Vol. 108, No. 2, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 30, 1982 Pages 797-803

THE EFFECT OF APHIDICOLIN ON DNA REPAIR IN RESTING AND MITOGEN STIMULATED HUMAN LYMPHOCYTES

Vilhelm Bohr and Lars Køber

Department of Biochemistry B, Panum Institute, Blegdamsvej 3 C, DK-2200 Copenhagen N, Denmark

Received August 17, 1982

Aphidicolin inhibits DNA repair in human lymphocytes as measured by unscheduled DNA synthesis and the rejoining of strand breaks. When the lymphocytes are mitogen stimulated, sensitivity of DNA repair towards aphidicolin decreases, possibly due to the induction of the beta DNA polymerase.

INTRODUCTION

The tetracyclic diterpenoid aphidicolin has been an important tool in investigations on eucaryotic DNA repair and replication (1). In studies on isolated DNA polymerases from a number of different eucaryotic cells, the compound has been shown to be a specific inhibitor of DNA polymerase alpha, but not to affect the beta and gamma enzymes (2,3,4). The inhibition by aphidicolin of DNA replication in cellular systems is consistent with the notion that DNA polymerase alpha is the replicative enzyme (5). It is of considerable interest to study DNA repair in eucaryotic cells in the presence of aphidicolin since presumably the beta enzyme, and not the alpha polymerase is responsible for repair. In many cellular systems (6,7) aphidicolin has not affected DNA repair, and in a recent review by Spadari (8) it is forwarded that aphidicolin does not affect DNA repair in any known cellular systems.

Abbreviations: Phytohaemagglutinin - PHA
Phosphate buffered saline - PBS.

0006-291X/82/180797-07$01.00/0

Some investigators have, however, reported on an inhibitory effect of aphidicolin on DNA repair (9,10). The methods used for measuring DNA repair have mainly been indirect such as thymidine incorporation into replication arrested cells, i.e. unscheduled DNA synthesis. Aphidicolin has been reported to inhibit unscheduled DNA synthesis in human lymphocytes (9). In only one case (10), using human fibroblasts, has inhibition by aphidicolin been determined by measuring the rejoining of strand breaks and the release of pyrimidine dimers.

Further clarification of this issue seems essential to the understanding of the role of the different DNA polymerases in cellular functions.

We here report on the inhibition of DNA repair in human lymphocytes by aphidicolin. This was assessed not only by measuring unscheduled DNA synthesis but also the rejoining of strand breaks. DNA damage was done by the use of short wave UV-light or by treatment with dimethylsulphate.

Normally human lymphocytes are resting cells. They can be stimulated to proliferate by the use of mitogens such as phytohaemagglutinin (PHA), thus representing a valuable system for direct comparison of drug effects on DNA repair in quiescent and replicative cells. By the use of aphidicolin we have attempted to evaluate the involvement of the alpha DNA polymerase in DNA repair under these different cellular conditions.

MATERIALS AND METHODS

Human lymphocytes were isolated on ficoll-isopaque gradients and washed in phosphate buffered saline (PBS). When stimulated with PHA they were resuspended to 10^6 cells/ml in MEM containing 20% donor serum and incubated for 3-4 days at 37°. Prelabelling was done by adding 10 µCi of ^{3}H-thymidine and 1 µmole of cold thymidine per ml of cells, 24 hours before harvesting. L1210 cells were grown in culture flasks in RPMI medium supplemented with 10% fetal calf serum. They were inoculated biweekly and grown to a concentration of approximately 0.4 x 10^6 cells/ml. Aphidicolin was from ICI. PHA was from Difco.

