Eur J Cancer Clin Oncol, Vol. 23, No. 7, pp. 973–977, 1987.
Printed in Great Britain

0277-5379/87$3.00+0.00

Tetracycline in the Treatment of Malignant Effusions: Evidence for a Cytostatic Action of the Decomposed Drug

CHRISTIAN SAUTER* and MARIANNE COGOLI

Division of Oncology, Department of Medicine, University Hospital, 8091 Zürich, Switzerland

Abstract—*During the* in vitro *growth of human carcinoma cell lines an inhibition of cell growth in media containing tetracycline was observed at a concentration which was about* 1000 *times below the one reached in the pleural fluid of malignant effusions after tetracycline instillation. During short time drug–cell contact only previously heated tetracycline showed growth inhibition like doxorubicine, a substance of similar structure and origin. Freshly prepared tetracycline inhibited cell growth only after several minutes of drug cell contact. Our observations* in vitro *suggest that decomposed tetracycline plays an important role in the control of malignant effusions.*

INTRODUCTION

THE TREATMENT of choice in managing malignant pleural effusions today is tube drainage followed by instillation of tetracycline [1]. It has been postulated that the low pH of the tetracycline solution is responsible for the sclerosing effect [2]. A randomized study comparing tetracycline with an acidified multivitamin solution of similar pH did not substantiate this hypothesis [3]. We noticed a constant growth inhibition of human cell lines by media containing tetracyclines. Our experiments were designed to study the cytostatic action of tetracycline *in vitro* and its relevance to the treatment of malignant effusions.

MATERIAL AND METHODS

Drugs

The drugs were obtained from the following sources: ascorbic acid (Redoxon): Roche, Basel, Switzerland; doxorubicine (Adriblastin); Farmitalia Carlo Erba, Freiburg, F.R.G.; minocycline (Minocin) and tetracycline (Achromycine): American Cyanamid Company, Lederle Laboratories Division, U.S.A. Decomposition of tetracycline and minocycline was done by heating the dissolved drugs for 10 min at 100°C.

Cells

A human hypernephroma line [4] as previously described [5] and a human breast cancer line (BT 20 [6]) were used.

Inhibition of cell growth

The cells were incubated with tetracycline, minocycline, doxorubicine or ascorbic acid in the following way: to 1 ml of medium (RPMI 1640 supplemented with 8% fetal bovine serum) containing a given drug concentration 1 ml of cell suspension with about 120,000 cells freshly prepared by trypsinization of a cell monolayer was added. This mixture was incubated at 37°C for various periods of time in sterile 7-ml screw-capped centrifuge tubes. After incubation the cells were centrifuged at 200 ***g*** for 10 min at 4°C, and then resuspended in 4.2 ml of fresh medium; 1.0 ml of the cell suspension was put in each of four wells of sterile flat bottom plastic plates (24 wells, dia. 16 mm, Costar, Cambridge, Mass., U.S.A.). The plates were then incubated at 37°C in a 5% CO_2 atmosphere. After 4 days of incubation—at this time the control wells (only medium without drugs) showed a complete monolayer containing about 1.3 million cells per well—the cells were stained by methylene blue/parafuchsin [7] and the plates evaluated. In the case of continuous drug contact to 1 ml of the original cell suspension (about 120,000 cells) 1 ml of medium and 2 ml of drug dilution were added, thoroughly mixed, and also distributed into four wells.

The evaluation of the stained plates was done over a neon screen with a photographic light meter

Accepted 16 January 1987.
*Author to whom correspondence and reprint requests should be addressed.
Supported by the "Stiftung für angewandte Krebsforschung", Zürich. We thank Mrs Elisabeth Sauter for linguistic assistance.

Table 1. Growth inhibition of hypernephroma cells by tetracycline or doxorubicine

Time of contact (min)	Inhibition of growth (IG) by Tetracycline*				Doxorubicine*			
	Fresh		Heated		Fresh		Heated	
	IG† complete	IG detectable	IG complete	IG detectable	IG complete	IG detectable	IG complete	IG detectable
1	‡	‡	225*	112	17*	1.7	17	1.7
5	‡	112	225	112	17	0.86	17	0.86
15	225	22	112	11	17	0.17	17	0.17
60	112	22	112	11	8.6	0.09	8.6	0.09
120	112	22	112	11	8.6	0.09	8.6	0.09
Continuous (4 days)	22	11	22	11	0.86	0.017	0.86	0.017

*Numbers in the table represent minimal drug concentrations in μM for production of inhibition of growth (IG).
†Definitions of complete and detectable inhibition of growth (IG): see Materials and Methods.
‡No inhibition of growth (IG) with 225 μM of freshly prepared tetracycline.

