented with C-Med-100® 350 mg/day. There is a statistically significant main effect (* = $p < 0.05$) of supplement as by MONOVA Repeat Measurement (SPSS, Inc.).

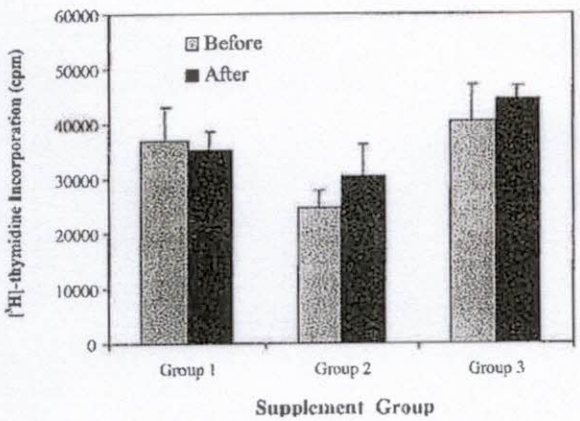

Fig. 3. The effect of C-Med-100® on human mononuclear leukocyte (HML) proliferation induced by PHA. Freshly prepared HML were cultured in microtiter plates at 25 000 cells/well in 200 µl RPMI 1640 - 10% FCS - 10 µl PHA at 37 °C and 5% CO_2 for 5 days then pulsed for 6 hours with [³H]-thymidine (2 Ci/mmole, final concentration 0.5 µCi/ml). Labeled nuclear material was collected on glass fiber filters in a microtiter plate cell harvester, dried and counted in scintillation fluid. Group 1 is controls, group 2 is supplemented with C-Med-100® 250 mg/day and group 3 is supplemented with C-Med-100® 350 mg/day. Data are expressed as mean ± SD (n = 4).

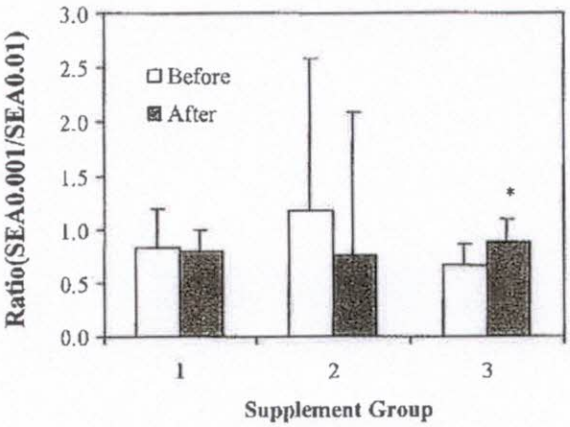

Fig. 4. The effect of C-Med-100® on human mononuclear leukocyte (HML) proliferation induced by staphylococcal enterotoxin A (SEA). Freshly prepared HML were cultured in microtiter plates at 25 000 cells/well in 200 µl RPMI 1640 - 10% FCS - SEA 0.001 ng/ml or SEA 0.01 ng/ml at 37 °C and 5% CO_2 for 5 days then pulsed for 6 hours with [³H]-thymidine (2 Ci/mmole, final concentration 0.5 µCi/ml). Labeled nuclear material was collected on glass fiber filters in a microtiter plate cell harvester, dried and counted in scintillation fluid. Group 1 is controls, group 2 is supplemented with C-Med-100® 250 mg/day and group 3 is supplemented with C-Med-100® 350 mg/day. Data are expressed as mean ± SD (n = 4). * = $p < 0.05$ compared to before treatment.

showed the same lymphocyte proliferation but stimulated by staphylococcal enterotoxin superantigen (SEA) and expressed as ratio of SEA 0.001/ SEA 0.01 µg/ml. There was a significant difference before and after C-Med-100® supplement for group 3 (supplement with 350 mg/day). Since the proliferation index is a ratio between thymidine incorporation (growth) and cell number over a defined period, there could be both an increase of lymphocyte proliferation stimulated by SEA at 0.001µg/ml (lower dose), or a decrease of lymphocyte proliferation when stimulated by SEA at 0.01µg/ml (higher dose) when compared to control levels. One possible explanation for these data would be that C-Med-100® increased the low dose SEA stimulated lymphocyte proliferation, while the high dose SEA stimulated lymphocyte proliferation had already passed the peak value of maximum response, and hence was in a declining phase at the fixed cell culture time used for evaluation.

Discussion

C-Med-100® is a hot water extraction of plant parts (primarily inner and outer bark sources) from *Uncaria tomentosa* (Willd.) DC, commonly called Una de Gato

or Cat's Claw. Cat's Claw has been used historically as a medicinal plant source by the Native Indians of South America for a long time and its products have been offered commercially in the USA by more than 50 companies (Keplinger, et al., 1999). However, scientific evaluation and publication about the efficacy and toxicity of these natural products, especially human data, are still rare. Although these natural products are classified as nutritional and food supplements, it would be valuable both scientifically and commercially to evaluate them both in animal and human studies. This is also in accordance with the guideline of newly-formed National Center for Complementary & Alternative Medicine (NCCAM Director's Testimony, March 2, 2000).