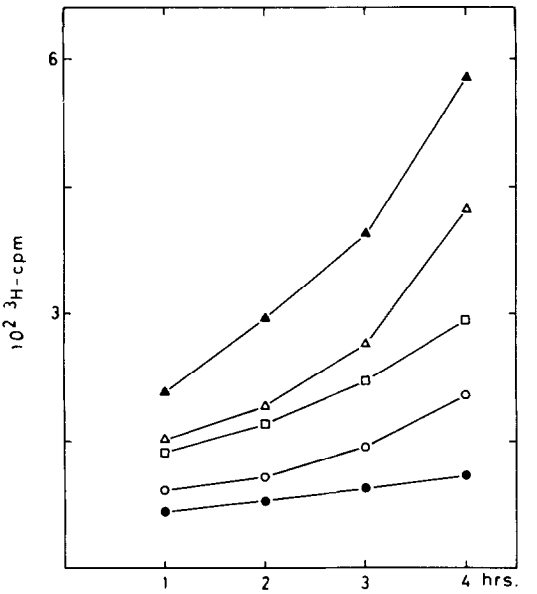

Fig. 1.

Effect of aphidicolin on unscheduled DNA synthesis in resting human lymphocytes. Incubation of ^{3}H-thymidine in the presence of 7 mM hydroxyurea.
●-● Untreated cells.
▲-▲ Irradiated cells (20 J/m^2)
The remaining incubations have also been UV-irradiated and contain different concentrations of aphidicolin. Δ-Δ 0.07 µg/ml aphidicolin, □-□ 0.13 µg/ml aphidicolin and o-o 0.7 µg/ml aphidicolin.

Measuring unscheduled DNA synthesis. Cells were irradiated with 20-50 J/m^2 of short wave UV-light (254 nm) at a concentration of 10^7 cells/ml PBS. They were then mixed with an equivolume of serum and three volumes of medium. Incubation for repair was at 37°C in volumes of 1.5 ml containing 20 µCi ^{3}H-thymidine (25 Ci/mmol (NEN)). At time points, 100 µl samples were lysed in 30 µl lysis solution containing 1% sodium dodecyl sulphate, 0.4 mM thymidine, 0.7 mg protease K and 0.1 mg sodium polyphosphate. After 16 hours at 20° they were spotted on Whatman filters, washed in 0.1 M HCl, and radioactivity was determined. Cells were counted twice in each incubation mixture.

Alkaline elution. The procedure was a modification of the one used by Kohn (11). DNA was damaged by suspending the prelabelled cells for 3-5 min. in medium containing 0.50 mM dimethylsulphate at 37°C. The suspension was then diluted with 3 volumes of PBS, isolated by centrifugation and resuspended in medium containing 10^6 cells. Time samples after repair were resuspended in cold PBS. They were then applied to the alkaline elution apparatus, caught on 2 µm PVC filters (Millipore) and washed thoroughly in cold PBS. Lysis was done by slowly passing 4 ml of 0.2% sarcosyl (Ciba-Geigy), 2 M NaCl, 0.04 M Na_2EDTA at pH 10.0 through the filters. The lysate was washed in 4 ml 0.01 M Na_2EDTA at pH 10.0. Alkaline elution was carried out in the dark by pumping elution solution (20% tetraethylammoniumhydroxide, 0.01 M ammoniumhydroxyde, 0.01 M Na-free EDTA, pH 12.2) through the filters at a speed of 1.8 ml/hour using a peristaltic pump. Radioactivity determination of the eluate and the filters were done as described by Kohn (11). Results were expressed as percentage of total counts added to the filters.

