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Effects of Sulfhydryl Compounds on Cancer Cell Lines: I: N-(2-Mercaptopropionyl)-glycine Exerts Antiproliferating Effects and Antagonizes the Stimulating Effect of Prolactin on MCF-7 Human Breast Cancer Cells

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Abstract. *The effects of prolactin (PRL) and N-(2-mercaptopropionyl)-glycine (MPG) on the growth of MCF-7 human breast cancer cells, and the influence of MPG on PRL-induced MCF-7 cell proliferation, when added simultaneously to the culture medium, were examined. Prolactin at concentrations of 200 ng/ml - 350 ng/ml enhanced the growth of MCF-7 cells, while at lower and higher concentrations its action was diminished. MPG alone at concentrations above 2 mM caused a decrease in cell proliferation. When PRL and MPG were added simultaneously to the culture medium, an inhibitory effect on PRL-induced MCF-7 cell proliferation was observed, at concentrations of MPG lower than 2 mM.*

The role of hormones, like estrogens, in breast cancer is well established and the contribution of prolactin (PRL) to the pathogenesis and progression of the disease is being increasingly appreciated. Although PRL is clearly involved in normal breast growth and differentiation during puberty, pregnancy and lactation, its role in the development of human breast cancer has been obscure. Many epidemiological studies failed to establish a definite connection between PRL levels and breast cancer risk, and PRL-lowering drugs, such as bromocriptine, have been ineffective in the treatment of breast cancer patients. In contrast, its role in rodent mammary carcinoma and transgenic mice overexpressing the *prl* gene has been well established (1, 2). At the cellular level, PRL and its receptor are expressed in many normal and cancer breast cell lines, as well as in human breast tumors (3, 4). Breast cancer cells secrete prolactin (5, 6), which has been shown to act as a mitogen for these cells (7). Antibodies against prolactin inhibit the proliferation of breast cancer cell lines (5), while PRL receptor antagonists antagonize the effect of PRL on cell proliferation (8).

Since cancer is often an incurable disease the prevention of the disease is of great importance. Antioxidants are among the many substances that have been used in experimental and epidemiological studies for cancer prevention, since oxidative stress and reactive oxygen species (ROS) contribute to the pathogenesis of cancer.

N-(2-Mercaptopropionyl)-glycine (MPG), or tiopronin, is a sulphydryl compound and chelator. It is given in the management of cystinouria (9), in rheumatoid arthritis, and has been tried in heavy-metal poisoning and hepatic disorders (10). In women in the puerperium the use of MPG caused suppression of lactation (11).

In this study we examined the influence of MPG on MCF-7 human breast cancer cell proliferation, and also tested the effect of MPG on PRL-induced MCF-7 cell proliferation, when both were added simultaneously to the culture medium.

Materials and Methods

Substances. DMEM-high glucose (D5671), DMEM without phenol red (D1145), human prolactin (L4021), human transfering (T8158), human insulin (I9278), human albumin (A6909) and MCF-7 human breast cancer cells (86012803) were obtained from SIGMA-ALDRICH, Germany. N-(2-Mercaptopropionyl)-glycine (63794) was obtained from Fluka (SIGMA-ALDRICH, Germany).

Cells were cultured in DMEM-high glucose, supplemented with 10% fetal bovine serum, L-glutamine (0.584 g/L), penicillin (100 U/ml) and streptomycin (100 μg/ml). Cells were maintained at 37˚C, in a 5% CO_2 enriched, humidified air atmosphere. When 70-80% confluent, cells were trypsinized and seeded in 24-well culture dishes at an initial density of 35,000 cells/well, with 1 ml of medium/well. Cells were allowed to attach for 24 h and then were washed twice with 37˚C PBS, and medium was replaced with DMEM without phenol red, supplemented with D-(+) glucose (4.5 g/L), L-glutamine

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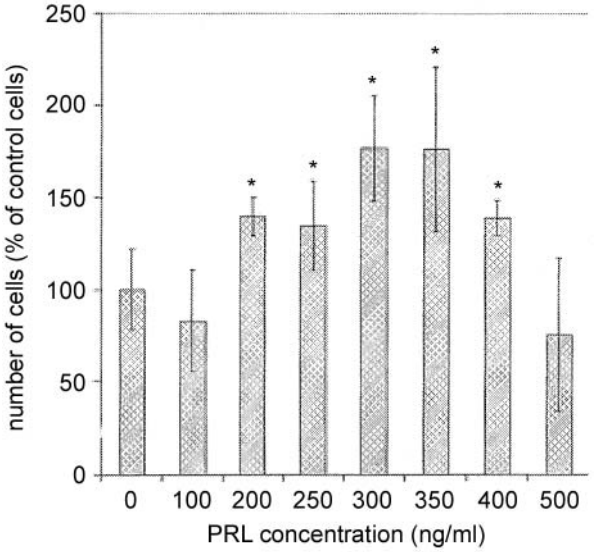

