A 7700/131

SGAE Ber. No. 2895 BL-221/78 März 1978

Berichte der
Österreichischen Studiengesellschaft
für Atomenergie Ges. m. b. H.

Forschungszentrum Seibersdorf

THE INFLUENCE OF SOME PROSTAGLANDINS ON DNA SYNTHESIS AND DNA EXCISION REPAIR IN MOUSE SPLEEN CELLS 'IN VITRO'

WOLFGANG KLEIN
HANS ALTMANN
FRANZ KOCSIS
DIETMAR EGG
ROBERT GÜNTHER

SGAE Ber.No. 2895 BL- 221/78 März 1978

THE INFLUENCE OF SOME PROSTAGLANDINS ON DNA SYNTHESIS AND DNA EXCISION REPAIR IN MOUSE SPLEEN CELLS 'IN VITRO'

Wolfgang Klein
Hans Altmann
Franz Kocsis
Dietmar Egg *
Robert Günther *

Erscheint in "Prostaglandins"

Österreichische
Studiengesellschaft für Atomenergie
Ges.m.b.H.
Lenaugasse 10 A-1082 Wien

INSTITUT FÜR BIOLOGIE
Forschungszentrum Seibersdorf

* Universitätsklinik Innsbruck

SGAE Ber.No. 2895 BL- 221/78 März 1978

THE INFLUENCE OF SOME PROSTAGLANDINS ON DNA SYNTHESIS AND DNA EXCISION REPAIR IN MOUSE SPLEEN CELLS 'IN VITRO'

ABSTRACT

'In vitro' experiments were performed on mouse spleen cells to establish possible influences of some naturally occuring prostaglandins on DNA synthesis and DNA excision repair. The prostaglandins A_1, B_1, E_1, E_2 and $F_{2\alpha}$ were tested in concentrations of 10 pg, 5 ng and 2,5 /ug per ml cell suspension.

DNA synthesis was significantly increased by $PgF_{2\alpha}$ in all the three concentrations tested, while the other tested prostaglandins were essentially ineffective.

DNA excision repair was significantly inhibited by PgE_1 and PgE_2 at 5 ng/ml and at 2,5 /ug/ml but increased by $PgF_{2\alpha}$ in the two lower concentrations.

The rejoining of DNA-strand breaks after gamma-irradiation was slightly reduced by PgE_1, PgE_2 and $PgF_{2\alpha}$ at 2,5 /ug/ml.

Key words: PROSTAGLANDINS/SPLEEN CELLS/DNA SYNTHESIS/DNA EXCISON REPAIR/

DER EINFLUSS NATIVER PROSTAGLANDINE AUF DIE DNA-SYNTHESE UND DIE DNA-REPARATUR IN MAUSMILZZELLEN 'IN VITRO'

KURZFASSUNG

Eine mögliche Beeinflußung der DNA-Synthese und der DNA-Exzisionsreparatur durch natürlich vorkommende Prostaglandine wurde an Mausmilzzellen 'in vitro' untersucht. Dabei kamen die Prostaglandine A_1, B_1, E_1, E_2 und $F_{2\alpha}$ in Konzentrationen von 10 pg, 5 ng und 2,5 /ug/ml Zellsuspension zum Einsatz.

PgF_2 bewirkte in den drei untersuchten Konzentrationen eine signifikante Erhöhung der semikonservativen DNA-Synthese, während die

anderen Prostaglandine keine Wirkung zeigten.
Durch die Prostaglandine E_1 und E_2 kam es zu einer signifikanten Unterdrückung der DNA-Exzisionsreparatur bei 5 ng/ml und 2,5 µg/ml. Durch Prostaglandin $F_{2\alpha}$ in einer Konzentration von 10 pg/ml und 5 ng/ml ergab sich dagegen eine gesteigerte DNA-Exzisionsreparatur. Die Heilung von Gamma-Strahlen induzierten Strangbrüchen an der DNA der Zellen war durch PgE_1, PgE_2 und $PgF_{2\alpha}$ bei einer Konzentration von 2,5 µg/ml verzögert.

