PROFLAVINE INHIBITION OF PROTEIN SYNTHESIS IN MALIGNANT HAMSTER MELANOMA

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Received 1 October 1970

1. Introduction

The amelanotic hamster melanoma is a very rapidly growing tumor which leads in less than 18 days after subcutaneous transplantation to the death of the animal. The rapid growth of this tumor implies a high nucleic acid and protein synthesizing activity. Studies have been undertaken to find agents which can influence this hyperactivity and reduce the tumor growth without affecting the host cells. Several reports have been published on growth retardation of melanoma by drugs [1–4] but only a few have been concerned with the influence of these drugs on nucleic acid and protein synthesis [5, 6]. Preliminary experiments from our laboratory indicated that neither chloramphenicol nor D-penicillamine affected the tumor. However, using the acridine dye proflavine we found some remarkable growth retardation [7]. Our recent studies on the incorporation rate of ^{3}H-thymidine into nuclear and mitochondrial DNA of the melanoma in the presence of proflavine let us assume that this dye has an inhibitory effect on the DNA synthesis [8]. Inhibition of RNA-polymerase from this tumor by proflavine *in vitro* and *in vivo* could also be demonstrated [9].

The effects of acridine dyes on the metabolism of animal cells are well documented and broadly resemble those produced by actinomycin D; the synthesis of RNA is strongly inhibited and the synthesis of protein relatively unaffected [10–13]. However, we suspected that the inhibition of DNA and RNA synthesis in the melanoma by proflavine may not completely account for the observed growth retardation. Therefore the purpose of the experiments described in this paper was to investigate the influence of proflavine on the protein synthesizing machinery of the melanoma cells.

2. Materials and methods

The amelanotic hamster melanoma, described by Fortner [14] was generously supplied by Priv.Doz.Dr.D. Gericke (Leiter der Abteilung für Tumorforschung der Farbwerke Hoechst AG., Frankfurt am Main). The tumors were serially transplanted subcutaneously into female Syrian hamster weighing 60–80 g. On the tenth day after transplantation 100 mg proflavine (Fluka, Buchs, SG., Schweiz) per kg bodyweight dissolved in isotonic NaCl solution was applied intraperitoneally to a group of 4 animals. After 60 min the proflavine treated animals together with 4 control hamsters received 20 μCi/animal of ^{3}H-phenylalanine (Radiochemical Centre, Amersham England; specific activity 21 Ci/mmole). A chase of a 5000 fold excess of cold phenylalanine was applied after one or 3 hr respectively. The animals were killed 20 min after injection of the chase. Tumors and livers of all animals were carefully dissected, washed and homogenized. Nuclei and mitochondria of both sources were prepared and purified as previously described [7]. The mitochondrial supernatant was centrifuged for 1 hr at 144,000 *g*. The pellet was regarded as the microsomal fraction and the supernatant of the microsomes as cytoplasm. In all these cell fractions the specific radioactivity was determined and the inhibition of the incorporation rate by proflavine was calculated.

Table 1
Incorporation rate of ^{3}H-phenylalanine after 60 min into nuclear, mitochondrial, microsomal and cytoplasmic proteins of melanoma and liver in the presence and absence of proflavine.

Cell fraction	Condition	Melanoma		Liver	
		Specific radio-activity	% Inhibition by proflavine	Specific radio-activity	% Inhibition by proflavine
Nuclei	untreated	185.0	–	450.0	–
	proflavine treated	16.0	91.5	335.0	25.0
Mitochondria	untreated	210.0	–	1050.0	–
	proflavine treated	22.0	89.5	840.0	20.0
Microsomes	untreated	140.0	–	480.0	–
	proflavine treated	13.7	90.2	445.0	7.0
Cytoplasm	untreated	165.0	–	478.0	–
	proflavine treated	19.8	88.0	405.0	15.0

3. Results and discussion

The incorporation of ^{3}H-phenylalanine during a 60 minute period into nuclear, mitochondrial, microsomal and cytoplasmic proteins from melanoma and liver is shown in table 1. The cell fractions of the liver exhibit a 2.5 fold higher incorporation rate than the tumor fractions except for the mitochondria. The labelling of the tumor mitochondria is only one fifth of that of the liver mitochondria. This might be due to differences in the extrinsic and intrinsic contribution to the formation of intact mitochondria of melanoma or liver. Preliminary studies of the electrophoretic pattern of the mitochondria, showing significant differences between the mitochondria of both sources [15], support this view.

Treatment of the tumor-bearing animals with proflavine 60 minutes before injection of the radioactivity, results in an inhibition of the incorporation rate in all cell components of the melanoma. The highest inhibition (91.5%) is found for the nuclear protein but the inhibition was about the same in the cell components studied. However, the incorporation rate of phenylalanine into the liver fractions was dramatically less inhibited by the proflavine treatment. The highest inhibition was found in the nuclear protein (25%), the lowest in the microsomal fraction (7%) (see also table 1).

