GP4G STIMULATES HUMAN HAIR FOLLICLE CELLS METABOLISM AND GROWTH IN ORGAN CULTURE

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Introduction

It is well known that metabolic processes are involved in embryonic development, in genome activation and contribute to large increase of protein synthesis and cell division. The GP4G or diguanosine-tetra-phosphate, is considered as a precursor of the ATP. It is described to activate G protein in skin (Casemelle et al, 1982). The binding activity of GP4G in brain increases with time of rat nervous system development (Grau et al, 1982). The aim of this study is to check whether this molecule, can stimulate the metabolism of hair dermal papilla cells in vitro and the hair growth ex vivo. Oxygen consumption is an ubiquitous parameter which can provide valuable information on metabolic mechanism. We assessed oxygen consumption following treatment with GP4G of cultured human hair follicle dermal papilla (HDPC) cells by using electrochemical technique. We used HDPC as a useful model for evaluating metabolic activity of GP4G and a-tocopherol nicotinate. Time courses of survival of HDPC were measured colorimetrically by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) test. In this study, we also presented the kinetic aspect of hair shaft elongation ex vivo after hair follicle treatment with GP4G.

Materials and methods

Dermal papilla cell culture

Dermal papillae were isolated from the lower part of human hair follicles from the occipital region of scalp. Several papillae were placed in 35mm culture dishes containing 2ml of DMEM supplemented with 20% foetal bovine serum, penicillin (100units/ml) and streptomycin (100µg/ml). Medium was changed every 3 days. All cell cultures were incubated at 37°C in a humidified 5% CO2 atmosphere.

Isolated hair follicle culture

Hair follicles were obtained from the occipital region of human hair. After removing the fat, the hair follicles were placed on 24-well (1 intact follicle/mL/well) at 37°C in a humidified atmosphere containing 5% CO2. The hair follicles were maintained in Dulbecco's modified Eagle's medium containing insulin at 10µg/mL, transferrin at 10µg/mL, hydrocortisone at 10µg/mL, 1% of penicillin-streptomycin, fungizone at 2.5µg/mL and 1mM-glutamin. The length of hair follicle was measured immediately before treatment with GP4G at 5%, α-tocopherol-nicotinate at 100µg/ml or 24µM Minoxidil, and after 3, 6 and 9 days of culture. Images were taken by a digital camera coupled to an image analysis software. The percentage of daily hair growth was then calculated and the activity of treatment was evaluated at each point of hair culture.

MTT assay

To examine cell viability, an MTT assay was used. Briefly, HDPC were seeded in 96-well cell culture plates at a density of $1x10^6$/ml cells in DMEM supplemented with 25 µl MTT solution (5mg/ml). 25µl of GP4G at 5% or a-tocopherol nicotinate at 100µg/ml was added to each well for 30min and 2hours. Then the MTT solution was removed, and the blue MTT formazan precipitate was dissolved in 100 µl DMSO. The optical density of samples was measured at 540 nm using an enzyme-linked immunosorbent assay plate reader.

Measurment of oxygen consumption

By using Clark oxygen electrodes, the oxygen consumption rates of HDPC was determined. The protocol used for measurement of oxygen consumption is as follow: a) cells were incubated for 24h with DMEM (negative control), DMEM + calf serum (positive control) or GP4G at 1% and 5%. b) cells were collected by centrifugation in Hank's buffer c) oxygen consumption was monitored for 10min at 25°C.

Statistical analysis

Comparisons were performed by the Student t-test, with the level of significance set at 0.05. Results are reported as means ± SD.

Results

Cell viability increases after treatment

After treatment with different concentrations of GP4G (1%, 3% and 5%) or a-tocopherol nicotinate at 100µg/ml, HDPC were assayed to detect cell viability compared to control groups. As shown in Figure 1, cell viability increased after 30 min and 2h of treatment with both components. GP4G stimulated cell viability prominently and significantly after 2h culture, the percent of cells metabolising MTT vs control was (+33%,+33% and +36% for GP4G at 1%, 3% and 5% respectively) compared to the a-tocopherol nicotinate groups (+30%).

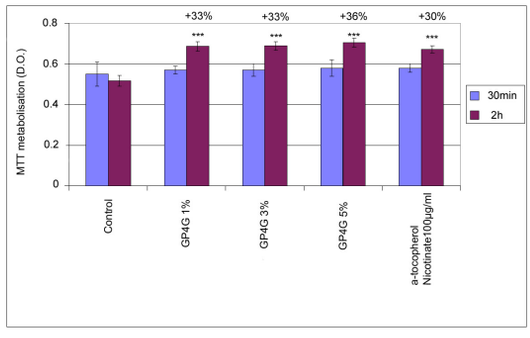

Figure 1. MTT analysis of cell metabolism after GP4G or a-tocopherol nicotinate treatment

A small increase of cell viability could be seen after treatment for 30min with GP4G or a-tocopherol nicotinate and was more prominent after 2h treatment. The error bars represent the standard deviation. Results of statistical comparisons to the control group are denoted by asterisk ***($p<0.05$).

Oxygen consumption

GP4G tested at 1 and 5% increased the oxygen consumption of cultured hair dermal papilla cells in a dose responsive manner. The oxygen values in GP4G 1% and 5% treated group were (0.07), (0.1) nmoles/sec/mL respectively. The value of 0.05 was obtained for the negative control group.

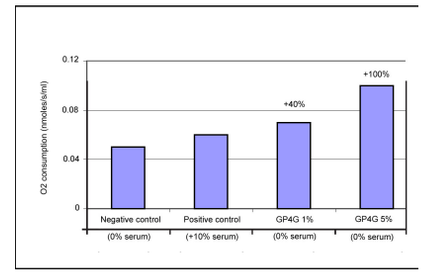

Figure 2: cultured HDPC oxygen consumption in the presence of GP4G.

Treated cells with GP4G display more oxygen consumption than control without serum. The % of increase of oxygen consumption related to the negative control is about +40% and +100% in the presence of GP4G at 1% and 5% respectively.

Hair growth ex vivo :

Ex vivo, the hair grow faster in the presence of GP4G at 5%.
The mean rate at day 6/day0 was +110% for treated follicles with GP4G vs +17% for control follicles, +36% for minoxidil group and +20% for the group treated with a-tocopherol nicotinate.

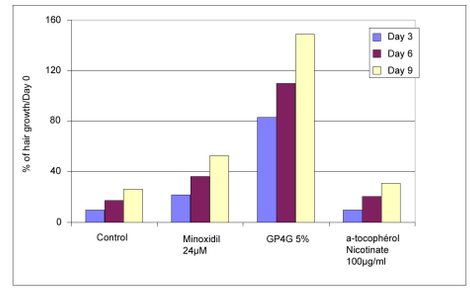

Figure 3. Time course of hair follicle growth ex vivo

Hair follicles are maintained in culture in a defined medium and hair elongation was measured at 3, 6 and 9 days in the presence of GP4G at 5%, a-tocopherol nicotinate at 100µg/ml or 24µM Minoxidil.

Conclusion

The ability of GP4G to stimulate hair follicle cells metabolism in vitro and the hair growth ex vivo suggests that hair follicles maintained in organ culture are able to respond to this metabolic promoter.

References

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Grau VP, Günter-Sillero MA, Sillero A. Binding of P1,P4 (5'-guanosyl) tetraphosphate to brain.1982;257:11848-11851

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