SCHLÜSSELWORTE: PROSTAGLANDINE/MILZZELLEN/DNA SYNTHESE/DNA EXZISIONS-REPARATUR/

THE INFLUENCE OF SOME PROSTAGLANDINS ON DNA SYNTHESIS AND DNA EXCISION REPAIR IN MOUSE SPLEEN CELLS 'IN VITRO'

Due to their manyfold pharmacological properties naturally occuring prostaglandins as well as their synthetic analogues have been under experimental clinical investigation for many years. It is obviously of increasing interest to extend the range of therapeutical application of these biologically highly active substances. However, until now, mainly the use in therapeutic abortion and labour induction has proved to be especially promising. Ever since zhe first successful abortions through intravenous infusions of $PgF_{2\alpha}$ (1) attempts have been made to minimize the often serious side effects. Thus, the systemic stress of organism could be reduced predominantly by better forms of application, e.g. vaginally, extraamniotically, intraamniotically (2,3,4), however only at the expense of an increased local stress of tissue.
Recently it has been shown that other naturally occuring substances like steroids may inhibit DNA synthesis and DNA excision repair (5,6). Similar effects have been reported for drugs even in therapeutical concentrations (7,8). Additionally to toxicological (9,10) and teratological (11) investigations this preliminary study is concerned with effects of some prostaglandins on DNA synthesis and DNA excision repair on mouse spleen cells 'in vitro'.

MATERIAL AND METHODS

Spleens of SPF (specified pathogen free) mice were carefully homogenized and the cell suspension was twice washed with Hanks medium (12). The cell content of the suspension used in all the experiments was

set to about 10^7 cells/ml. Prostaglandins A_1, B_1, E_1, E_2 and $F_{2\alpha}$ were used in concentrations of 10 pg, 5 ng and 2,5 /ug per ml cell suspension. To determine semiconservative DNA synthesis cell samples were preincubated for 60 min at 37°C, washed with Hanks medium and thereafter 5 /uCi ^{3}H-thymidine (NEN, spec.activity 50 Ci/mmole) per ml cell suspension was added. The incorporation of labeled thymidine into the DNA was stopped after 60 min incubation at 37°C by addition of ice cold perchloric acid to a final concentration of 6%. Unincorporated ^{3}H-thymidine was washed out and the DNA hydrolysed at 90°C. After centrifugation the DNA in the supernatant was quantitated by the diphenylamine reaction of Burton (13) and the radioactivity measured in a liquid scintillation counter.

For the estimation of DNA excision repair semiconservative DNA synthesis was depressed by hydroxyurea (10^{-2}m). After addition of hydroxyurea prostaglandin containing samples as well as control samples were preincubated for 30 min at 37°C followed by an irradiation with 4000 erg/mm^2 UV light (254 nm). The ^{3}H-thymidine incorporation was measured in the same way as described for DNA synthesis.

The rejoining of strand breaks after gamma-irradiation in the presence of the various prostaglandins (2,5 /ug/ml cell suspension) was investigated by gradient centrifugation in alkaline sucrose. For this purpose cells were prelabeled with 5 /uCi ^{3}H-thymidine per ml suspension, incubated for 60 min at 37°C and then irradiated with 30 krad in a 12 kCi ^{60}Co-source (0,94 Mrad/h). Immediately after irradiation (0 min) as well as after an incubation period of 30 min aliquots of samples were harvested and washed with Hanks medium containing 50 /ug inactive thymidine per ml. Cells were lysed with NaOH (0,25 n), the lysate applied on the top of a 5 - 20% alkaline sucrose gradient and centrifuged in a swing-out rotor at 180 000 g for 300 minutes. Equal fractions were collected automatically and the radioactivity counted in a liquid scintillation counter (14,15).

All experiments were carried out in quadruplicate and expressed as per cent of controls.

Statistical calculations were made by the Student's t-test.

RESULTS

Semiconservative DNA synthesis in the presence of various prostaglandins

in three different concentrations compared with control samples is graphically illustrated in Figure 1.