Prolongation of the incorporation time of ^{3}H-phenylalanine from 60 to 180 min resulted in an increase of the specific activity in all cell components of the melanoma and the liver, although the increase was remarkably higher in the tumor (table 2). The inhibition by proflavine remained the same in the melanoma whether measured 120 or 240 min after injection of the drug. However, an increase in proflavine inhibition was observed for the liver fractions after 240 min of treatment. This effect corresponds to an increase in the proflavine content of the liver fractions. The proflavine concentration, as estimated by the absorption of this dye at 450 nm, was 120 minutes after application 100 nmoles/mg protein in the liver nuclei and about 10 nmoles/mg protein in the other cell components as mitochondria, microsomes and cytoplasm. After 240 minutes the proflavine concentration increased in the mitochondria, microsomes and cytoplasm to somewhat more than twofold. It should also be mentioned that the actual proflavine content in the cell fractions of the melanoma at both times was less than 2.5% of that found in the liver fractions. If we assume proportionality of the proflavine effect, the inhibition of phenylalanine incorporation into the liver would be even smaller than stated in table 1. When considered for the same proflavine content as that found in the tumor the proflavine inhibition in mitochondria, microsomes and cytoplasm of liver might be calculated as 0.5%. These results suggest that a protein synthesizing system exists in the melanoma, which can be differentiated from the system normally found in eucaryotic cells by its proflavine sensitivity.

The highly proflavine-sensitive protein synthesis in

Table 2

Incorporation rate of ^{3}H-phenylalanine after 180 min into nuclear, mitochondrial, microsomal and cytoplasmic proteins of melanoma and liver in the presence and absence of proflavine.

Cell fraction	Condition	Melanoma		Liver	
		Specific radioactivity	% Inhibition by proflavine	Specific radioactivity	% Inhibition by proflavine
Nuclei	untreated	1410.0	–	1380.0	–
	proflavine treated	80.0	94.5	650.0	53.0
Mitochondria	untreated	1580.0	–	1920.0	–
	proflavine treated	174.0	89.0	920.0	52.0
Microsomes	untreated	1730.0	–	3460.0	–
	proflavine treated	176.0	89.8	1580.0	54.0
Cytoplasma	untreated	1550.0	–	1400.0	–
	proflavine treated	145.0	90.6	740.0	47.0

the melanoma might be a tumor-specific one which may be coded for by tumor-specific RNA species, derived from a messenger of foreign origin. A search is now being made to identify tumor-specific proteins in the melanoma by their proflavine sensitivity, to characterize their functions and to find out in which cell components they are localized.

Acknowledgements

We wish to thank Prof. Dr. h. c. Th. Bücher for helpful advice and discussion and for reviewing this manuscript. The skilful technical assistance of Miss Schroeder is gratefully acknowledged. This work was supported by a grant of Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 51).

References

[1] B.T.Hourani and H.B.Demopoulos, Lab. Invest. 21 (1969) 434.
[2] M.H. Van Woert and S.H.Palmer, Cancer Res. 29 (1969) 1952.
[3] H.B.Demopoulos and G.Kaley, J. Natl. Cancer Inst. 30 (1963) 611.
[4] H.B.Demopoulos, M.A.Gerving and H.Bagdoyan, J. Natl. Cancer Inst. 35 (1965) 823.
[5] B.R.Balda and G.D.Birkmayer, Arch. Expti. Pathol. Pharmakol. 266 (1970) 291.
[6] H.S.Schwartz and J.E.Sodergren, Cancer Res. 28 (1968) 445.
[7] G.D.Birkmayer and B.R.Balda, Arch. Exptl. Pathol. Pharmakol. 266 (1970) 294.
[8] B.R.Balda and G.D.Birkmayer, Arch. Klin. Exptl. Derm. (1970) in press.
[9] B.R.Balda and G.D.Birkmayer, in preparation.
[10] E.Reich, R.M.Franklin, A.J.Shatkin and E.L.Tatum, Proc. Natl. Acad. Sci. U.S. 48 (1962) 1222.
[11] R.Weisner, G.Acs, E.Reich and A.Shafiq, J. Cell Biol. 27 (1965) 47.
[12] C.Scholtissek and R.Rott, Nature 204 (1964) 39.
[13] J.W.Watts and M.A.F.Davis, Biochem. J. 100 (1966) 467.
[14] J.G.Fortner, A.G.Maky and G.R.Schrodt, Cancer Res. suppl. 161 (1961).
[15] G.D.Birkmayer and B.R.Balda, in preparation.