CYTOTOXICITY OF SEVERAL MARKETED ANTIBIOTICS ON MAMMALIAN CELLS IN CULTURE

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The cytotoxicity and biochemical effects of several marketed antibiotics on four mammalian cell lines were determined. Several metabolites of clindamycin and several clinically useful anticancer drugs were also included in this study. The four cell lines were mouse leukemia L1210, human oral carcinoma KB, human acute myelogenous leukemia RPMI 6410, and human lymphocyte RPMI 1788. At concentrations of 0.4 μmole/ml, ampicillin, lincomycin and penicillin G were not lethal for L1210 cells, nor had they any significant inhibitory effects on the growth of the other cell lines. Tetracycline was the most cytotoxic antibiotic tested, followed by clindamycin and erythromycin, cephaloglycin, and chloramphenicol. In general, inhibition of cell growth paralleled lethality for L1210 cells. Tetracycline was also found to have a stronger inhibitory effect than clindamycin on DNA, RNA, and protein synthesis in human RPMI 6410 cells. Drug effects on macromolecular synthesis correlated closely to those on cell growth. Among the clindamycin metabolites, clindamycin sulfoxide and clindamycose were nontoxic and only N-demethyl clindamycin had an activity equivalent to that of clindamycin. The structure-activity relationships of these metabolites are briefly discussed. None of the antibiotics tested, with the possible exception of tetracycline, however, approached the potency exhibited by most anticancer drugs. The lack of potential for these antibiotics as anticancer drugs is discussed.

The biological and biochemical effects on mammalian systems observed with clindamycin are discussed in terms of the possible relation to pseudomembranous colitis.

This investigation was undertaken to determine the cytotoxicity and biochemical effects of several clinically useful antibiotics on normal and neoplastic mammalian cells in culture and to evaluate the potentiality for using these drugs as anticancer agents. Several classes of clinically useful anticancer drugs were also included in this study for comparison purposes.

Materials and Methods

Materials.

The labeled precursors; [Methyl-^{3}H] thymidine (2.2 Ci/mmole), [5-^{3}H] uridine (27 Ci/mmole) and DL(1-^{14}C) leucine (40~60 mCi/mmole) were purchased from New England Nuclear, Boston, Massachusetts. RPMI 1629 and 1634 media were supplied by Associated Biomedic Systems, Inc., Buffalo, New York. EAGLE's basal medium (BME) was obtained from Microbiological Associates, Bethesda, Maryland.

Mammalian Cell Growth in Culture.

The basal media for growing mouse leukemic L1210 cells, human acute myelogenous leukemia RPMI 6410 and human lymphocyte were RPMI 1634 with 5% fetal calf serum (FCS)[1], RPMI 1634 with 10% FCS[2], and RPMI 1629 with 10% FCS and 1% human serum (a modification of GLICK *et al.*[3]), respectively. Sodium bicarbonate (0.075%), penicillin (0.1 mg/ml of medium), and streptomycin (50 μg/ml of medium) were added to each of the basal media. KB cells were cultured in BME with 10% FCS, bacitracin (100 μg/ml), streptomycin (100 μg/ml), and peptone (500 μg/ml)[4]. Cells used for the experiments were incubated at 37°C in the logarithmic phase of growth.

Growth Inhibition Studies.

For KB cells, 3.8 ml of cell suspension (approximately 2.5×10^4 cells/ml) and 0.2 ml of drug were pipetted in plastic culture tubes with screw caps (16 mm × 125 mm; Falcon Plastic Co., Oxnard, California). After 3 days incubation, the medium was decanted and cells were detached with 0.05% trypsin (10 min). Cells were counted with a Coulter counter (Coulter Electronics, Hialeah, Florida). For the other three cell lines, 4 ml of cell suspension (approx. 5×10^3 cells/ml for L1210 cells and 1×10^4 cells/ml for either RPMI 6410 or RPMI 1788) were incubated with 1 ml of drug or solvent for 3 days and cell number was determined. The ID_{50} (concentration for 50% inhibition of cell growth) was calculated[1].

For time-course studies, either RPMI 6410 or RPMI 1788 (approx. 5×10^5 cells/ml) was incubated with drug for 4 and 24 hours, following which the cells were washed twice and then resuspended in fresh medium at a concentration of 1×10^4 cells/ml. Cells were counted after 3 days growth.

L1210 Cell Survival Studies.

The cloning method for determining cell survival has been described[5]. In all cases we used duplicate cultures per sample and cells in exponential growth at the time of drug exposure. Briefly, drug-treated L1210 cells were centrifuged, washed, and resuspended at 10^5 cells/ml. The cells were serially diluted in medium, and the final dilution was made in RPMI 1634 medium containing 20% serum and 0.15% agar. Cells were dispensed into three culture tubes per sample and incubated for 8~10 days, following which colonies were visually counted. To calculate percentage survivals, the control (no drug treatment) samples were normalized to 100% survival. The coefficient of variation in determining cell survival was about 15%, the coefficient of variation being the standard deviation expressed as a percentage of the mean.