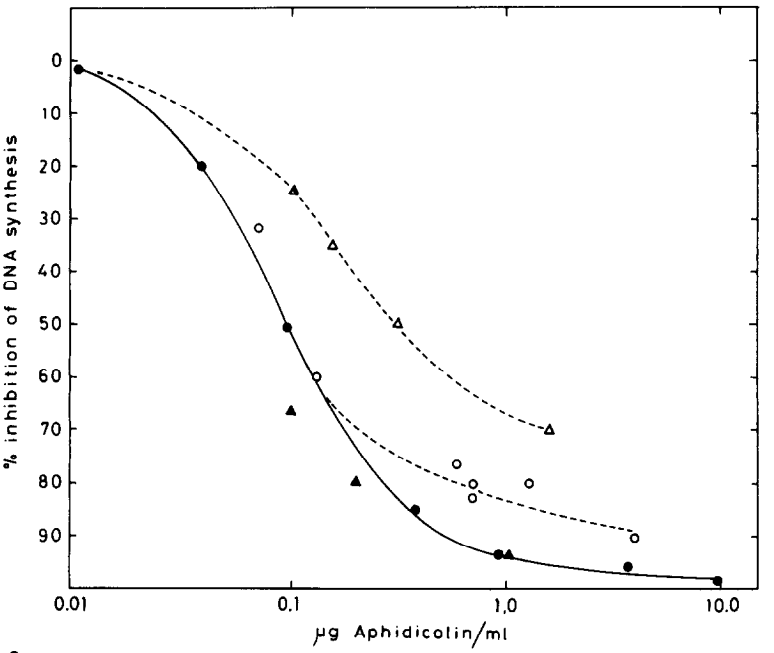

Fig. 2.

Inhibition of replication and unscheduled DNA synthesis by aphidicolin.
●-● Untreated L1210 cells. ▲-▲ Untreated PHA stimulated lymphocytes. o-o Unscheduled DNA synthesis in resting human lymphocytes and in 6 days PHA stimulated lymphocytes Δ-Δ, both in the presence of 7 mM hydroxyurea and after damage with 20 J/m^2 short-wave UV-light.

RESULTS

Fig. 1 shows the effect of aphidicolin on unscheduled DNA synthesis in human lymphocytes. This was measured in the presence of 7 mM hydroxyurea. The strong inhibition of unscheduled DNA synthesis by aphidicolin suggests that the alpha DNA polymerase plays a major role in this synthesis. This is further established by the data shown in fig. 2. Here, the effect of aphidicolin on replication in L1210 mouse leukaemia cells has been compared to the effect of the drug on unscheduled DNA synthesis in resting and PHA stimulated human lymphocytes. It is seen that the inhibition of unscheduled DNA synthesis is stronger in the resting cells. At concentrations of aphidicolin lower than 0.2 µg/ml (∿ 60% inhibition of DNA synthesis), effects on replication (L1210 cells) and on unscheduled DNA synthesis in

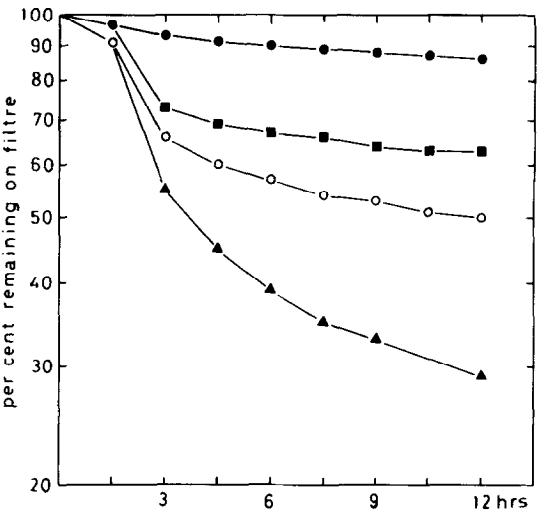

Fig. 3.

The effect of aphidicolin on the rejoining of strand breaks in 3 day PHA stimulated human lymphocytes. Alkaline elution. ●-● Untreated cells. ▲-▲ cells treated with 0.5 mM dimethylsulphate for 4 min. and incubated at 37° for 15 min. ■-■ Incubation for 2½ hours after damage. o-o Incubation for 2½ hours in the presence of 20 µg/ml aphidicolin after damage.

resting human lymphocytes are very similar. At higher concentrations of the drug inhibition of unscheduled DNA synthesis in the resting lymphocytes is less than that of replication in the L1210 cells. This difference of approximately 10% may be ascribed to a beta-DNA polymerase activity in the resting lymphocytes. The major repair synthesis is then likely to be carried out by the alpha-DNA polymerase.

PHA stimulated lymphocytes.

The effect of aphidicolin on untreated PHA stimulated lymphocytes is similar to its effect on the L1210 cells (Fig.2). Unscheduled DNA synthesis is clearly much less sensitive towards aphidicolin in PHA stimulated lymphocytes than in the resting ones (Fig. 2).

