Iraqi Journal of Cancer and Medical Genetics

Cytotoxicity of purified methionine γ- lyase produced by *Pseudomonas putida* on several cell lines

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Abstract:

The cytotoxicity of different concentrations of purified methionine γ- lyase from Pseudomonas putida on cancer cell lines (RD, AMN3 and AMGM) at 96 hr was studied. The bacterial enzyme with concentration 1000µg/ml was revealed highly cytotoxicity against cancer cell lines in comparison with other concentrations whereas slight cytotoxicity was observed on normal cell (REF).

Key words: *Methionine γ- lyase, Pseudomonas putida, Cytotoxicity .*

Introduction:

Many human malignant cell lines and primary tumours have absolute requirements for L-methionine (1). Under normal circumstances methionine comes from dietary proteins, most normal tissues can also synthesize methionine from either homocysteine or methylthioadenosine (2). Upon L-methionine depletion, L-methionine-dependent cancer cells are not able to divide and became arrested in the late S/G2 phase of the cell cycle (3).Methionine cleaving enzyme (methionine γ- lyase) has been found to be an effective antitumour agent in vitro as well as in vivo (4). Methionine γ-lyase is a pyridoxal 5-phosphate dependent enzyme that catalyzes the α, γ- elimination of L-methionine to α-ketobutyrate, methanethiol and ammonia (5).

Materials and Methods:

Methionine γ- lyase (MGL)

Methionine γ- lyase was extracted from Pseudomonas putida by sonication and purified by DEAE-Sephadex ion exchange chromatography and Sephacryl S-300 gel filtration as previously described (6).

Cells and cell culture

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Rhabdomyosarcoma (RD) cell line at passage 65, Ahmed-Mohammed-Nahi-2003(AMN3) cell line at passage 180, AMGM (Ahmed – Majeed-Glioblastoma-Multiform) cell line at passage 65 and normal Rat Embryo Fibroblast (REF) cell line at passage 56, kindly provided by Iraqi center for cancer and medical genetics research (ICCMGR) were cultured in PRMI 1640 medium supplement with 10% heat inactivated fetal calf serum and incubated at 37 ºC with 5% CO2.

Examination of cytotoxicity of methionine γ- lyase

Cytotoxicity was determined with the crystal violet stain as previously described (7). In briefly , 1x105 cell/ml was seeded onto 96-well culture plates in 200 µl RPMI 1640 medium and incubated until the cell reached confluent monolayer(vary according to the kind of cell-line). After incubation period the medium was removed and 200 µl of various concentrations (1000, 500,250 and 125 µg/ml) from methionine γ- lyase were added to the plate by using 3- well replicates for each concentration. The plates were incubated at 37ºC for 96 hr. The control (cancer cell lines without treatment with enzyme) (3-well replicates) was treated with 200 µl of serum free medium and incubated at 37ºC for 96 hr. At the end of incubation period, the enzyme and medium was removed from plate and washed with PBS to remove unattached (dead) cells. Two hundred µl of crystal violet was added to each well and left for 20 min at 37ºC.The stain was removed by washing with tap water several times, and the plates were left to dry. After drying, each plate was read by using ELISA microplate spectrophotometer at 492nm wave length. The percentages of Inhibitory Rate (IR %) were estimated (8).

Statistical analysis

Experimental data were analyzed using ANOVA at probability level P< 0.05.

Results

Cytotoxicity of purified methionine γ- lyase on several cell lines

Cytotoxic activity of methionine γ- lyase on cell lines was increased gradually with increasing concentration. The results showed that all concentrations of methionine γ- lyase had a slight effect on the viability of normal cell line (REF) after 96 hr of exposure time (Figure 1), the inhibition rate (IR %) at 1000 µg/ml was 37.49 % with significant difference at P≤ 0.05 in comparison with control while the inhibition rate (IR%) at 500, 250 and 125 µg/ml was 21.72% ,5.05 % and 4.46% respectively with no significant difference P>0.05 in comparison with control

