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INHIBITION OF PROTEINASE 3 (PR3) BY SURAMIN AND FETAL CALF SERUM (FCS): EFFECT OF PR3 AND SURAMIN ON CHINESE HAMSTER OVARY CELLS (CHO-CELLS)

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Background: Proteinase 3 (PR3) is a lysosomal protease that is stored in azurophilic granules neutrophilic granulocytes and monocytes. A number of inhibitors for this proteinase are reported. Comprehensive studies on the inhibitory effect of suramin and heat treated fetal calf serum (ΔFCS) on PR3 have not been reported. It has been reported that PR3 is able to destroy the cytoskeletal integral proteins, but we have not find any reports which showed the effect of this protease on Chinese hamster ovary cells (CHO-cells) in culture medium. Suramin has proven to be useful as an antitumor drug, but there was not any report on the effect of suramin on CHO-cells.
Methods: The effects of various concentrations of ΔFCS (from 0.5% up to 10%) and suramin (from 0.8 μM up to 100 μM) on PR3 and different concentrations of suramin (from 0.8 μM up to 1000 μM) on CHO-cells were investigated. Data analysis were performed by, Kolmogorov-Smirnov test, ANOVA test and Tukey HSD post tests.
Results: Results showed that ΔFCS and suramin have an inhibitory effect on PR3 and these effects increased with increasing the concentration significantly ($p < 0.01$). PR3 with the concentration of 2.2 Unit/ml has no effect on CHO-cells. Although suramin with the concentration of less than 125 μM cell growth retarded for only a few hours, but with the concentration of 125 to 250 μM inhibit the cell growth for a week, and after that cells gain normal growth gradually. Suramin with concentration of more than 500 μM inhibited the cell growth completely.
Conclusions: Although suramin reversibly inhibit the PR3 activity but in concentration of less than 250 μM it had no long-term effect on CHO-cells. Therefore it can be used in the investigation of proteases. There were unknown components in ΔFCS, which cause the inhibition of PR3 activity. This finding is very important in PR3 production in culture medium. However CHO-cells are resistant to PR3 and suramin in low concentration.

Keywords: Proteinase 3, suramin, FCS, CHO-cells.

INTRODUCTION

Proteinase 3 (PR3) is a human neutrophil serine protease that is a target of classical antineutrophil cytoplasmic autoantibodies (c-ANCA) in sera from patients suffering from autoimmune systemic vasculitis conditions such as Wegener's Granulomatosis (Rao *et al*. 1996; Rao *et al*.1991). This enzyme is a lyzosomal proteinase stored in azurophilic granules, neutrophilic granulocytes and monocytes (Rao *et al*.1991). This enzyme is able to destroy the cytoscletal integral proteins (Rao *et al*. 1996; Leid *et al*.1993). The natural inhibitor of PR3 in human body is found to be α-1-antitrypsin (Jenne,1994). In addition a number of synthetic inhibitors for this proteinase have been reported (Kam *et al*.1992). The inhibitory effect of suramin as an antitumor drug has been described by prevention the growth factors binding to the cancerous cell surface (Voogd *et al*., 1993). Cadene *et al* (1997) explained reversible inhibitory effect of suramin on PR3 activity. Although Ballieux *et al*. (1994) investigated the inhibitory effect of heat treated fetal calf serum (ΔFCS) on PR3 binding to human umblical vein endothelical cells (HUVEC) , but there was no report on the effect of ΔFCS on PR3 which may be important on the culture condition specially when PR3 is being cloned and expressed in eukaryotic cells such as Chinese Hamster Ovary cells (CHO-cells). The aim of this study was to evaluate the following: 1) Can ΔFCS inhibit the proteinase activity of PR3 ? 2) How is inhibitory effect of different concentration of suramin on PR3 activity ? 3) What is the effect of PR3 as a proteinase on growth and proliferation of CHO-cells ? 4) In what manner CHO-cells behavior is in presence of different concentration of suramin ? In order to find possible answers to the above questions, we determined the inhibitory effect of different concentration of suramin and ΔFCS on PR3 activity and different concentration of suramin on the growth and

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proliferation of CHO-cells in culture medium. We simultaneously measured the amount of suramin required to inhibit PR3 in such a way that minimum effect on CHO-cells are observed.

