DEPRESSION OF THE INTRACELLULAR HISTONE MESSENGER RNA CONTENT BY THE ALKYLATING AGENT 2,3,5-TRISETHYLENEIMINOBENZOQUINONE

Gerhard RAYDT+, Friedrich HOLZWEBER, Bernd PUSCHENDORF and Hans GRUNICKE++
Institut für Medizinische Chemie der Universität Innsbruck, A-6010 Innsbruck, Postfach, Austria

Received 20 December 1976

1. Introduction

Previous publications of our group [1,2] have demonstrated that 5×10^{-7} mol/kg of the alkylating agent 2,3,5-trisethyleneiminobenzoquinone (Trenimon), a concentration which does not inhibit DNA synthesis but blocks cell multiplication, causes a drastic inhibition of [^{3}H]lysine incorporation into all histone fractions of Ehrlich ascites tumor cells. At the same Trenimon concentration, the [^{3}H]lysine incorporation into chromosomal non-histone proteins and into total cellular proteins is unaffected. Based on these data it is concluded that the drug selectively inhibits histone biosynthesis. Riches and Harrap [3] have observed similar effects with chlorambucil. Since DNA synthesis remains unaffected, Trenimon uncouples DNA and histone biosyntheses – two processes which are normally tightly co-ordinated. The inhibition of histone biosynthesis may be caused by a decrease of biologically active histone messenger RNA. The studies presented here demonstrate that this is indeed the case.

2. Materials and methods

L-[5-^{3}H]Arginine (17 Ci/mmol), L-[4,5-^{3}H]leucine (35 Ci/mmol), L-[4,5-^{3}H]lysine (20 Ci/mmol) and [5,6-^{3}H]uridine (35 Ci/mmol) were obtained from The Radiochemical Centre (Amersham, UK). All unlabelled amino acids were purchased from Boehringer Mannheim (Mannheim, FRG). CM-Cellulose type CM-52 was obtained from Whatman. ATP, GTP, creatine phosphate and creatine kinase were purchased from Boehringer Mannheim (Mannheim, FRG). All other reagents were from Merck (Darmstadt, FRG). 2,3,5-Trisethyleneiminobenzoquinone (Trenimon = triaziquone, WHO recommended) was kindly donated by Farbenfabrik Bayer (Leverkusen, FRG).

Various groups of 30 Ehrlich ascites tumor-bearing male NMRI/Han mice received a single injection of Trenimon as indicated, 3–4 days after tumor-transplantation. Control animals received a corresponding volume of 0.14 M NaCl. After 1 h all animals were injected with 0.1 mCi uridine; 30 min later all mice were killed and the cells were harvested as described previously [4]. The cells were lysed in 5 mM $MgCl_2$ with 20 strokes in a tightly fitting Dounce homogenizer. An isotonic solution was obtained by adding a corresponding volume of 1.5 M sucrose–0.15 M KCl. The lysate was centrifuged at 20 000 × *g* for 20 min and the polysomes were pelleted from the resulting supernatant by centrifugation through 36% sucrose at 105 000 × *g* for 120 min. Polysomal RNA from Ehrlich ascites tumor cells was prepared and fractionated by the method described by Breindl and Gallwitz [5].

Gel electrophoresis of fractionated polysomal RNA was performed on 3.5% polyacrylamide gels. The reticulocytes were prepared from rabbits according to the method described by Evans and Lingrel [6]; cells were lysed according to [5]. The composition of the cell-free system is described by Lockard and Lingrel [7]. For fractionation of the products formed in vitro, the method of Breindl and Gallwitz [5] was used with

+Present address: Institut für Physiologische Chemie und Physikalische Biochemie der Universität München, D-8000 München, Goethestr. 33, FRG.

++To whom correspondence should be addressed.

the exception that the polyacrylamide gel electrophoresis was performed at pH 3.2 in 15% gels containing 6.25 M urea. For radioactivity measurements sliced gels were solubilized in NCS and after addition of 10 ml scintillator [8] counted in a liquid scintillation spectrophotometer.

3. Results

A fraction of polysomal RNA enriched in 9 S RNA was prepared as described under Materials and methods. The effect of Trenimon on the radioactive labelling of this RNA is shown in fig.1. In the control, the polyacrylamide gel electrophoretic pattern has a radioactivity peak in the region 3.0–4.5 cm from the start. This peak corresponds to the 9–11 S region and should contain, among other RNA species, histone messenger RNA [5]. Histone messenger RNA from HeLa cells (kindly donated by Dr D. Gallwith) migrates into this region. After treatment of Ehrlich ascites tumor cells with Trenimon less radioactivity is observed in this area. The radioactivity in the region corresponding to the 4–5 S and 18 S RNA does not represent the total intracellular 4–5 S and 18 S RNA as a variable loss of these RNA species occurs during the isolation of the 9 S RNA.

