Final Report

Immunomodulatory and antioxidant activities of Ge132+Natural ™

VivaCell Biotechnology GmbH

Final report (Draft 1)

Immunomodulatory and antioxidant activities of Ge132+Natural ™

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2. Aims of project (objectives)

The aims of this project were the following:

(1) To study potential anti-inflammatory effects of **Gel132+Natural™** on the release of LPS-induced cytokines IL-6, IL-8 and TNF and the release of LPS-induced in primary human monocytes.

(2) To study the anti-oxidant effects of **Gel132+Natural™**

3. Study protocol

1) Measurement of prostaglandin and isoprostane release in primary human monocytes

Human primary monocytes were isolated from buffy coats of healthy human blood donors and seeded in 24-well-plates (approx. 500 000 cells/ml in 1 ml) for ELISA experiments.

Cells were incubated without (unstimulated control) or with LPS (10 ng/ml) for 24 h. **Gel132+Natural™** was added 30 min before LPS treatment in five doses. After 24 h, supernatants are removed, centrifuged and investigated for TNFalpha (TNFalpha), interleukin-6 (IL-6) and interleukin-8 (IL-8) concentrations in ELISA using manufacturer's protocol. Each dose was investigated 6 times in buffy coats from three different blood donors.

2) Antioxidant activity

Detection of Reactive Oxygen Species. Intracellular production of ROS was measured by using 2,7 dichlorofluoresceindiacetate (H2DCF-DA; Molecular Probes). This nonpolar compound is converted to the membrane-impairment polar derivative H2DCF by esterases when it is taken up by the cell. H2DCF is non-fluorescent but is rapidly oxidized to the highly fluorescent DCF by intracellular H2O2 and other peroxides. Stocks of H2DCF-DA (5 mM) were made in DMSO and stored in the dark at 80°C. HaCaT cells (100 cell/well) were preincubated during 30 minutes with the test compounds at the doses indicated and stimulated with a ROS inductor (TBHP). After the period of incubation = 3 h, cells were washed with PBS and incubated with 1 mM of H2DCF-DA during 20 minutes at 37ºC, the fluorescente will be determined at 460/520 nm em/ex in a plate reader TriStar LB 941 (Berthold Technologies, GmbH & Co. KG).

Activity scavenger DPPH assay. The radical DPPH is the most widely reported method for screening of antioxidant activity of many drugs. The DPPH assay method is based on the reduction of methanolic solution of coloured free radical DPPH by free radical scavenger. The procedure involves measurement of decrease in absorbance of DPPH at its absorption maxima of 516 nm, which is proportional to concentration of free radical scavenger added to DPPH reagent solution. GE132+ Natural with DPPH 1 mM in a methanolic solution (final volumen 4,5 ml). After stirring, the mix was incubated during 30 min at room temperature. As positive control Trolox (200 µM) was used. The decrease in absorbance was determined in a plate reader TriStar LB 941 (Berthold Technologies, GmbH & Co. KG).

4. Results

1) Anti-inflammatory effects of **Gel132+Natural™**

We studied the effects of GE132+ on cytokine release in primary human monocytes. As shown in Fig. 1, GE132+ inhibited LPS induced IL-6 release starting with 50 µg/ml and maximal inhibition using 250 µg/ml, which prevented IL-6 synthesis of about 80%. LPS-induced IL-8 release was inhibited starting with 100 µg/ml and maximal effects in the higher dose of 250 µg/ml. TNFalpha synthesis induced by LPS was blocked by GE132+ in the concentration of 250 µg/ml.

Our data show that GE132+ Natural has a strong anti-inflammatory potential and thus is an useful therapeutic to treat diseases with an inflammatory background. The mechanisms underlying these effects still need to be elucidated.

