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SHORT COMMUNICATION

The Synthesis of Rantes, G-CSF, IL-4, IL-5, IL-6, IL-12 and IL-13 in Human Whole-blood Cultures is Modulated by an Extract from *Eleutherococcus senticosus* L. Roots

M. W. Schmolz,[1]* F. Sacher[2] and B. Aicher[2]

[1]EDI (Experimental & Diagnostic Immunology) GmbH, Markwiesenstr. 55, D-72 770 Reutlingen, Germany
[2]Pharmaton GmbH, Birkendorfer Str. 65, D-88 386 Biberach/Riß, Germany

An ethanol extract derived from the roots of *Eleutherococcus senticosus* was found to influence markedly the cytokine synthesis of activated whole blood cultures of ten healthy volunteers. Whereas the synthesis of Rantes was increased over a wide range of concentrations, the release of IL-4, IL-5 and IL-12 was significantly inhibited. An inhibition at higher concentrations, switching to a stimulation at lower doses of the extract was seen with G-CSF, IL-6 and IL-13. From these particular immuno-pharmacological effects of *Eleutherococcus senticosus* we suggest this herbal preparation possesses immuno-modulatory potency, rather than just being immuno-suppressive or -stimulating. Copyright © 2001 John Wiley & Sons, Ltd.

Keywords: Eleutherococcus senticosus; immune system; immuno-modulation; cytokines; whole-blood cultures; herbal extract.

INTRODUCTION

Eleutherococcus senticosus is the Russian 'relative' of the Chinese plant *Panax ginseng* which is the source of the *Ginseng* root extract. The widespread traditional use of extracts from *Eleutherococcus* roots, mainly taken to improve mental and physical conditions, has resulted in many papers on preclinical and clinical trials during the past decades (Bohn *et al.*, 1987; Winterhoff *et al.*, 1993; Elkin *et al.*, 1986; Novozhilov and Sil'chenko 1985). Double-blind, placebo-controlled studies revealed an increase in resistance to infections (Vereshchagin, 1978). An excellent review on the pharmaceutically used parts of the plant is given in *Hagers Handbuch* (Aicher and Wosniewski, 1998).

Therefore, we investigated how Eleu-Kokk®, a pharmaceutical based on *Eleutherococcus* extract, affects the immuno-regulatory capacities of human peripheral blood leukocytes, using cytokine syntheses as a measure.

MATERIALS AND METHODS

Test sample. Eleu-Kokk® (lot no. 50002) was provided by the manufacturer, Pharmaton GmbH, Germany. 5 mL of this pharmaceutical contains 0.98 g of a crude ethanol extract from the roots of *Eleutherococcus senticosus*. The final dilutions in culture were 1:125; 1:500; 1:2.000; 1:8.000; 1:32.000 (C1–C5).

* Correspondence to: Dr M. W. Schmolz, EDI (Experimental & Diagnostic Immunology) GmbH, Markwiesenstr. 55, D-72 770 Reutlingen, Germany.

Volunteers. The blood of ten healthy volunteers, seven female, three male, 24–45 years old, was used to perform the cultures in this study the exclusion criteria used to preclude any negative interference by drugs, alcohol and inflammatory diseases (either acute or chronic) were chronic diseases involving the immune system; any symptoms of infectious or inflammatory diseases during the past 3 weeks and intake of antiinflammatory, analgesic or anti-pyretic drugs within the past 2 weeks before blood donation.

Cell cultures. Whole-blood cultures, adapted from a method described by DeForge *et al.* (1992) were used, as this system most closely resembles the natural conditions *in vivo*. Experimental stimuli were either opsonized Zymosan (obtained from Sigma-Aldrich, Germany), phyto-haemagglutinin (PHA) or PHA + anti-CD28 (from Murex and Pharmingen, Germany). Controls included non-stimulated as well as stimulated cultures with Hanks' buffered salt solution. Cytokines were measured with commercially available ELISA kits from Pharmingen and R&D Systems (both Germany) and Endogen (Belgium).

Statistics. Descriptive statistical evaluation and Student's *t*-test were performed using Excel 5.0 and the Excel-Add-In Astute 1.51 (DDU Software, UK).

