Effect of Human Uraemic Plasma on Prostaglandin (PGA₂) Stimulated ^3H-Uridine Incorporation by Mouse Erythroid Cells

J LEVI, HANNA BESSLER, M DJALDETTI
Hasharon Hospital, Petah-Tiqva and Tel-Aviv University Medical School, Israel

Summary

Prostaglandin A₂ (PGA₂) is shown to stimulate RNA synthesis in 12-day embryonic mouse liver cells, thus expressing an erythropoietin-like effect. This effect was found to be dose-dependent. No significant difference was observed in the results obtained with patients' plasma before and after dialysis, with and without addition of PGA₂. The almost equal potentiation by PGA₂ of RNA synthesis in erythroid cells incubated with both normal and uraemic plasma favours a direct, and not an erythropoietin-mediated effect of this substance.

Introduction

Anaemia is almost invariable in patients with chronic renal failure (CRF) and frequently complicates the course of a wide spectrum of other chronic diseases. Reticulocytopenia, bone marrow histology, and ferrokinetic data suggest that the anaemia in chronic renal failure is due primarily to underproduction of red cells. This conclusion is further supported by the low erythropoietin (EP) level in CRF.

The presence of a serum factor in patients with CRF, toxic to erythropoiesis, has been suggested by inhibition of erythroblast maturation and decreased ^3H-thymidine incorporation into erythroblasts cultured with serum from uraemic patients, decreased response to EP in uraemic animals and the ability of chronic haemodialysis to increase haematocrit (Wallner et al, 1976).

During the past fifteen years an intensive study of the prostaglandins has indicated that they are omnipresent in mammalian tissues and have potent physio-
logical activities, probably through an as yet incompletely characterised interaction with adenyl cyclase. This mechanism was demonstrated in the endocrine, reproductive, nervous, digestive, haemostatic, respiratory, cardiovascular and renal systems.

It is evident from the numerous proved and suggested physiologic activities of the prostaglandins that they may be of widespread and profound clinical importance.

Schooley and Mahlmann (1971) reported that redistribution of the renal plasma flow may lead to increased EP production.

Later Fisher and co-workers (Fisher, 1972) found that PGA may exert direct action on the kidney to stimulate EP production.

Dukes et al (1973) have found that PGA₂ was the most effective in stimulation of mouse erythropoiesis, when compared with other prostaglandins. It was shown that PGA₂ stimulates erythropoiesis in mice, both by a direct effect on the EP target cell and by stimulation of erythropoietin production.

Dukes and co-workers demonstrated in 1971 (Dukes, 1971) that PGA potentiated the effect of EP on marrow cell culture. Since these studies suggested a relationship between PGA and EP a system was designed for examination of the activity of both hormones.

The aim of the present work was to examine the effect of PGA₂ on the RNA synthetic capacity of 12-day embryonic mouse liver erythroid cells. This model was found to be suitable for examination of the EP effect on ³H-uridine incorporation and RNA synthesis in these cells (Djaldetti et al, 1972).

MATERIAL AND METHODS

Samples

Control sera were taken from 10 normal volunteers. Renal failure sera were obtained from 10 stable patients on the chronic haemodialysis programme at Hasharon Hospital. Blood samples were taken pre- and post-dialysis. Most patients were anaemic with mean haematocrits of 26% (range 18–40%). Pre- and post-dialysis urea concentrations were 130–260 and 35–110 mg/100 ml, and creatinine concentrations 6.5–12.5 and 3.9–9 mg/100 ml respectively.

Tissue Culture

Erythroblasts from 12-day embryonic livers were obtained from C57 Bl/GJ mice. One ml of cell suspension was incubated with and without the patients' and controls' plasma as well as with and without PGA₂. In addition cell suspensions were incubated with urea and creatinine. At the end of the incubation ³H-uridine was added, and the incorporation of the ³H-uridine into RNA was detected as described previously.
Prostaglandins

Prostaglandin A₂ (u-25286) was supplied by Upjohn International Inc. Different amounts of PGA₂ were incubated with 10⁶ erythroid cells/ml of culture medium under the described conditions and the effect on the ³H-uridine uptake into RNA was measured.

RESULTS

The effect of PGA₂ on erythroid cell RNA synthesis was dose dependent and is shown in Figure 1. An inhibitory effect was observed upon increase of PGA₂

![Bar graph showing effect of different concentrations of PGA₂ on RNA synthesis by fetal liver erythroid cells. Vertical bars indicate ± standard error of the mean (SEM).](image)

Figure 1. Effect of different concentrations of PGA₂ on RNA synthesis by fetal liver erythroid cells. Vertical bars indicate ± standard error of the mean (SEM)

concentration. Figure 2 shows the effect of normal and uraemic plasma, without PGA₂. Control plasma caused a 31.0% ± 1.74 (SE) increase of RNA synthetic activity (P < 0.001), whereas uraemic plasma caused a 6.0% ± 3.8 (SE) decrease (P > 0.1). Addition of PGA₂ to control and uraemic plasma caused a 56.0% ± 6.6 (SE) (P < 0.001) and 60.0% ± 12.6 (SE) (P < 0.01) increase in
RNA synthetic activity, respectively (Figure 3). There was no significant difference in the results obtained with patients’ plasma before and after dialysis, with and without the addition of PGA₂.

Addition of urea or creatinine to the erythroid cell suspension did not affect synthetic activity.
CONCLUSION

It is shown that prostaglandin A₂ (PGA₂) stimulates RNA synthesis in 12-day embryonic mouse liver cells, thus demonstrating an erythropoietin-like effect.

The almost identical increase in RNA synthesis in erythroid cells incubated with normal and uraemic plasma obtained with PGA₂ is in favour of a direct, and not an erythropoietin-mediated effect of this substance on the erythroid cells. There was no significant difference in the results observed with patients’ plasma before and after dialysis, with and without addition of PGA₂.

The elevated urea and creatinine levels in the plasma of the uraemic patients could not be blamed for the lack of increase in RNA synthesis, since the same plasma obtained after haemodialysis with lower urea and creatinine levels failed to potentiate the erythroid cells. Furthermore, addition of urea and creatinine to the incubation mixture did not alter the erythroid cell capacity for RNA synthesis.

Since EP was shown to stimulate haemoglobin synthesis in cultured fetal mouse erythroid cells (Chui et al, 1971; Djaldetti et al, 1970) and since PGA₂ exhibits an erythropoietin-like effect, it is conceivable that PGA₂ will also stimulate haemoglobin synthesis, thus suggesting a new approach for the treatment of anaemia in uraemic patients.

References

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Open Discussion

BLUMBERG (Aarau) Did you study the effect of adding erythropoietin plus prostaglandin to your system?

LEVI There are a lot of publications on the subject of adding erythropoietin to this culture, but we have not used both in the same medium.

DANDONA (London) Does anyone know anything about PGA₂ levels in uraemic
plasma or in transplanted patients? I wondered whether the PGA₂, the renal
anaemia, and the erythrocytosis following transplantation could be correlated
on this basis.

LEVI As far as I know the prostaglandin level is normal in uraemic patients; the
only exception is renal failure due to pre-eclamptic toxaemia where some abnor-
mality in the concentration was found.