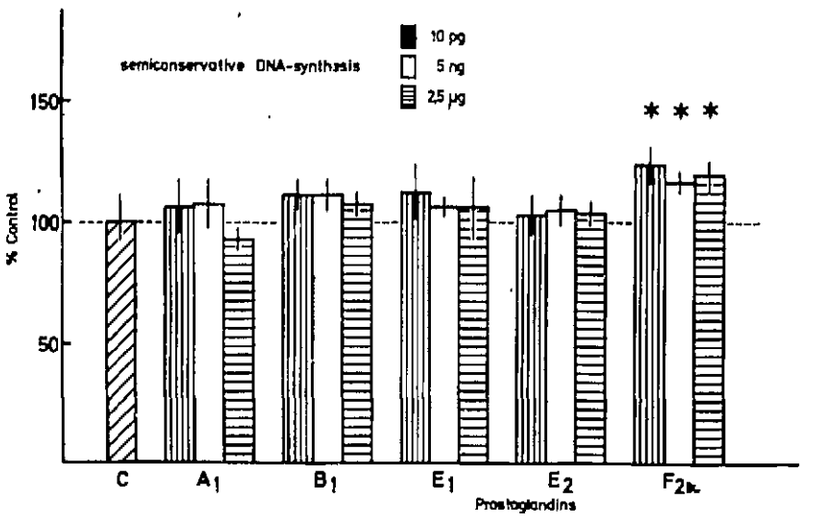

FIGURE 1: Semiconservative DNA synthesis in mouse spleen cells 'in vitro' in the presence of various amounts of prostaglandins (*P 0,001).

None of the prostaglandins tested showed an inhibitory effect, but $PgF_{2\alpha}$ significantly increased the incorporation rate of ^{3}H-thymidine into the DNA in all three tested concentrations (P 0,001).

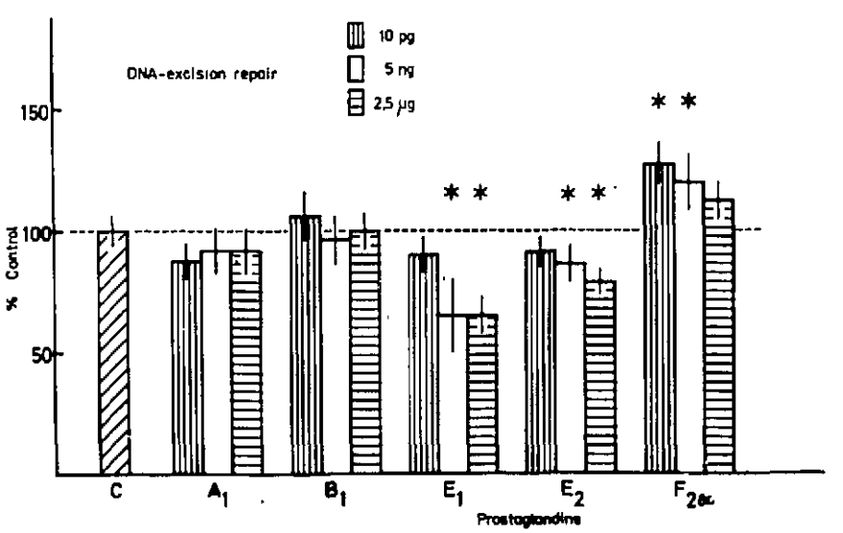

FIGURE 2: DNA excision repair in mouse spleen cells 'in vitro' after UV irradiation in the presence of various amounts of prostaglandins (* P 0,001).

The excision repair of prostaglandin containing samples compared with control samples is demonstrated in Figure 2. The ^{3}H-thymidine

incorporation into cell DNA of control samples after UV irradiation at suppressed semiconservative DNA synthesis by hydroxyurea is set as 100%. In the presence of $PgF_{2\alpha}$ at 10 pg/ml and 5 ng/ml the incorporation rate is significantly increased (P 0,001). PgA_1 and PgB_1 treated samples did not differ from controls.

The sedimentation profiles of DNA after gamma-irradiation and gradient centrifugation in alkaline sucrose are shown in Figure 3. Immediately after centrifugation (0 min) the DNA maximum was found in fraction 20 and after a 30 minutes incubation period in fraction 17 under control conditions. In the presence of PgE_1, PgE_2 and $PgF_{2\alpha}$ the DNA maxima after 30 minutes incubation was found in fraction 18.