Previously, it has been shown that a proprietary hot water extract of C-Med-100® has lower or no toxicity compared with some other extracts of *Uncaria tomentosa*. It has also been shown that C-Med-100® could increase DNA repair capacity in a rat model and enhance the recovery of leukopenia induced by doxorubicin (Sheng et al, 2000a; 2000b). In this study, the fact of DNA repair enhancement by C-Med-100® in vivo was tested by a pilot volunteer study. Although the number of participants was small, it showed that after 8 weeks of C-Med-100® supplementation at doses of 250 mg/day or 350 mg/day, the subjects statistically significantly increased the DNA repair capacity as shown in Figure 1 and Figure 2. This increase of repair is not attributed to decreased DNA damage by a standardized dose of hydrogen peroxide since only the last week (blood sampling time point 6) data in Panel A of Figure 1 showed that tendency. Moreover, DNA repair also increased after 6 weeks of supplement (time point 4) even though the levels of induced DNA damage between groups were the same, and the repair increase continued until week 8 (time point 6). Although there was significant increase of DNA repair for both 250 and 350 mg/day groups compared to control group ($p < 0.05$), there was no significant difference between the two doses used. This probably was due to the small population used since there was a dose dependent tendency in Panel 2, Figure 1. Furthermore, no adverse effects, in terms of symptoms and signs, whole blood hematological analysis, or blood chemistry analysis have been found in this volunteer study supplemented with C-Med-100® at doses of 250 mg or 350 mg for 8 weeks. These results are in accordance with another human study conducted in New York, the population of which was supplemented with C-Med-100® at a higher dose of 2×350 mg/day for 2 months (Lamm et al., 2000).

The extracts of *Uncaria tomentosa* have been postulated to increase the immune response (Keplinger, 1982; Sheng et al, 2000a) but most of these data are from animals. Although the results are not conclusive due to the small population and high variation of the lymphocyte proliferation index, these data supported an increase in the immune response after C-Med-100® supplementation, which have also been demonstrated in vivo rodent studies (Sheng et al, 2000a and 2000b) and the New York human trial (Lamm et al, 2001).

The mechanism of DNA repair enhancement by C-Med-100® supplementation is still not clear. Here we present several likely possibilites for the modes of action: (1) C-Med-100® prevents or decreases the DNA damage. Since the DNA repair measured in this study (30 minutes after induction of DNA damage) depends on both the original damage level and the individual's cellular ability to defend against and/or repair DNA damage, the decrease of DNA damage level will also result in an increased DNA repair level. This is confirmed by that at the last supplement week (time point 6) the DNA damage induced by a standardized dose of hydrogen peroxide is significantly decreased (Panel A, Figure 1). (2) There is a close correlation between DNA damage, DNA repair and immune responses for lymphocytes. T cells in vivo have been shown to accumulate DNA damage and mutations over time. Since T cells are required to undergo extensive clonal expansion upon antigenic stimulation, DNA damage and mutations may result in a failure of T cells to proliferate, because of DNA damage-mediated cell cycle arrest, and also decreased rates of proliferation, as a consequence of selection in vivo against cells carrying certain mutations and/or apoptosis, triggered by critical levels of DNA damage. Insufficient number of T cells may be produced in this case and so the immune response would be sub-optimal (Barnett & Barnett, 1998). On the other hand, decreased DNA damage and increased DNA repair are often accompanied with faster recovery of immune cell number and enhanced immune function (response) (Sheng et al., 1998; 2000a; 2000b). (3) Inhibition of NF-kB which has been reported in extracts of *Uncaria tomentosa* by Sandoval-Chacon, et al (1998).

As already pointed out, C-Med-100® is depleted of the already known immune modulating class of agents known as oxindole alkaloids (less than 0.05%), but contains at least 8–10% of a new class of agents preliminarily identified as carboxy alkyl esters (CAE) that can also enhance immunity. This is a very general classification based on the identification of at least one carboxyl ester function. Furthermore, these type of compounds have been identified in extracts of *Uncaria tomentosa*. Aquino and colleagues (Cerri et al 1988; Aquino et al 1989) have identified carboxy esters of quinovic acid and its glycosides to be present in Uncaria, and also to possess antiinflammatory activity. Whether the active ingredient(s) of C-Med-100® are quinovic acid analogs has not as yet been determined but is the subject of our latest research efforts.

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Address

Yezhou Sheng, Department of Cell and Molecular Biology, Section of Tumor and Immune Biology, University of Lund, BMC: 1-12, S-221 84 Lund, Sweden.
Phone: 46 46 2223625; Fax: 46 46 2224624;
e-mail: Yezhou.Sheng@wblab.lu.se