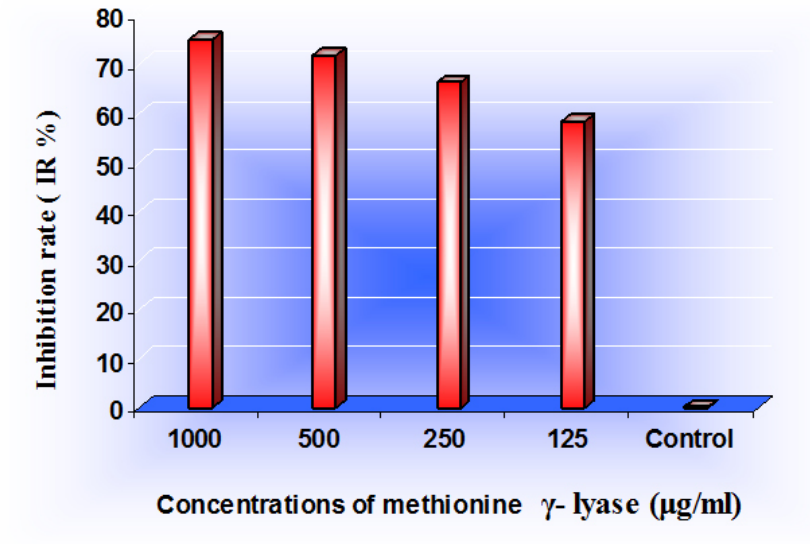

Figure 2: Section of ovary for mouse treated with 17β-estradiol show ill defined ova seen inside the secondary follicle ( ) and primary follicle ( ) (H&E 200 X).

Whereas increasing the concentration of methionine γ-lyase result in an increasing the inhibitory effects of this enzyme on the viability of RD cells ; thus the concentration 1000, 500,250,125 µg/ml of this enzyme caused a significant inhibition rate 75.05%, 72%, 66, 40% and 58.26% respectively with high significant differences at P≤ 0.05 in comparison with control.(Figure 2)

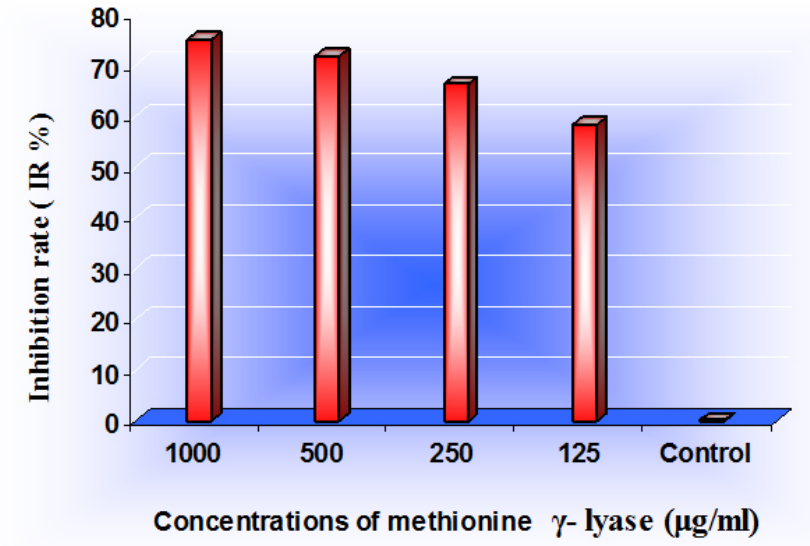

Figure 2: Cytotoxic activity of different concentrations of purified methionine γ- lyase produced by *P.putida* on RD cells.

The effect of AMN-3 cells with purified methionine γ-lyase produced by P.putida was shown in figure (3) .The methionine γ- lyase showed a significant toxic effect started from the concentration 125 µg/ml till the concentration 1000 µg/ml with high significant differences at P≤ 0.05 in comparison with control. The highest concentration of methionine γ-lyase exhibited inhibition rate 61.51% against AMN-3 cells .

