RESEARCH PAPER

Accepted 19 June 2002
Revised 31 May 2002
Received 31 July 2001
Indian J. Pharm. Sci., 2002, 64(4): 386-388

Influence of Light on the Production of Urokinase from Mesangio Proliferative Glomerulo Nephritis Patient's Kidney Cells

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The effect of light of different coloured filters (blue, violet, green, yellow, pink and red) with various λmax (640, 635, 630, 457, 570 and 567 nm) was studied on the secretion of urokinase from mesangio proliferative glomerulo nephritis patient's kidney cells. The cells were grown on Dulbecco's medium. The maximum secretion of urokinase was observed in the presence of blue coloured filter (152.3 Plough units, control 80.42 Plough units) with an increase of 89.38% over control.

Urokinase is an endogenous enzyme which is involved in many physiological processes. It is a 54 kDa serine protease[1-3], which is used in the treatment of many cardiac disorders. It is used in the treatment of pulmonary embolism, myocardial infarction, deep vein thrombosis and many other disorders requiring dissolution of blood clots[4]. The urokinase receptor was found on the surface of monocytes and many other cell types[4]. Urokinase is prepared from cultures of human embryonic kidney cells[5,6]. It is secreted from cells as a single chain proenzyme (SCU-PA) from which the active two chain enzymatic plasminogen activator (TCU-PA) is derived by proteolysis[7,8]. The urokinase which is used clinically is a TCU-PA type[9]. It functions as a thrombolytic, which specifically cleaves the zymogen, plasminogen at the Arg-Val bond to form the active enzyme plasmin. Plasmin degrades the fibrin polymers of blood clots[10-14].

It was observed that the light intensity, spectral quality and the duration of exposure influence the production of plant or other biological metabolites[15]. There are no reports available in the literature about the effect of light on the production of urokinase. An attempt has been made to study the effect of normal light and different spectral composite lights (blue, violet, green, yellow, pink and red) on the production of urokinase from mesangio proliferative glomerulo nephritis [MPGN] patient kidney cells.

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MATERIALS AND METHODS

Urokinase, fibrinogen and thrombin were obtained from Sigma Chemical Co., St. Louis, Mo, USA. Dulbecco's medium was procured from HIMEDIA, Mumbai. All other chemicals used were of analytical grade obtained from various chemicals suppliers.

Isolation of cell line:

Biopsy tissues of patients with glomerulonephritic conditions were tested for the urokinase production by fibrin plate method[16]. The biopsy tissues of patients with the following disease conditions were studied: mesangio proliferative glomerulo nephritis (MPGN), focal segmental glomerulo nephritis (FSGN), diffuse proliferative glomerulo nephritis (DPGN), minimal change glomerulo nephritis (MCGN), necotizing glomerulo nephritis (NGN) and cresentic glomerulo nephritis (CGN). The tissues obtained from MPGN patients showed a maximum urokinase production. Fresh biopsy tissue from a MPGN patient was taken and trypsinized. [125 mg/50ml phosphate buffered saline (PBS) with sodium citrate 3 mg/ml pH 7.4]. The trypsinized cell suspension was plated on Dulbecco's medium (HIMEDIA, Mumbai) with 2% agar and incubated at 37°.

After 1 mo, colonies were selected and isolated based

on morphological differences. The colonies were inoculated into Dulbecco's medium, incubated at 37° in a shaker incubator as suspension cultures with 70 % air space.The colonies were tested for their urokinase production capabilities. One of the colonies, which showed maximum urokinase production was designated as AUB 101 and used for urokinase production studies. The Dulbecco's medium was used as the production medium.

Effect of light of different colored filters on the secretion of urokinase:

To study the effect of light of different spectral colour region with various λmax on the production of urokinase, different transparent coloured papers (blue, violet, green, yellow, pink and red) of same thickness with various λmax (640, 635, 457, 570, 567 nm, respectively) were used as a filters. The above papers were wrapped around the 100 ml EM flasks, such that it covers the flask completely. A plain flask without coloured paper was taken as a control.

To each flask, 25 ml of Dulbecco's medium was added and sterilized. One millilitre of AUB 101 cell culture from the stock was inoculated into 10 ml of Dulbecco's medium and incubated at 37° for 8 d, which served as an inoculum. A 10% level of inoculum was added to each flask and kept on a rotary shaker (220 rpm), at 30°, such that the flasks were exposed to the regular white light. The samples were collected regularly at 8 d interval for 32 d. The activity of urokinase was determined by the fibrin plate method. The cell count was also determined by haemocytometer.

Assay of urokinase:

The fibrin plate method was used for the estimation of urokinase activity[15]. A thin and stable fibrin clot was obtained by mixing of 0.1 ml of thrombin solution (100 NH units /ml), 0.1 ml of fibrinogen solution (5 mg/ml) and 0.6 ml of phosphate buffer (pH 7.5). For the assay, discs with 5 mm diameter were prepared from Whatman No. 1 filter paper and sterilized in a hot air oven. The discs were dipped in the urokinase solution for 15 sec and then placed on the surface of the clot in the plate. The plates were incubated at 37° for 6 h and the area of lytic zones were measured with the help of planimeter. The concentration of urokinase was calculated from the standard graph. The cell count was determined by counting in haemocytometer[17]. The activity of urokinase was expressed in Plough units/10x10^6 cells/ml. All the experiments were conducted in duplicate and their average results were recorded.

RESULTS AND DISCUSSION

The production of urokinase in the presence of coloured filters with various λmax was studied. The maximum activity of urokinase was obtained on the d 24 and the results are tabulated in Table 1. The percentage of increase in the production of urokinase over control was calculated and the results are shown in Table 1.

From this study it is clear that the activity of urokinase produced by control was 80.4 Plough units. The maximum urokinase production was obtained in the presence of blue coloured filter (λmax of 640 nm) with 152.3 plough units (89.38% increase over control), followed by violet filter (λmax 635 nm) with 141.6 Plough units (77.3% increase over control). The lowest production of urokinase was obtained in the presence of red coloured filter (λmax 567 nm) with 87.1 Plough units (only 8.3% increase over control).

Light showed to have effect on the secretion of biologi-

TABLE 1: PRODUCTION OF UROKINASE IN THE PRESENCE OF DIFFERENT COLOURED FILTERS (AFTER 24 D).

Type of filter	λmax (nm)	Cell count	pH	Production of urokinase/ml (Plough units)	Urokinase activity for 10x10^6 cells/ml (Plough units)	% increase over control
Blue	640	11.15x10^6	~7.5	169.8	152.3	89.4
Violet	635	13.15x10^6	~7.5	186.2	141.6	77.3
Green	630	12.85x10^6	~7.5	147.9	115.1	43.1
Yellow	457	12.15x10^6	~7.5	128.8	106.0	31.8
Pink	570	12.8x10^6	~7.5	117.5	91.9	14.1
Red	567	12.3x10^6	~7.0	107.2	87.1	8.3
Control	-	12.15x10^6	~7.5	97.7	80.4	-

cal metabolites. Jain *et al.*[15] observed that light had an effect on the synthesis of plant metabolites. They reported a maximum increase in the secretion of secondary metabolites in the presence of blue coloured light followed by green light. Our results are in agreement with their findings. From the present study, it is clear that the maximum activity of urokinase was obtained in the presence of the coloured light. Thus, the light quality and intensity may play an important role in the secretion of biological metabolites.

ACKNOWLEDGEMENTS

One of the authors (V. Saisha) thank CSIR, New Delhi for awarding CSIR-JRF and for the financial assistance provided.

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