The inhibition by aphidicolin on unscheduled DNA synthesis in PHA stimulated lymphocytes was studied in different donors and on each day from day 1 to day 7 after PHA stimulation. With a concentration of aphidicolin of 1.7 µg/ml, and in the presen-

ce of 7 mM hydroxyurea, inhibition was typically about 65% on day one and two and decreased to about 50% on the following days from day 3 to day 7. Inhibition of aphidicolin on unscheduled DNA synthesis in lymphocytes was similar when the damage was caused by UV-light or by dimethylsulphate.

Rejoining of strand breaks.

The effect of aphidicolin on the rejoining of strand breaks after dimethylsulphate damage and 2½ hours repair in PHA stimulated human lymphocytes is shown in Fig. 3. Using concentrations of aphidicolin of about 10-20 µg/ml, inhibition of strand rejoining was consistently found in different experiments and donors. Inhibition was detectable at concentrations of aphidicolin down to 2 µg/ml.

DISCUSSION

By the use of two different techniques for the assessment of DNA repair, we have found inhibition by aphidicolin of this process in resting and proliferating human lymphocytes.

The much stronger inhibitory effect of aphidicolin on unscheduled DNA synthesis in resting than proliferating lymphocytes is in accordance with previous findings (12) that a beta DNA polymerase activity is being induced a few days after PHA stimulation. It does, however, also suggest that repair polymerase activity in the resting cells is primarily due to the alpha-DNA polymerase which is not normally regarded as a repair enzyme. The polymerizing step of DNA repair does, therefore, not appear to be a specialized function of the beta DNA polymerase.

In PHA-stimulated lymphocytes, a ten times higher concentration of aphidicolin is needed for the inhibition of strand ligation as compared to inhibition of unscheduled DNA synthesis. This suggests that ligation takes place even under conditions

of strong inhibition of strand elongation and polymerisation, indicating that a considerable diversification exists between the techniques, and none of them may be directly identifiable with the repair process.

Most of the cell types in which the effect of aphidicolin has been studied have been actively growing cell lines (8), and it is possible that the involvement of the different DNA polymerases in repair depends upon the level of cellular proliferation. When cells are in quiescence and possibly also under other conditions the alpha-DNA polymerase may be able to carry out functions usually undertaken by the beta-DNA polymerase.

ACKNOWLEDGMENTS

Technical assistance was provided by Anna Johannesen. We wish to thank the Danish Cancer Society, The Hasselbach Foundation and the Hertz Foundation for support. We thank Peter Nielsen and Hans Klenow for valuable discussions.

REFERENCES

1. Huberman, J.A., Cell 23, 647-48 (1981)
2. Ikegami, S., Taguchi, T., Ohashi, M., Ogura, M., Nagano, H. and Mano, Y. (1978) Nature (London) 275, 458-460
3. Ohashi, M.,Taguchi, T. and Ikegami, S. (1978) Biochem. Biophys. Res. Commun. 82, 1084-1090
4. Pedrali-Noy, G. and Spadari, S. (1979) Biochem. Biophys. Res. Commun. 88, 4, 1194-2002
5. Ikegami, S., Taguchi, T., Ohashi, M., Nagano, H. and Mano, Y. (1979) Develop. Growth and Differ. 21, 2, 119-127
6. Pedrali-Noy, G. and Spadari, S. (1980) Mut. Res. 70, 389-394
7. Hanaoka, F., Kato, K., Ikegami, S., Ohashi, M. and Yamada, M. (1979) Biochem. Biophys. Res. Commun. 87, 575-580
8. Spadari, S., Sala, F. and Pedrali-Noy, G. (1982) Trends in the Biochemical Science. January 1982, 29-31
9. Berger, N., Koruhara, K., Petzold, S. and Sikorski, G. (1979) Biochem. Biophys. Res. Commun. 89, 218-225
10. Snyder, R.D. and Regan, J.D. (1981) Biochem. Biophys. Res. Commun. 99,4, 1088-1094
11. Kohn, K.W. (1979). In Methods in cancer research vol 16, Ch. 7 pp 309-331
12. Bertazzoni, V., Stefanini, M., Pedrali-Noy, G., Guilotto, E., Nuzzo, F., Falaschi, A. and Spadari, S. (1976). Proc. Nat. Acad. Sci. USA 73, 785-789