Figure 2 shows that this 9 s RNA-enriched preparation stimulates the incorporation of amino acids into acid-soluble proteins. These proteins are retained by carboxymethylcellulose and, after elution with 0.25 N HCl–6 M urea, are recovered in fractions No. 24–30 of the eluate (peak B of fig.2). The proteins of peak A

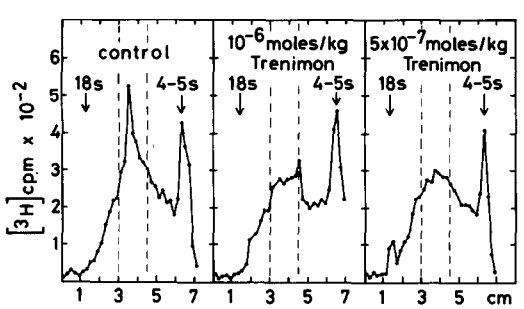

Fig.1. Polyacrylamide gel electrophoresis of fractionated (9 S rich) polysomal RNA. Electrophoresis was performed with 0.45 A_{260} RNA/gel.

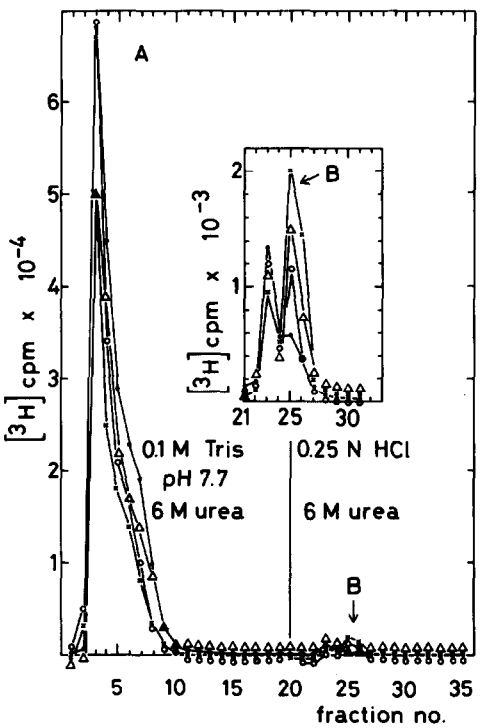

Fig.2. Separation of the translation products of 9 S rich polysomal RNA by carboxymethylcellulose chromatography. 3.2 A_{260} of each RNA preparation were added to a cell-free system from rabbit reticulocytes. The labelled proteins were extracted by 0.5 N HCl and separated on CM-cellulose. Peak A represents globin. (• - •) without exogenous RNA, (x - x) control, (△——△) 10^{-6} moles/kg Trenimon, (○ - ○) 5 × 10^{-7} moles/kg Trenimon.

and of the fractions No. 21–23 are mainly coded by endogenous messenger RNA of the reticulocyte lysate. As can be seen, the amount of protein obtained in peak B is reduced if RNA from Trenimon treated cells is employed. Gel electrophoresis of the translation products of peak B fig.2 exhibits a typical histone pattern (fig.3). Only small amounts of H1 histones seem to be formed in the reticulocyte system; this is in accordance with observations of other authors [5]. In agreement with the authors cited above [5] we assume that the radioactivity in the region 5.0–5.5 cm from the start corresponds to incomplete histones. Table 1 shows that in the in vitro system, the synthesis of histones is reduced by approximately 60%, if mRNA from Trenimon treated cells is used.

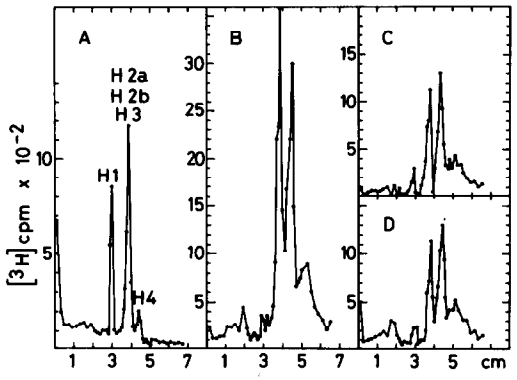

Fig.3. Characterization of the translation products of peak B (fig.2) by polyacrylamide gel electrophoresis. (A) [^{3}H]lysine labelled histones prepared from Ehrlich ascites tumor cells. (B) proteins of peak B (control). (C) Proteins of peak B (10^{-6} moles/kg Trenimon). (D) Proteins of peak B (5×10^{-7} moles/kg Trenimon). Without exogenous RNA there is no radioactivity in the region 3–7 cm from the start.