RESULTS

Our results show a moderate stimulation of Eleu-Kokk® on G-CSF synthesis (C2 1:500) although not statistically

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Table 1. IL-6 Synthesis in human whole blood cultures incubated with C1–C5

	C1	C2	C3	C4	C5
Mean difference	−0.369	0.207	−0.032	−0.043	−0.029
95% CI upper limit	−0.268	0.402	0.059	0.030	0.045
95% CI lower limit	−0.470	0.013	−0.122	−0.116	−0.103
p-level	<0.001	0.039	0.466	0.231	0.422

Values are the mean difference of each concentration tested compared with the buffer control as well as upper and lower limits of the confidence intervals; *p* levels were calculated using the Student's *t*-test.

Table 2. IL-12 synthesis in human whole blood cultures incubated with C1–C5

	C1	C2	C3	C4	C5
Mean difference	−0.821	−0.474	−0.118	−0.111	−0.019
95% CI upper limit	−0.734	−0.372	0.020	0.022	0.116
95% CI lower limit	−0.907	−0.576	−0.255	−0.243	−0.155
p-level	<0.001	<0.001	0.088	0.096	0.764

Values are the mean difference of each concentration tested compared with the buffer control as well as upper and lower limits of the confidence intervals; *p* levels were calculated using the Student's *t*-test.

Table 3. Rantes synthesis in human whole blood cultures incubated with C1–C5

	C1	C2	C3	C4	C5
Mean difference	1.195	0.861	0.220	−0.017	−0.089
95% CI upper limit	1.800	1.280	0.327	0.104	−0.012
95% CI lower limit	0.590	0.442	0.113	−0.138	−0.167
p-level	0.002	0.001	<0.001	0.766	0.027

Values are the mean difference of each concentration tested compared with the buffer control as well as upper and lower limits of the confidence intervals; *p* levels were calculated using the Student's *t*-test.

significant ($p = 0.081$, data not shown). However, IL-6 synthesis was clearly inhibited by the highest concentration (C1) of Eleu-Kokk® ($p < 0.001$, Table 1) switching to a stimulation at C2 ($p < 0.04$). The release of IL-12 was significantly suppressed over a wide concentration range (C1 to C4, $p < 0.001$ to <0.1; Table 2), whereas Rantes was stimulated with Eleu-Kokk® (C1 to C3, $p = 0.002$ to <0.001, Table 3).

When looking at IL-4 and IL-5, Eleu-Kokk® C1 induced rather heterogeneous responses in the cultures of different donors (with some being stimulated and others being inhibited). However, the predominant activity was

Table 4. IL-4 synthesis in human whole blood cultures incubated with C1–C5

	C1	C2	C3	C4	C5
Mean difference	−0.191	−0.295	−0.091	−0.019	0.011
95% CI upper limit	0.044	−0.211	−0.013	0.054	0.092
95% CI lower limit	−0.426	−0.379	−0.169	−0.092	−0.071
p-level	0.100	<0.001	0.025	0.596	0.784

Values are the mean difference of each concentration tested compared with the buffer control as well as upper and lower limits of the confidence intervals; *p* levels were calculated using the Student's *t*-test.

Table 5. IL-5 synthesis in human whole blood cultures incubated with C1–C5

	C1	C2	C3	C4	C5
Mean difference	−0.466	−0.443	−0.099	−0.029	−0.017
95% CI upper limit	−0.284	−0.352	0.006	0.047	0.054
95% CI lower limit	−0.649	−0.533	−0.203	−0.105	−0.088
p-level	<0.001	<0.001	0.063	0.435	0.615

Values are the mean difference of each concentration tested compared with the buffer control as well as upper and lower limits of the confidence intervals; *p* levels were calculated using the Student's *t*-test.

an inhibition of the release of both cytokines. C2 and C3 suppressed the synthesis of IL-4 and IL-5 uniformly and significantly (Tables 4 and 5). A very similar dose-response relation was found for IL-13 ($p < 0.05$; data not shown).

DISCUSSION

Our results confirm previous studies showing the immuno-modulatory effects of *Eleutherococcus* extracts (Elkin *et al.*, 1986; Kozlov 1986). Moreover, the transition from inhibitory to stimulatory effects in the syntheses of different immuno-regulatory cytokines as a result of serial dilution clearly indicates the immuno-modulating properties of Eleu-Kokk®. Considering the pleiotropic nature of cytokines, Eleu-Kokk® when applied therapeutically can be expected to affect various different immuno-regulatory pathways, either directly or indirectly (Devergne *et al.*, 1994; Trinchieri and Gerosa, 1996; Zurawski and de Vries, 1994).

Having in mind the complexity of immune reactions, it is too soon to speculate on which clinical benefits Eleu-Kokk® will display in the therapy of infectious or allergic diseases. Nevertheless, the data derived from our study are promising and clinical studies are presently initiated to address these topics.

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