Influence of sodium hyaluronate - iodine complex on human keratinocytes and leukocytes

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INTRODUCTION

The complex of sodium hyaluronate with iodine (Hyiodine®) reveals excellent healing properties, especially in the healing of large and infected wounds. In this study we are showing the effect of Hyiodine® (HY) on the cells that play an important role in wound healing, such as epidermic cells (keratynocytes) and immune system cells (lymphocytes, monocytes and PMNL).

In order to prove that Hyiodine® is not cytotoxic and actually supports the wound healing process we determined LDH release from the cells (cytotoxic effect), IL6, IL8, IL12, TNFα production, expression of CD11b (adhesion, complement receptor) and CD62L (L-selectin, adhesion receptor) and production of free radicals.

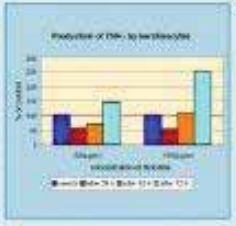
MATERIALS AND METHODS

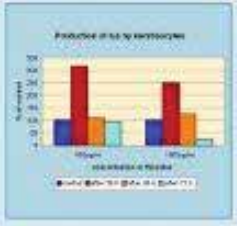
Primary human keratinocytes were isolated from donors after plastic surgery and cultivated according to Rheinwald and Green (1975). Heparinized blood samples were obtained from healthy volunteers. Immune cells were resuspended in RPMI-1640 supplemented with 10% heat inactivated human AB serum after the separation.

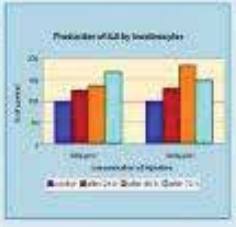
Cell viability was determined by XTT measurement (Roche France). Cytotoxicity was evaluated by the measurement of LDH release from the cells (Roche France). ELISA was used for the quantification of IL6, IL8, IL10 and TNFα production (DendermedSystems, Austria). Luminol-enhanced chemiluminescence of whole blood phagocytes was used for the phagocyte-derived free radicals quantification (Immunotech, Czech Republic). The expression of cell surface molecules was measured by flow cytometry using unfixed whole blood (Caltag Laboratories, USA).

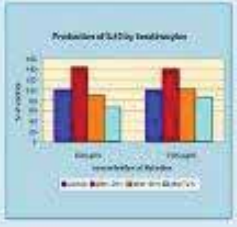
The Wilcoxon non-parametric tests were used for statistical analysis (Statistica for Windows 5.0, Statsoft, USA).

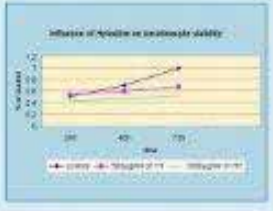
KERATINOCYTES





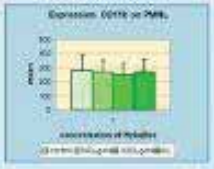


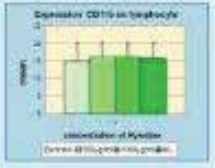


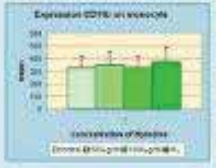


IMMUNE CELLS

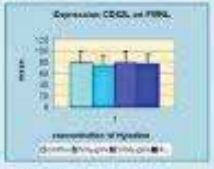
Expression CD11b

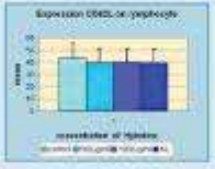


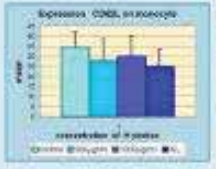




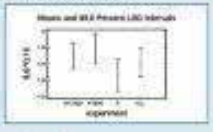
Expression CD62L

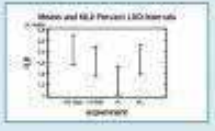


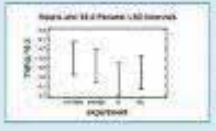




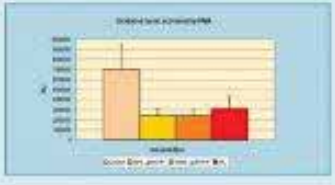
Production IL6, IL8, TNFα

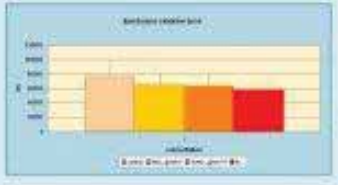






Oxidative burst





RESULTS

- Hyiodine® at concentration 500 µg/ml increased LDH release from keratinocytes for 17,86% and at concentration 1000 µg/ml for 9,02%.
- Hyiodine® influences keratinocytes viability (decreases their growth rate) particularly if they are in prolonged contact with Hyiodine®.
- Production of cytokines IL6 and IL12 by keratinocytes is effected by Hyiodine® mainly in early intervals (after 24 hours) in contrast to IL8 and TNFα which are influenced mainly in the later intervals (48 and 72 hours).
- Expression of CD11b (adhesive receptor, play a key role in cell interaction) and CD62L (play a primary role in mediating initial leukocyte interaction with activated vascular endothelium) on lymphocytes, monocytes and PMNL is not influenced neither by Hyiodine® at both concentration nor by LPS after their 3 hours incubation with whole blood.
- Hyiodine® increased production of IL6, IL8 and TNFα by isolated lymphocytes after 24 hours incubation with Hyiodine® at both concentration. This increase was not significant.
- The production of free radicals by blood phagocytes, which had been induced by PMA as well as at spontaneous oxidative burst, was significantly decreased by both concentrations of Hyiodine® and also by iodine-potassium iodide complex. It can be suggested that the presence of iodine-potassium iodide complex alone induced the observed decrease of free radical production and sodium hyaluronate does not have any further influence. The lack of effect of sodium hyaluronate on free radical production by blood phagocytes shown in this study has been already proven in our previous studies. The observed effect of iodine potassium iodide complex can be caused by direct effect of iodine-potassium iodide complex on phagocytes or by the interaction of iodine-potassium iodide complex with luminophor with consequent inhibition of CL.

CONCLUSION

- Hyiodine® is a non-cytotoxic product (if the increase of LDH cell release is higher than 30%, the product is decided to be cytotoxic).
- Hyiodine® influences keratinocytes viability.
- Hyiodine® influences pro-inflammatory cytokine production by keratinocytes. We can conclude that, through the increase of pro-inflammatory cytokines production, Hyiodine® can support the "cleaning phase" of wound healing.
- The ability of immune cells to penetrate into the wound is not effected by Hyiodine® as the expression of CD11b and CD62L on lymphocytes, monocytes and PMNL is not influenced.
- We did not prove that Hyiodine® is able to influence cytokines IL6, IL8 and TNFα production by lymphocytes.
- Free radicals production was significantly depressed by both Hyiodine® concentrations.

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