Macromolecular Synthesis.

The procedure used in this study has been described[1]. The specific activity for incorporation of a labeled metabolite was expressed as cpm/10^6 cells.

For the time-course studies, RPMI 6410 cells (approx. 5×10^5 cells/ml) were incubated with drug for 4 and 24 hours. The drug was then removed by centrifugation, cells were washed twice and resuspended in fresh medium. The cells were incubated with labeled metabolite for 40 minutes, following which the radioactivity was determined.

Results

Inhibition of Mammalian Cell Growth by Antibiotics and Antitumor Drugs

In view of the results (Table 1), we considered the antibiotic to be growth inhibitory when the ID_{50} was less than 0.4 μmole/ml. The results indicate that tetracycline was the most inhibitory antibiotic towards cell growth, followed by clindamycin and erythromycin. Cephaloglycin was much more growth inhibitory to KB and RPMI 1788 than to RPMI 6410. Chloramphenicol was much more inhibitory towards L1210 cells, but was nontoxic to all three human cell lines. The reasons for these differences remain obscure. Ampicillin, lincomycin, and penicillin G did not significantly inhibit any of the four mammalian cell lines ($ID_{50} > 0.4$ μmole/ml).

Of the clindamycin metabolites (Fig. 1), N-demethyl clindamycin was as active as clindamycin (Table 1). The other three metabolites; namely, clindamycose, clindamycin sulfoxide, N-demethyl clindamycin sulfoxide, were inactive. N-Demethyl lincomycin, an analog of lincomycin[6] was also inactive.

Several classes of anticancer drugs were also tested against three mammalian cell lines, and the results are summarized in Table 2. Actinomycin D and adriamycin, vincristine, and 5-fluorodeoxyuridine were most inhibitory to cell growth followed by the antimetabolites, methotrexate, cytosine arabinoside, and 5-fluorouracil, and two alkylating agents, BCNU (1,3-bis(2-chloroethyl)-1-nitroso-

Table 1. Inhibition of mammalian cell growth by several marketed antibiotics

Drug	ID_{50} (μmole/ml)[a]			
	Mouse cell L1210	Human cells		
		KB	RPMI 6410	Lymphocyte RPMI 1788
Ampicillin (trihydrate)	0.43	2.08	0.90	>1.35
Cephaloglycin (dihydrate)	0.39	0.16	>1.13[b]	0.11
Chloramphenicol	0.02~0.03	0.50	0.49~0.65	0.74
Clindamycin (HCl, hydrate)	0.15	0.22	0.16	0.29
N-Demethyl clindamycin (HCl)	0.07	0.29	0.13	0.32
Clindamycose	0.96	1.95	0.82	>1.27
Clindamycin sulfoxide (HCl)	1.72	2.09	>1.13	>1.13
N-Demethyl clindamycin sulfoxide	2.16	2.03	>1.08	>1.08
Erythromycin	0.18	0.16	0.18	0.42
Lincomycin (HCl)	4.42	3.32	>1.11	>1.11
N-Demethyl lincomycin (HCl)[c]	>4.57	>4.57	>1.14	>1.14
Penicillin G	>5.38	>5.38	>1.34	>1.34
Tetracycline (HCl)	0.02	0.08	0.05	0.08

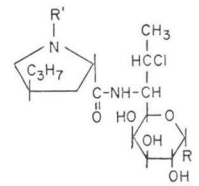
[a] Drug concentration required for 50% inhibition of cell growth in culture after incubation with drug for 3 days (37°C).
[b] Repeated twice.
[c] A probable metabolite although it has not been isolated in the biological systems.

urea), and chlorambucil. Hydroxyurea was least inhibitory. In most cases, RPMI 1788 was less sensitive to the antimetabolites than either RPMI 6410 cells or L1210 cells.

Lethality of Antibiotics for Mouse L1210 Cells

Survival of L1210 cells after 4, 6, or 24-hour exposure to the drug was determined by a cloning assay. Results (Table 3) indicate that ampicillin, lincomycin, and penicillin G were not lethal and more than 90% of the cells survived after a 24-hour exposure to the drugs (>2 μmole/ml). Tetracycline was the most cytotoxic antibiotic, followed by clindamycin, chloramphenicol, erythromycin, and cephaloglycin. For example, after a 4-hour exposure, the drug concentrations (μmole/ml) needed to kill 50% of the cells were 0.19 for tetracycline, 0.70 for erythromycin, 0.91 for clindamycin, 2.29 for chloramphenicol, and about 2.27 for cephaloglycin. The same order of cytotoxicity was seen after a 24-hour exposure. In comparison, cytosine arabinoside was much more lethal, since 0.0036 μmole/ml of the drug killed 100% of L1210 cells within 24 hours (Table 3).

Fig. 1. Structures of metabolites of clindamycin.