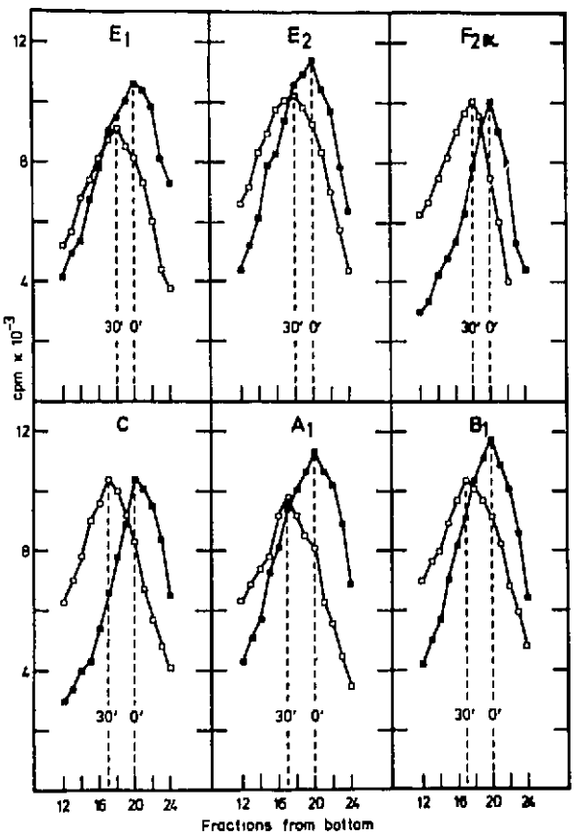

FIGURE 3: Rejoining of DNA-strand breaks caused by gamma-irradiation (30 krad, 12 kCi 60-Co-source, 0,94 Mrad/h). Radioactivity counted in automatically collected fractions of an alkaline sucrose gradient.

DISCUSSION

The generally accepted concept of a close interrelationship between

prostaglandins and cyclic nucleotides in cell function is based on measureable changes of cyclic nucleotide levels in different test systems caused by prostaglandins (16,17,18).
On the other hand there is much evidence to suggest that cyclic AMP and cyclic GMP seem to play an important role in the regulation of gene expression during the cell cycle. A reciprocal relationship between cyclic AMP and cyclic GMP in cell proliferation and cell differentiation, known as the Yin-Yang concept, has been postulated (19). Cyclic GMP is proposed to induce cell division and limit cell differentiation while cyclic AMP inhibits division and enhances differentiation.

According to the apparent relationship between prostaglandins and cyclic nucleotides on one hand and cyclic nucleotides and the cell cycle on the other effects of prostaglandins on the cell cycle, especially on the DNA synthesis and DNA excision repair may be conceivable.

'In vitro' experiments on thymus lymphocytes (20), bone marrow cells (21), mouse and human fibroblasts (22,23) suggest a stimulating effect of the used prostaglandins on DNA synthesis.

Moreover there is substantial evidence to suggest a direct correlation between Pg synthesis and DNA synthesis during liver -regeneration (24).

An inhibition of ^{3}H-thymidine incorporation into the DNA of suspended rat thymus cells at a concentration of 10 µg PgE_2/ml was observed by Toth et al. (25), but it was shown autoradiographically, that PgE_2 has no influence on the number of DNA synthesizing cells. Of great interest in this regard is the report by Sonis et al. (26) that treatment of EL-4 cells with PgE_2 resulted in a dose-dependent inhibition of ^{3}H-thymidine incorporation. Furthermore evidence has been presented by the same group that supernatants obtained from mouse fibrosarcoma cultures 48^{h} after the addition of fresh medium contained material which inhibited ^{3}H-thymidine incorporation in lymphoma cells 'in vitro'. Their results indicate that the inhibitor elaborated by fibrosarcoma cells may be PgE.