MATERIALS

Suramin was purchased from Bayer Co. Ltd Germany. FCS and RPMI1640 were obtained from Gibco BRL. Neutrophil purified PR3 was gift by Prof. M. R. Daha from Leiden University of Medical Sciences, the Netherlands. The synthetic substrate N-t-Boc-L-alanine p-nitrophenylester, dimethyl sulphonic acid (DMSO), dinitrophenol and other analytical reagents were purchased from Merck, Germany. Plastic vessels were obtained from Nunc, Denmark.

METHODS

Inhibition of PR3 using ΔFCS

The inhibitory effect of ΔFCS (FCS was incubated in 60^{o}C for 30 minutes) on the proteinase activity of PR3 in presence of 1mM of synthetic substrate N-t-Boc-L-alanine p-nitrophenylester in 50 mM phosphate saline buffer (PBS) pH 7.2 containing 2% DMSO after 3 hours at 37 ^{o}C was measured.

Inhibition of PR3 using suramin

A serial dilution of suramin from 0.8 up to 100 μM was prepared in PBS (50 mM, pH 7.2) and was added to wells containing fixed concentration of PR3 (2.2 Unit/ml).

Effect of PR3 on proliferation of CHO-cells

Cells were grown in 24 wells plate. A serial dilution of PR3 from 1.37x 10^{-2} up to 2.2 Unit/ml was added to each well in presence of 0.5% ΔFCS. Cells were then checked for viability at 1, 8, 24 and 48 hours.

Effect of suramin on proliferation of CHO-cells

CHO-cells were added to 24 well plates such that 1000 cells were added to each well. Various concentrations of suramin (0.8 up to 1000 μM) prepared in PBS (50 mM, pH 7.2) were added to each well. The cells were studied after 16 hours and every day up to one week.

Data analysis were performed by, Kolmogorov-Smirnov test, ANOVA test and Tukey HSD post tests.

RESULTS

The inhibitory effect of ΔFCS on PR3 showed that ΔFCS could inhibit the proteinase activity of PR3. Furthermore it was observed that degree of inhibitory effect increased in such way that ΔFCS of 0.5% concentration exerted minimum inhibitory effect on PR3 activity (fig. 1). In Kolmogorov-Smirnov test data distribution was normal and according to ANOVA test and Tukey HSD post tests, the effect of all concentrations of ΔFCS on PR3 activity was found significant ($p < 0.01$). When suramin was added in various concentrations it was found that a concentration of 100 μM could inhibit more than 72% of proteinase activity of PR3 (fig. 2). Kolmogorov-Smirnov test, ANOVA test and Tukey HSD post tests, for the effect of all concentrations of suramin on PR3 activity was also found significant ($p < 0.01$) as ΔFCS. The results obtained for the effect of PR3 on CHO-cells in presence of 0.5% ΔFCS was shown that increasing concentration of PR3 in presence of 0.5% ΔFCS had no adverse effect on the viability and proliferate state of CHO-cells. Finally in table 1 the results of the effect of suramin on CHO-cells are presented. The results showed that in concentrations of higher than 1.56 μM of suramin in the growth rate of CHO-cells decreased and the growth retardation increased as the concentration of suramin increased such that in concentrations of 500 μM to 1000 μM the growth of the cells was retarded completely and cells were not able to anchor to the plastic surface (table 1).

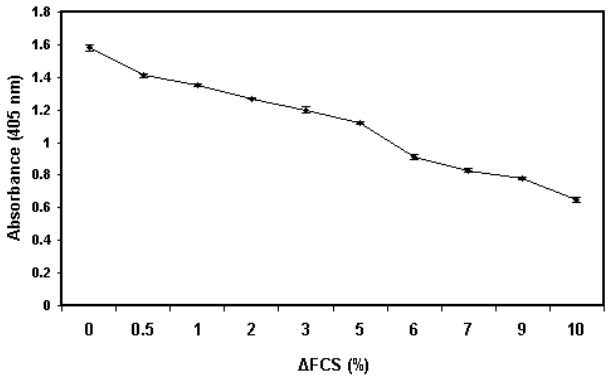

Fig. 1. Inhibition of PR3 using different amount of ΔFCS. Each data point represents the mean±SEM of 5 consecutive experiments.