Figure 1. *Effect of PRL on growth of MCF-7 cells after 72 h. Data shown are means. Bars are ±SE for triplicate experiments. *Significantly different compared to control ($p<0.05$).*

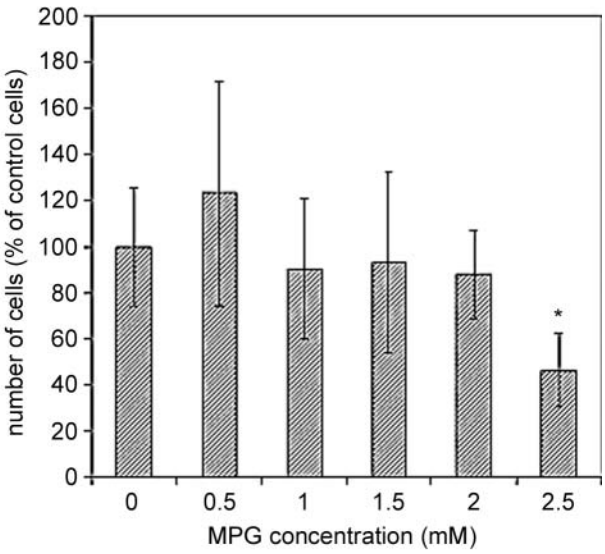

Figure 2. *Effect of MPG on growth of MCF-7 cells after 72 h. Data shown are means. Bars are ±SE for triplicate experiments. *Significantly different compared to control ($p<0.05$).*

(0.584 g/L), transfering (10 μg/ml), insulin (5 μM), human serum albumin (0.1%). Cells were allowed to grow for 48 h in this serum-free medium before the test substances (PRL and/or MPG) were added, in order to minimize the carry-over of fetal bovine serum lactogens. This serum-free medium was renewed using the following treatments: (i) PRL, at concentrations from 100-500 ng/ml, was added in order to estimate its dose-dependent effect on the proliferation rate of MCF-7 human breast cancer cells. (ii) MPG at concentrations from 0.5-2.5 mM was added and its dose-dependent effect on MCF-7 cell proliferation was also estimated. (iii) Prolactin, at a concentration found to enhance MCF-7 proliferation, from (i) above, and MPG, at concentrations found to not affect MCF-7 cell proliferation, from (ii) above, were added simultaneously to cell cultures as above, and the effects of MPG on PRL-induced proliferation on MCF-7 breast cancer lines were estimated.

In all culture modes, after 72 h cells were trypsinized, incubated with tryptan blue dye, and viable cells were counted using a hemocytometer. Results were expressed as the percentage of that of the control. In each experiment four separate wells were used for each concentration and all the experiments were performed at least three times.

Results of the above treatments of MCF-7 cells were compared to the controls, *i.e.* the proliferation rate of the MCF-7 cells, in serum free and phenol red free medium.

Statistical analysis. The significance of any differences between the two groups was determined using Student's *t*-test with $p<0.05$ considered as statistically significant.

Results

The effect of PRL on MCF-7 cell proliferation. PRL was added to the medium at concentrations ranging from 100-500 ng/ml. As shown in Figure 1, cell proliferation was enhanced with PRL stimulation, especially at concentrations above 200 ng/ml, with a maximum effect observed at 300-350 ng/ml. The effect was statistically significant up to and including 400 ng/ml when compared to the control group ($p<0.05$). At higher concentrations the effect of PRL on cell proliferation was diminished.

The effect of MPG on MCF-7 cell proliferation. At 0.5 mM, MPG has a slight enhancing effect on cell proliferation, which was not statistically significant. At 2.5 mM it caused a decrease in cell proliferation, which was statistically significant. MPG at concentrations between 1.5-2 mM did not cause any statistically significant reduction in cell number compared to the control. (Figure 2).