(Lunsix 3, Gossen, F.R.G.) containing an adapter piece fitting the 16-mm wells of the plastic plates. The results were recorded the following way. Complete growth inhibition (Fig. 1, A2) corresponded to a light intensity (measured in lux) equal to that of plates stained 2 hr after cell seeding; detectable inhibition of growth (Fig. 1, B3) corresponded to an increase of light intensity of at least 25% in each well over the control wells.

RESULTS

Table 1 shows the concentrations necessary to produce growth inhibition of hypernephroma cells by tetracycline and doxorubicine either prepared freshly or heated for 10 min at 100°C. The heated tetracycline was more active than the freshly prepared when the time of drug–cell contact was 15 min or less. This difference disappeared at 60 min drug–cell contact. The heating of doxorubicine did not change its growth inhibitory activity. Figure 1 illustrates the difference of growth inhibitory activity of heated and freshly prepared tetracycline solutions during short time drug–cell contact. The figure also demonstrates that which we photometrically define as complete inhibition of growth (corresponding to the light intensity of stained wells 2 hr after seeding 30,000 control cells; Fig. 1, A2), as detectable inhibition of growth (Fig. 1, B3) and as no inhibition of growth (corresponding to the light intensity of control cells forming a monolayer with 1.3 million cells after 4 days of incubation; Fig. 1, B4).

The tetracycline preparation (Achromycine) which is used in the treatment of pleural effusions contains ascorbic acid. To exclude any influence of ascorbic acid on the growth of the hypernephroma cells growth assays were done with a wide range of ascorbic acid concentrations (from 56 μM to 11.3 mM). No growth inhibition or growth enhancement could be observed. In addition experiments were performed with freshly prepared and heated minocycline (which does not contain ascorbic acid) on hypernephroma and mammary carcinoma cells. Inhibition of growth in both cell lines with freshly prepared and heated minocycline was observed at the following concentrations after 4 days of continuous drug–cell contact: complete inhibition of growth at 22 μM, detectable inhibition of growth at 2.2 μM, which is similar to tetracycline (see Table 1).

DISCUSSION

Effective control of recurrent malignant effusion greatly improves the quality of life of the cancer patient. Different techniques have been advocated for controlling this complication of neoplastic disease. The treatment of choice today seems to be tube drainage followed by instillation of tetracycline [1]. The common hypothesis is that its effectiveness is related to its low pH. Doubts about this hypothesis led to a randomized study comparing tetracycline to a solution of similar pH and appearance [3]. This study suggested that the efficacy of tetracycline is not related to its acidic pH.

An antitumor effect of tetracycline by inhibition of mitochondrial protein synthesis has been shown in several animal systems [8, 9]. Our observation demonstrating growth inhibition of human hypernephroma and mammary cancer cells *in vitro* by heated tetracycline and minocycline similar to doxorubicine adds another possibility of a mechanism of action: tetracyclines lose the activity classically attributed to them, i.e. the inhibition of protein synthesis at the level of the ribosomes, rapidly when heated [10] or more slowly when in solution [11, 12]. As tetracycline is known to bind to DNA [13] the possibility that the degradation products such as epitetracycline, anhydrotetracycline and epianhydrotetracycline (EATC) [14] bind even more efficiently to DNA and act like anthracyclines