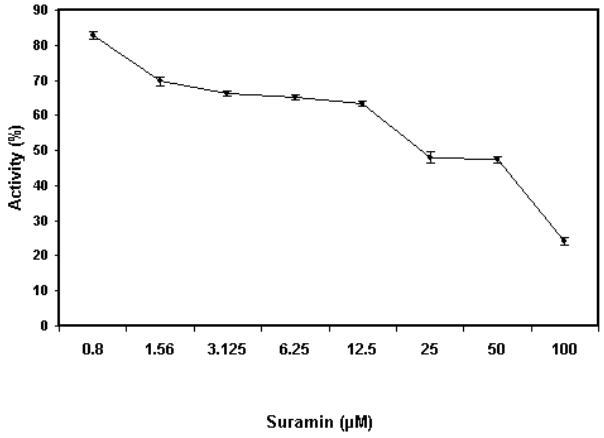

Fig. 2. Inhibition of PR3 using suramin from 0.8 up to 100 μM. Each data point represents the mean ± SEM of 5 consecutive experiments.

Table 1
Effect of different concentrations of suramin on the CHO-cells' growth rate, viability and proliferative state

Suramin concentration	Growth rate, viability and proliferate activity of CHO-cells		
	Up to 16	16 hour up to 7 days	After 7 days
≥ 500 μ M	- - - -	- - - -	- - - -
250 μ M	- - - -	- - - -	+ + - -
50 up to 125 μ M	- - - +	- - + +	+ + + +
0.8 up to 50 μ M	- - + +	+ + + +	+ + + +

- - - - = The cell growth, viability and proliferate activity of completely blocked.
- - - + = The cell growth, viability and proliferate activity blocked.
- - + + = The cell growth, viability and proliferate activity partially blocked.
- + + + = The cell growth, viability and proliferate activity affected very little.
+ + + + = There was no effect on cell growth, viability and proliferate activity

DISCUSSION

The results of these experiments showed that both suramin and ΔFCS in specific concentrations could inhibit proteinase activity of PR3. On the other hand PR3 in lower concentrations did not show any adverse effect on viability and proliferate activity of CHO-cells. However higher concentrations of suramin (higher than 250 μM) adversely affected the growth and proliferation of CHO-cells and long treatment of the cells in medium containing higher quantities of suramin caused a total destruction of the cells. Bollieux *et al*. (1994) reported that ΔFCS could inhibit the binding of PR3 to HUVEC cells. However our experiments focused on inhibition of PR3 proteinase activity using specific concentration of ΔFCS. These information will have important implications specially when large scale production and purification of recombinant PR3 is required. Previous studies on the effect of suramin on PR3 inhibition used lower concentration of chemical for inhibition (Cadene *et al*., 1997). We used higher concentration of suramin (higher than 12 up to 100 μM) which showed that increasing suramin concentration will effect on PR3 activity in proper manners and at a specific concentration it was possible to obtain the highest inhibition without much proteinase activity and cell destruction. Many reports discussed the adverse and destructive effect of PR3 on cytoskeletal proteins and cell adhesion molecules (Leid *et al*., 1993; Rao *et al*., 1991). However our results indicated that PR3 in concentrations used in this study had no adverse effect on proliferation of CHO-cells. Another point addressed in this work was the effect of suramin on CHO-cells. For this porpuse different concentrations of suramin were used the result of which showed that although a concentration of less than 250 μM affected the cells in first hours, however cells were adapted and recovered slowly. Althogh this adverse effect never reverted when higher concentration of suramin was used in culture medium. Finally it remains to be investigated that which component of ΔFCS does the inhibition of PR3 and whether this cell line would be resistance towards other proteinases. Another question would be why CHO-cells could resist higher concentration of suramin as compared to other cell lines. All these need more investigations.

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