The effect of MPG on PRL-induced MCF-7 cell proliferation. We tested the effect of MPG on PRL-induced MCF-7 cell proliferation when both substances were added simultaneously to the culture medium. MPG at concentrations between 1.5-2 mM did antagonize the proliferating effect of prolactin on cells, compared to PRL alone (300 ng/ml), (Figure 3).

Discussion

Antibodies against PRL inhibit the proliferation of breast cancer cell lines (5), while PRL receptor antagonists antagonize the effect of PRL on cell proliferation (8).

In our study, PRL at concentrations up to 100 ng/ml has no effect on growth of MCF-7 human breast cancer cell proliferation after 72 h, which is in agreement with the work

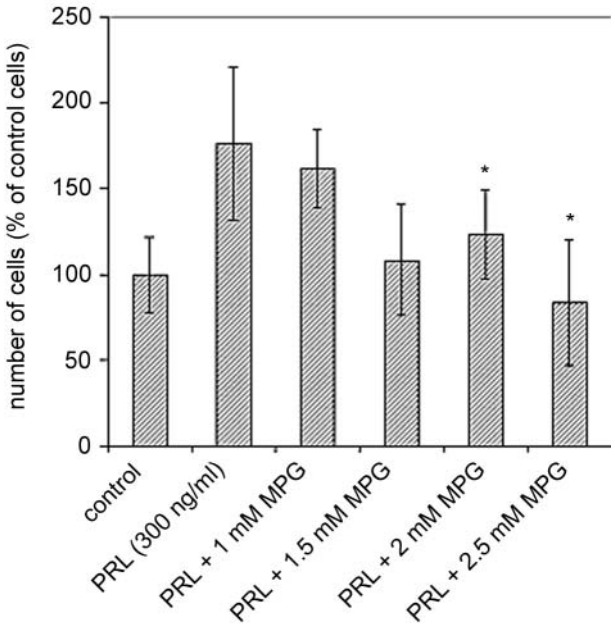

Figure 3. *Effect of prolactin on growth of MCF-7 cells after 72 h, in the presence of MPG. Data shown are means. Bars are ±SE for triplicate experiments. *Significantly different compared to prolactin alone ($p<0.05$).*

of Perks *et al.* (12). PRL at concentrations of 200 ng/ml - 350 ng/ml enhanced the growth of MCF-7 cells after 72 h, while at higher concentrations its action was diminished. The decrease in response at concentrations of 500 ng/ml and higher has been reported by others (7, 13). The proliferative action of prolactin on MCF-7 cell lines has been reported with a significant role of cyclin D1 (14, 15).

The fact that PRL in doses above 350 ng/ml had lower action on MCF-7 cell proliferation after 72 h, could be explained by decreased receptor homodimerization (16-18) and may also be related to down-regulation of the receptors by prolactin (7).

MPG caused suppression of lactation in a group of women in the puerperium (11). MPG in combination with antioxidants-anticarcinogens had a preventive and therapeutic effect on experimentally induced malignant tumours in Wistar rats (10). In regenerating rat liver a decrease of DNA synthesis due to MPG was reported (19). MPG was also reported to protect against radiation hazards (20).

It is well known that oxidative stress and reactive oxygen species (ROS) contribute to the pathogenesis of cancer. Antioxidants, like thiols, have been studied clinically and experimentally in the prevention of cancer disease.

MPG is a sulfhydryl compound (thiol) which possesses antioxidant properties.

This is the first time the influence of MPG on MCF-7 human breast cancer cell proliferation has been tested. The exact mechanism is not known yet. Furthermore, when MPG at concentrations between 1.5-2 mM, that do not affect the proliferation of MCF -7 human breast cancer cells, was used simultaneously with prolactin at a concentration of 300 ng/ml which accelerated proliferation rates, the stimulating effect of PRL on MCF-7 cell proliferation was significantly inhibited. It thus seems that MPG exerts an inhibitory action on PRL-induced MCF-7 cell proliferation. The later is exerted at concentrations lower than 2 mM, which are non-toxic for MCF-7 cells, which indicates that either MPG reacted with PRL in the culture medium, preventing PRL receptor dimerization or it affected DNA synthesis or PRL receptors.

In conclusion, MPG at pharmaceutical doses exerts antiproliferating effects on human breast cancer cells. It also antagonizes the mitogenic effect of PRL on the MCF-7 human breast cancer cell line, at non-toxic concentrations.

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