Harper (27) has studied the influence of various prostaglandins on

^{3}H-thymidine uptake into DNA of cultured human epidermal cells and has found that at higher concentrations, all the Pg's except $PgF_{2\alpha}$ showed a significant inhibition. At lower more physiological concentrations he could not find any inhibition in the ^{3}H-thymidine incorporation by the tested prostaglandins. This is in good agreement with the results of our 'in vitro' investigations in mouse spleen cells. Up to concentrations of 2,5 µg/ml PgE_1, PgE_2, PgA_1 and PgB_1 we could not find a significant influence on DNA synthesis. Interestingly $PgF_{2\alpha}$ caused a substantial alteration of DNA synthesis in all the three tested concentrations. This stimulating effect of $PgF_{2\alpha}$ may be explained by an increased cyclic GMP level, for considerable evidence has been presented that most of the actions of F prostaglandins seem to be mediated by intracellular accumulation of cyclic GMP (28). As mentioned earlier cyclic GMP is proposed to induce cell division, thus stimulating DNA synthesis.

The significant inhibition of DNA excision repair by E prostaglandins in higher concentrations may be of clinical interest, for any inhibition of the DNA repair process represents a potential cocancerogen factor involving a certain amount of risk to malignant degeneration of cells (29,30). In case of a suppressed DNA excision repair in proliferating cells, DNA damages have to be repaired by the imperfect postreplication repair process to ensure duration of daughter cells.

In regard to the inhibitory action of E prostaglandins on DNA excision repair the accumulation of large amounts of prostaglandins across biological membranes probably by an active transport mechanism in some tissues at least may be of great interest (31,32). On the other hand evidence has been presented that E prostaglandins increases cyclic AMP (33) and thereby stunts the growth of malignant cells (34) raising antitumor immunity (35), while F prostaglandins, by means of a higher cyclic GMP level may cause increased proliferation of malignant cells. The direct relation in cellular concentration of E and F prostaglandins may presumably be of importance too.

Contrary to E prostaglandins, the presence of $PgF_{2\alpha}$ resulted in a significant increase of DNA excision repair although gradient centrifuging disclosed a slight decrease in the rejoining of DNA strand

breaks caused by gamma-irradiation. At present direct evidence for this peculiar effect does not exist.

The clinical use of natural prostaglandins and various synthetical analogues has been extended rapidly within the last few years.

Already in the introduction we noted that various steroids are known to be inhibitors of DNA excision repair processes. Furthermore it is evident that long term treatment with estrogen drugs, such as oral contraceptives, increases the risk of endometrial cancer (36,37). In view of these facts the use of prostaglandins in the reproductive system should be deliberated critically.

In addition it is also known that the genetic material is attached constantly by various environmental factors and that these premutation defects must be corrected continuously by the natural repair system. Thus, any inhibition of the DNA repair system increases the risk of manifestation of mutations.

In cases of an inappropriate treatment, particularly with E prostaglandins, to induce abortion, the surviving foetus is endangered genetically, since a suppressed repair, especially in rapidly proliferating cells, may result not only in teratogenic but also in mutagenic damages.

Further experiments ought to demonstrate in how far these 'in vitro' results may be confirmed at 'in vivo' conditions.

REFERENCES

1. KARIM, S.M.M. and G.M. FILSHIE: Lancet 1, 157, 1970
2. KARIM, S.M.M. and S.D.SHARMA: J.Obstet.Gynecol., 78, 294, 1971
3. WIQVIST, N., BEGUIN, F., BYGDEMAN, M., FERNSTROM, I. and M. TOPPOZADA: Prostaglandins, 1, 37, 1972
4. CSAPO, A.I., KIVIKOSKI, A. and W.G. WIEST: Prostaglandins, 1, 305, 1972
5. GAUDIN, D., GREGG, R.S. and K.L. YIELDING: Biochem. Biophys. Res. Comm., 45, 630, 1971
6. SUCIU, D., ABRAHAM, A.D., SIMU, G. and Z. URAY: Endokrinologie, 67, 192, 1976