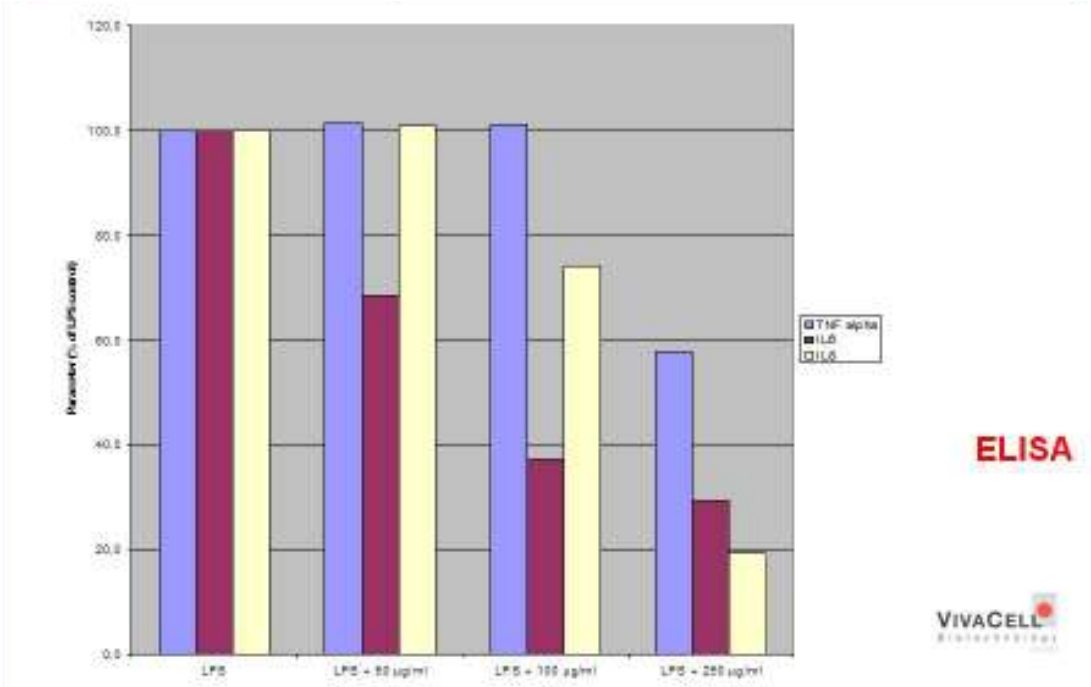

Fig. 1: Effects of Ge132+ on cytokine release in LPS-treated human monocytes.

2) Anti-oxidant effects of Gel132+Natural™

We used two different methods to study the antioxidant activity of GE132+: the effects on ROS (reactive oxidant species) and the protection from free radicals in the DPPH radical scavenger assay.

As shown in Fig 7, our data demonstrate that the G132+ compound strongly decreased the production of ROS induced by TBHP (second column from left). ROS formation is inhibited with the low dose of 1 µg/ml GE132+, maximal inhibition is achieved using 25 µg/ml of GE132+. This dose was even more potent as the positive control, the known anti-oxidant N-acetyl cystein (column 3 from left).

Using the DPPH radical scavenger assay, we are able to demonstrate that GE132+ starting with 5 µg/ml and maximal effect using 100 µg/ml, which was almost as effect as the known and strong anti-oxidant Trolox C (200 µM) (Fig. 8).

Our data clearly demonstrate that GE132+ Natural is a potent anti-oxidant preventing the the formation of free radicals in the same potency as known anti-oxidants. By preventing free radical formation GE132+ Natural is a potent therapeutic in disease where free radicals are known to be involved, such as the aging process.