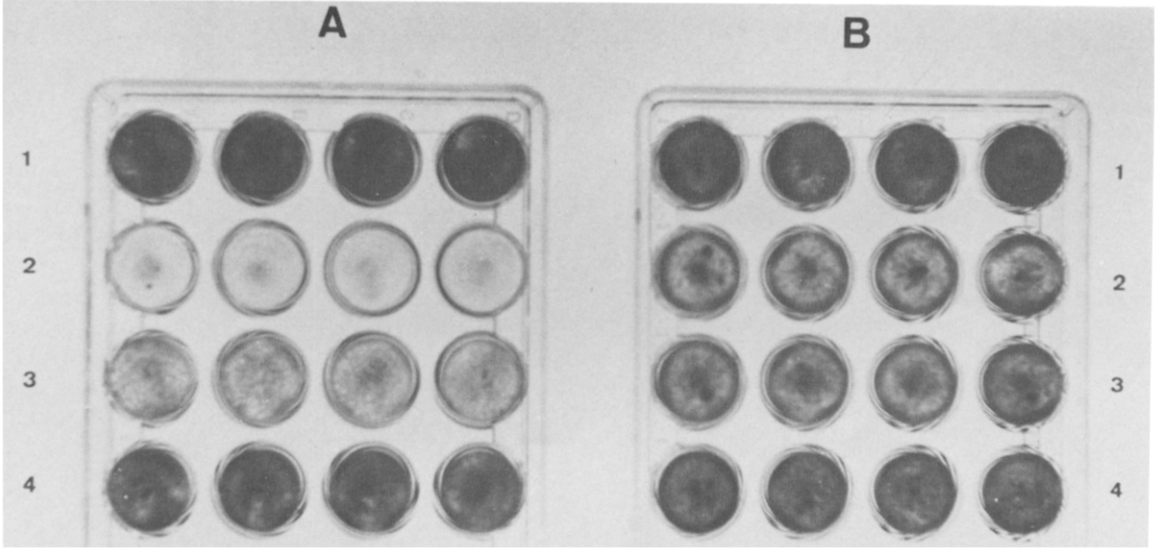

Fig. 1. *Example of growth inhibition of hypernephroma cells by tetracycline*–HCl. *Time of drug contact* 5 *min. A*1 *and B*1: *medium control* (*complete monolayer*). *A*2–*A*4: *decreasing concentrations* (μM) *of heated tetracycline*: 225, 112, 22 (*decreasing inhibition of growth*). *B*2–*B*4: *decreasing concentrations* (μM) *of freshly prepared tetracycline*: 225, 112, 22 (*just detectable inhibition of growth at* 225 *and* 112 μM).

as intercalating agents should be considered. These degradation products resemble in structure the anthracyclines being of a biosynthetic origin similar to the tetracyclines [15].

In our *in vitro* system the tetracycline exerts its growth inhibitory activity more rapidly after being heated to 100°C (see Table 1 and Fig. 1). The decomposition products of tetracyclines increase with temperature: at 40°C 80% of the tetracycline is present after 24 hr, while at 50°C only 60% remains. Increasing amounts of degradation products can be found [12]. Since EATC e.g. appears at 40°C after only a few hours of incubation [12] it must also be present in substantial amounts in the pleural cavity during tetracycline treatments.

In conclusion: to prove our suggestion that decomposed tetracycline plays a therapeutic role in the control of malignant pleural effusions the next obvious step will be the separation of the different tetracycline degradation products and their testing for cytostatic activity.

REFERENCES

1. Hausheer FH, Yarbro JW. Diagnosis and treatment of malignant pleural effusion. *Sem Oncol* 1985, **12**, 54–75.
2. Sahn SA, Good JT. The pH of sclerosing agents. *Chest* 1979, **76**, 198–200.
3. Zaloznik AJ, Oswald SG, Langin M. Intrapleural tetracycline in malignant pleural effusions. A randomized study. *Cancer* 1983, **51**, 752–755.
4. Groscurth P, Kistler GS. Human renal cell carcinoma in the nude mouse: long term observations. *Beitr Pathol* 1977, **160**, 337–360.
5. Sauter Chr, Cogoli M, Arrenbrecht S. Interactions of cytotoxic and other drugs: rapid cell culture assay. *Oncology* 1986, **43**, 46–49.
6. Sauter Chr, Baechi T, Lindenmann J. Human mammary carcinoma cell line: infection by an avian myxovirus as a prerequisite for immunopotentiation. *Eur J Cancer* 1975, **11**, 59–63.
7. Kistler GS, Bischoff A. Zur exfoliativen Zytologie kleiner Flüssigkeitsmengen. *Schweiz med Wschr* 1962, **92**, 863–965.
8. Van den Bogert C, Dontje BHJ, Wybenga JJ, Kroon AM. Arrest of *in vivo* proliferation of Zajdela tumor cells by inhibition of mitochrondrial protein synthesis. *Cancer Res* 1981, **41**, 1943–1947.
9. Van den Bogert C, Dontje BHJ, Kroon AM. The antitumor effect of doxycycline on a T-cell-leukemia in the rat. *Leukemia Res* 1985, **9**, 617–623.
10. Weiss PJ, Andrew ML, Wright WW. Solubility of antibiotics in twenty-four solvents: use in analysis. *Antibiot Chemother* 1957, **7**, 374–377.
11. Sande MA, Mandell GL. Tetracyclines. In: Goodman Gilman A, Goodman LS, Rall TW, Murad F, eds. *The Pharmacological Basis of Therapeutics*, 7th edn. New York, Macmillan, 1985, 1170–1179.
12. Thomson HJ, Merani S, Miller SS. Storage of tetracycline solutions for peritoneal lavage. *J R Coll Surg Edinb* 1984, **29**, 379–380.
13. Kohn KW. Mediation of divalent metal ions in the binding of tetracycline to macromolecules. *Nature* 1961, **191**, 1156–1158.
14. Walton VC, Howlett MR, Selzer GB. Anhydrotetracycline and 4-epianhydrotetracycline in market tetracyclines and aged tetracycline products. *J Pharm Sci* 1970, **59**, 1160–1164.
15. Hutchinson CR. The biosynthesis of tetracycline and anthracycline antibiotics. In: Corcoran JW, ed. *Antibiotics*. Berlin, Springer, 1981, Vol. IV, 1–11.