7. DEWSE, C.D.: J. Pharm. Pharmac., 28, 596, 1976
8. KLEIN, G. and A. WOTTAWA: Symposium on DNA repair and late effects, Vienna, 1973 Rötzerdruck Eisenstadt, Ed.: H.ALTMANN
9. GANESAN, P.A. and S.M.M. KARIM: Prostaglandins, 7, 215, 1974
10. SMITH, E.R. and M.M. MASON: Prostaglandins, 7, 247, 1974
11. PERSAUD, T.V.N., MANN, R.A. and K.L.MOORE: Prostaglandins, 4, 343, 1973
12. HANKS,H.J. and E.R.WALLACE: Proc.Soc.Exp.Biol.Med., 71, 196, 1949
13. BURTON, K.: Biochem. J., 62, 315, 1956
14. KOCSIS, F., KLEIN, W. and H. ALTMANN: Z. Naturforsch., 28c, 131, 1973
15. KLEIN, W., KOCSIS, F. and H.ALTMANN: Drug Res., 25, 1623, 1975
16. RAMWELL, P.W. and B.B. PHARRISS: Prostaglandins in Cellular Biology, Plenum Press, New York - London, 1973
17. KAHN, R.H. and W.E.M. LANDS: Prostaglandins and Cyclic AMP. Academic Press, New York - London, 1973
18. KUEHL, F.A.Jr.: Prostaglandins, 5, 325, 1974
19. GOLDBERG, N.D., O'DEA, R.F. and M.K. HADDOX: Advances in Cyclic Nucleotide Research, 3, 155, 1973
20. FRANKS, D.J., MacMANUS, J.P. and J.F. WHITFIELD: Biochem. Biophys. Res. Comm., 44, 1177, 1971
21. FEHER, I. and J. GIDALI: Nature, 247, 550, 1974
22. JIMENEZ De ASUA, L., CLINGAN, D. and P.S. RUDLAND: Proc.Nat. Acad. Sci., 72, 2724, 1975
23. MANGANIELLO, V.C. and J.BRESLOW: Biochem. et Biophys. Acta, 362, 509, 1974
24. MacMANUS, J.P. and B.M. BRACELAND: Prostaglandins, 11, 609, 1976
25. TOTH, M., ZAKAR, T. and F. ANTONI: Acta Biochim. et Biophys. Acad. Sci. Hung., 10, 161, 1975
26. SONIS, S.T., STELOS, P., STYLOS, W.A. and R.E. WILSON: Prostaglandins, 13, 87, 1977
27. HARPER, R.A.: Prostaglandins, 12, 1019, 1976
28. KUEHL, F.A.Jr., CIRILLO, V.J., HAM, E.A. and J.L.HUMES: in Advances in Biosciences, 9, International Conference on Prostaglandins, Bergström and Bernhard, Eds., Pergamon Press, Vieweg, Braunschweig, p. 155, 1973

29. LIEBERMAN, H., SELL, S. and E.FARBER: Proc. Am. Assoc. Cancer Res., 12, 5, 1971
30. WITSCHI, H., EPSTEIN, S.M. and E. FARBER: Cancer Res., 31, 270, 1971
31. BITO, L.Z.: J. Physiol., 221, 371, 1972
32. BITO, L.Z., WALLENSTEIN, M. and R. BAROODY: Advances in Prostaglandin and Thromboxane Research, Vol 1, Raven Press, New York, 1976
33. BUTCHER, R.W. and C.E. BAIRD: J. Biol. Chem., 243, 1213, 1968
34. SHEPPARD, J.R.: Nature, 236, 14, 1972
35. RIGBY, P.G.: Cancer Res., 32, 455, 1972
36. ZIEL, H.K. and W.D. FINKLE: N. Engl. J. Med., 293, 1167, 1975
37. SMITH, D.C., PRENTICE, R., THOMPSON, D.J. et al.: N. Engl. J. Med., 293, 1164, 1975

Diese Arbeit wurde im Rahmen des IBL Projektes Nr. 6302 "Medizinische Aspekte der DNA-Reparatur" durchgeführt.

SGAE-Berichte
Eigentümer, Herausgeber, Verleger und Druck:
Österreichische Studiengesellschaft für Atomenergie Ges.m.b.H.
Nach dem Pressegesetz verantwortlich: Prof. Dr. Hans GRÜMM,
alle Lenaugasse 10, 1082 Wien, Tel. (0222) 42 75 11, Telex 7-5400.