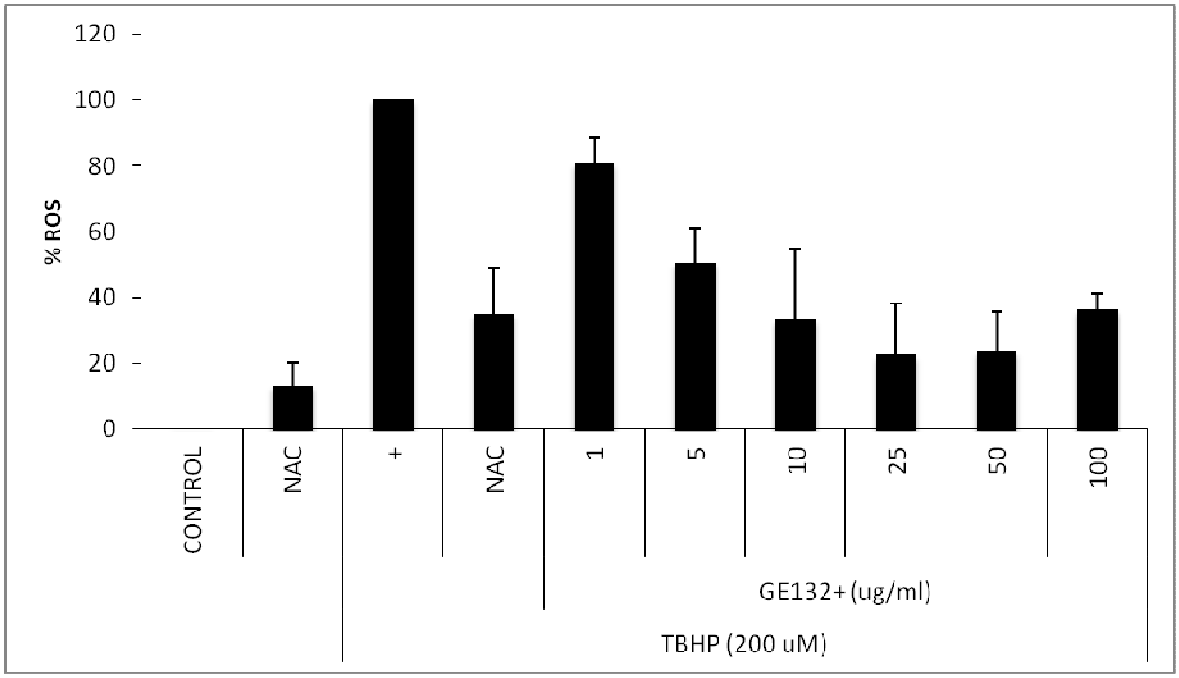

Fig. 7: Effects of GE132+ on TBHP induced ROS (reactive oxidant species) formation compared with the anti-oxidant N-acetyl cystein (NAC).

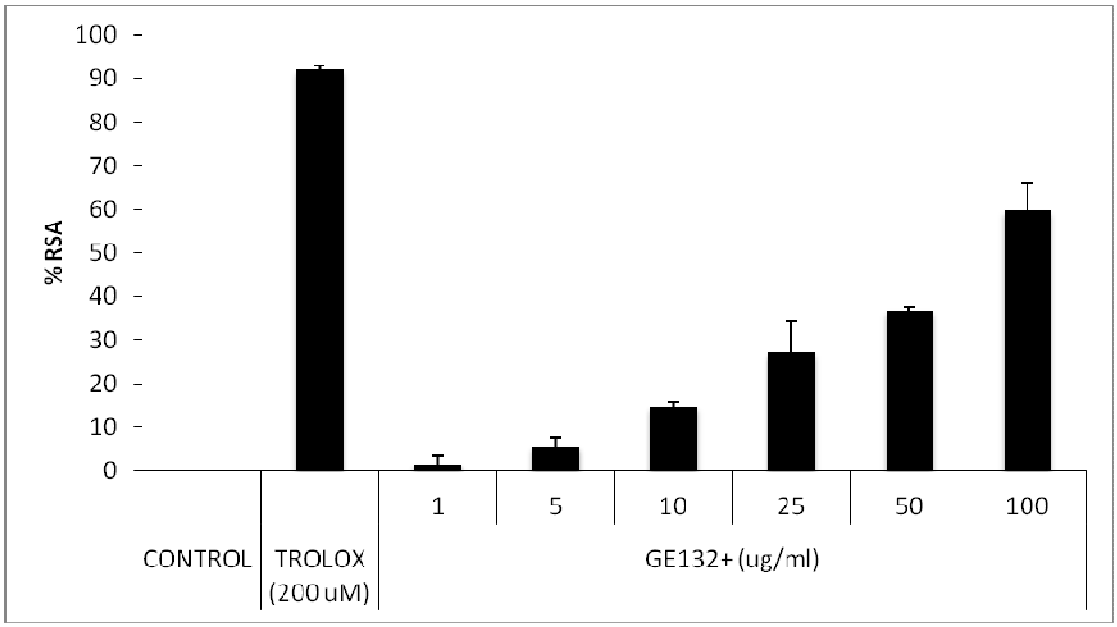

Fig. 8: Radical scavenging effects of GE132+ in the DPPH radical scavenger assay compared with the anti-oxidant Trolox C.

5. Signatures

Testing facility:

Principal investigator:

.....23.1.2012..... .....

Date: Dr. B. L. Fiebich (CEO/CSO, VivaCell)

Sponsor:

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Date:

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Date:

Submitted, Freiburg 23.1.2012
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