Table 1
Effect of trisethyleneiminobenzoquinone (Trenimon) on histone biosynthesis in vitro. For experimental details see Materials and methods. The translation products corresponding to peak B of fig.2 were subjected to polyacrylamide gel electrophoresis. The gels were scanned for radioactivity. Peaks corresponding to histones were evaluated planimetrically.

Trenimon (moles/kg)	Histone biosynthesis in vitro (% of control)
5×10^{-7}	44
10^{-6}	41
6×10^{-6}	29

4. Discussion

Previous findings from our group indicate that low, but chemotherapeutically active, doses of Trenimon produce modifications of the nucleoprotein including an inhibition of the template activity for DNA-dependent RNA synthesis [4,9]. Preceding studies have demonstrated [1,2] that Trenimon selectively inhibits the incorporation of [^{3}H]lysine into all histone fractions of Ehrlich ascites tumor cells. The studies presented here indicate that the inhibitory effect of the drug on histone biosynthesis can be explained by a decrease of the polysomal histone messenger RNA content. Ninety minutes after administration of the alkylating agent the amount of functionally active histone messenger RNA is reduced to approximately 40% of the control. Considering the lifetime of histone messenger RNA in mouse L cells of 11 h [10], it seems unlikely that the observed effect is due to an inhibition of the transcription of histones genes. A drug induced enhancement of the degradation of histone messenger RNA seems to be a more adequate explanation of the phenomenon. This assumption is in accordance with the effect of hydroxyurea on histone messenger RNA in synchronised HeLa cells [11]. As the [^{3}H]lysine incorporation into other proteins is not inhibited [1] it must be concluded that the histone messenger RNA is preferentially affected by Trenimon.

Is there any explanation for this specifity? It has been suggested that poly(A) protects messenger RNA against degradation by exonucleases [12]. Histone messenger RNA lacks poly(A) and is characterized by a shorter lifetime than other messenger RNA molecules [10,11]. We suggest that the drug activates a non-specific RNAase. Due to the absence of the poly(A) protection, histone messenger RNA will suffer most from this activated nuclease, whereas the cleavage of the poly(A)$^+$ messengers should occur at a much slower rate. However, the lifetime of histone messenger RNA in the cells used in our studies is unknown. It cannot be excluded, therefore, that the decrease in the amount of histone messenger RNA is caused by an inhibition of the formation of histone messenger RNA. A specific impairment of the histone genes by the alkylating agent is difficult to envisage. But, as a consequence of the relatively short lifetime of histone messenger RNA, a general inhibition of transcription will affect the biosynthesis of histones much earlier than the formation of proteins translated from more stable messengers.

The concentrations used in these studies of the alkylating agent Trenimon inhibit the biosynthesis of the histones but do not affect DNA replication [2]. Thus, the drug produces an uncoupling of DNA and histone biosyntheses. This effect offers an explanation for the arrest of the tumor cells in the G_2-phase which is observed after treatment with low doses of Trenimon [2] and other alkylating agents [13].

References

[1] Wolf, H., Raydt, G., Puschendorf, B. and Grunicke, H. (1973) FEBS Lett. 35, 336–340.
[2] Grunicke, H., Hirsch, F., Wolf, H., Bauer, U. and Kiefer, G. (1975) Exp. Cell Res. 90, 357–365.
[3] Riches, P. G. and Harrap, K. R. (1973) Cancer Res. 33, 389–393.
[4] Grunicke, H., Bock, K. W., Becher, H., Gäng, V., Schnierda, J. and Puschendorf, B. (1973) Cancer Res. 33, 1048–1053.
[5] Breindl, M. and Gallwitz, D. (1973) Eur. J. Biochem. 32, 381–391.
[6] Evans, M. J. and Lingrel, J. B. (1969) Biochemistry 7, 3000–3005.
[7] Lockard, R. E. and Lingrel, J. G. (1969) Biochem. Biophys. Res. Commun. 37, 204–212.
[8] Cain, D. F. and Pittney, R. E. (1968) Anal. Biochem. 22, 11–20.
[9] Puschendorf, B., Wolf, H. and Grunicke, H. (1971) Biochem. Pharmacol. 20, 3039–3050.
[10] Perry, R. P. and Kelley, D. E. (1973) J. Mol. Biol. 79, 681–696.
[11] Gallwitz, D. (1975) Nature 257, 247–248.
[12] Darnell, J. E., Philipson, L., Wall, R. and Adesnik, M. (1971) Science 174, 507–510.
[13] Wheeler, G. P. (1967) Fed. Proc. 26, 885–892.