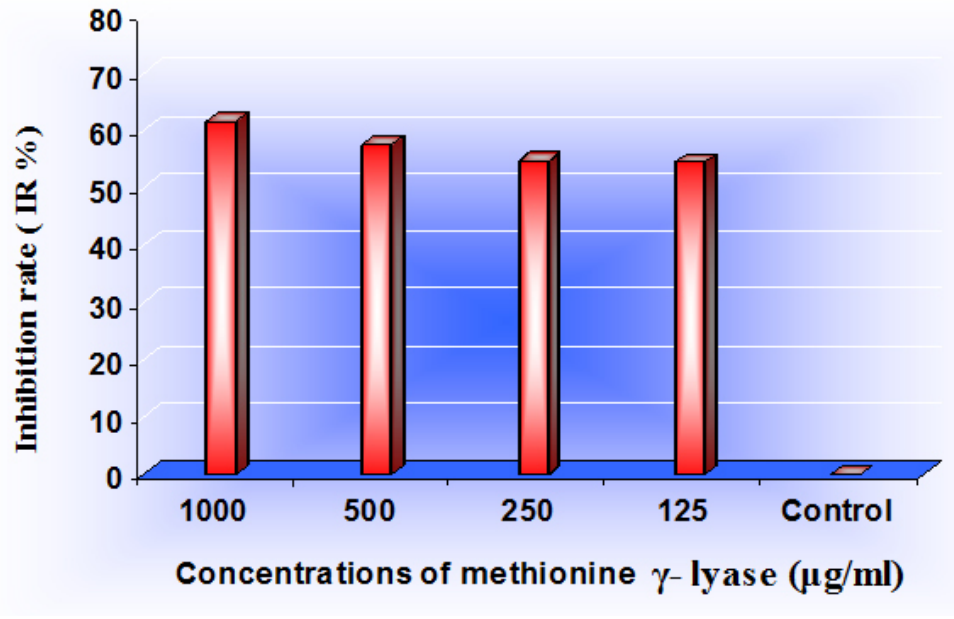

Figure 3: Cytotoxic activity of different concentrations of purified methionine γ- lyase produced by *P.putida* on AMN-3 cells

The cytotoxic activity of purified methionine γ- lyase on AMGM cells was examined. The enzyme had inhibitory effect against AMGM cells at low concentration; the IR% was increased with increasing concentration of enzyme with high significant differences at P ≤ 0.05 in comparison with control as shown in figure (4).

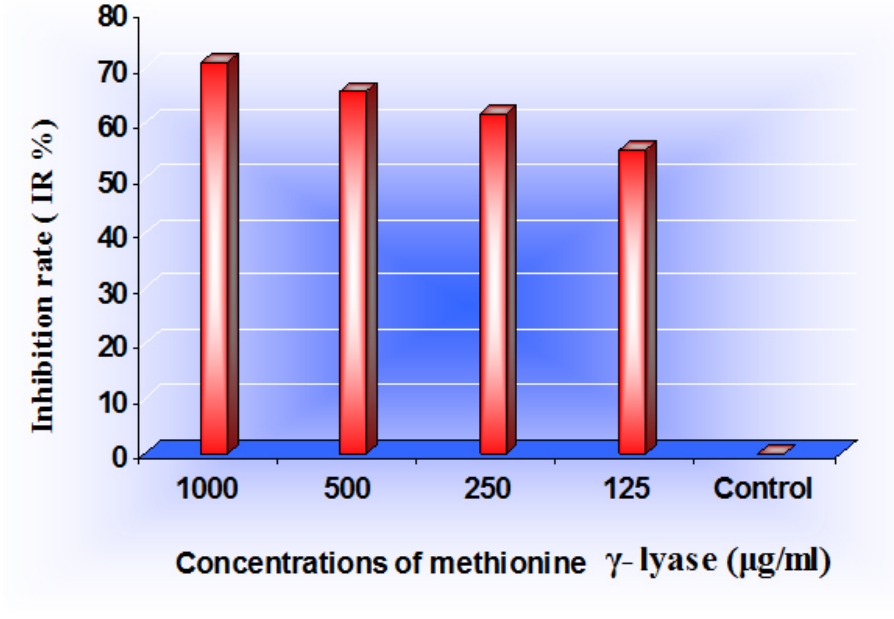

Figure 4: Cytotoxic activity of different concentrations of purified methionine γ- lyase produced *P. putida* on AMGM cells.

Discussion:

The cytotoxicity results indicated that all concentrations of methionine γ- lyase possess a cytotoxic effect toward cancer cell lines but the severity of cytotoxity of concentration was varied from one concentration to another and from one cell line to another. The concentration 1000µg/ml of enzyme have efficient toxicity against cancer cell lines RD, AMGM and AMN3 cell lines respectively thus this result can be concluded that methionine γ- lyase have antitumour activity against cancer cells. Methionine is the chief methyl donor for the methylation of DNA, RNA, protein and a large number of other biological substances (9).Methionine-dependent cells demonstrated reduced of S-adenosylmethionine (SAM) the universal donor methyl group donor and the growth ceases in methionine starvation due to the accumulation of methyl deficient nucleic acid so these condition would be caused late S/G2 arrest of tumour cells (10), also methionine is the precursor of glutathione that reduced reactive oxygen species thereby protecting cells from oxidative stress (11).

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السمية الخلوية لانزيم methionine γ- lyase المنتج من بكتريا Pseudomonas putida بعض على الخطوط الخلوية

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الخلاصة:

درست التأثيرات السمية لتراكيز مختلفة من أنزيم methionine γ- lyase المنتج من بكتريا *Pseudomonas putida* في الخلايا السرطانية من نوع REF,RD,AMGM and AMN3 . أظهر التركيز 1000 مايكرو غرام/ مل فعاليه سميه عاليه في الخلايا السرطانية مقارنة مع بقيه التراكيز بينما كانت التأثيرات السميه لهذا الانزيم طفيفة على الخلايا الطبيعية (RFE).