R' = CH_3, R = SCH_3 Clindamycin
R' = H, R = SCH_3 N-Demethylclindamycin
R' = CH_3, R = $-\overset{O}{\overset{\uparrow}{S}}-CH_3$ Clindamycin sulfoxide
R' = H, R = $-\overset{O}{\overset{\uparrow}{S}}-CH_3$ N-Demethylclindamycin sulfoxide
R' = CH_3, R = -OH Clindamycose

Of the clindamycin metabolites, N-demethyl clindamycin was the only cytotoxic compound (Table 3). Clindamycose, clindamycin sulfoxide, and N-demethyl clindamycin sulfoxide, N-demethyl lincomycin, and lincomycin were not lethal (>90% survival at 2 μmole/ml).

Table 2. Effects of several classes of anticancer drugs on growth of human and mouse cells in culture

	Drug	ID_{50} (nmole/ml)[a]		
		L1210 Mouse leukemia	RPMI 6410 (Human AML)	RPMI 1788 (Human lymphocyte)
Antimetabolite	Cytosine arabinoside	0.028	0.081	0.143
	5-Fluorouracil	0.82	0.49	2.82
	5-Fluorodeoxyuridine	0.0008	0.0024	2.033
	Methotrexate	0.048	0.066	0.032
Alkylating agent	BCNU	2.1	1.8	2.6
	Chlorambucil	5.5	4.7	8.7
Antibiotic	Actinomycin D	0.0009	0.0004	0.0006
	Adriamycin	0.005	0.0027	0.0029
Plant alkaloid	Vincristine sulfate	0.0023	0.0014	0.0002
Miscellaneous	Hydroxyurea	24.5	53.3	34.2

[a] ID_{50}=drug concentration required for 50% inhibition of cell growth (3 day assay).

Inhibition of Macromolecular Synthesis in RPMI 6410 Cells

Since RPMI 6410 is a human tumor cell line and was slightly more sensitive to these antibiotics than KB and RPMI 1788, it was chosen to study the inhibition of DNA, RNA, and protein synthesis (Table 4). Clindamycin was not inhibitory at 0.23 μmole/ml but showed significant inhibition of macromolecular synthesis at 1.08 μmole/ml. N-Demethyl clindamycin was at least as active as clindamycin. Tetracycline, at 0.24 μmole/ml, inhibited approximately 80%, 40%, and 70% of DNA, RNA, and protein synthesis, respectively (data not presented). Lincomycin had no significant effect at 1.1 μmole.

Effects of clindamycin and tetracycline were further evaluated by removal of drug after a 4- and 24-hour incubation period. Cell growth was determined at the end of 3 days and macromolecular syn-

Table 3. Lethality of several marketed drugs towards L1210 cells in culture[a]

Drug	Concentration (μmole/ml)	Cell survival (%)	
		4- or 6-hour exposure	24-hour exposure
Ampicillin (trihydrate)	2.70	116±11[b]	90±13
Cephaloglycin (dihydrate)	2.27	55	1
	0.68	—	64
Chloramphenicol	1.55	25	0
	0.77	97	105
Clindamycin (HCl, hydrate)	1.08	34.1	0
	0.54	86.5	28.2
	0.27	—	120
N-Demethyl clindamycin (HCl)	1.12	2[b]	0
	0.22	108	90
Clindamycose	2.53	97[b]	90
Clindamycin sulfoxide (HCl)	2.27	119[b]	103
N-Demethyl clindamycin sulfoxide (HCl)	2.16	102[b]	108
Erythromycin	1.29	3	<0.001
	0.65	64	<0.001
Lincomycin (HCl)	2.21	106	104
N-Demethyl lincomycin (HCl)	2.28	109[b]	79
Penicillin G	2.70	95[b]	100
Tetracycline (HCl)	0.47	1.6	0
	0.23	37.3	3.2
	0.12	93	43
Cytosine arabinoside	0.0036	17	0

[a] Drug was washed off after 4~6 or 24-hour incubation with cells and the cells planted in soft agar medium. Surviving colonies were counted after 8-day incubation.

[b] Six-hour exposure.

Table 4. Time course study of the inhibition of growth and macromolecular synthesis of human RPMI 6410 cells by antibiotics[a]

Drug	Concentration (μmole/ml)	Contact time (Hour)	% Inhibition macromolecular synthesis			
			Cell growth	DNA	RNA	Protein
Clindamycin (HCl)	0.022	4	0	0	0	0
		24	0	0	0	0
	0.23	4	0	0	0	0
		24	4.3	0	0	0
	1.08	4	20.2	7.2	4.2	10.9
		24	85.8	91.9	78.6	83.1
N-Demethyl clindamycin (HCl)	1.12	4	—	57.2	46.7	77.2
Lincomycin (HCl)	1.10	4	—	13.0	0	0
Tetracycline (HCl)	0.023	4	0	0~4.5	0	0
		24	16.1	0~30.2	0	17.0
	0.12	4	35.8	25.7	25.5	11.4
		24	89.1	96.8	78.5	82.9
	0.47	4	86.2	84.3	90.6	59.1
		24	85.2	93.1	81.6	85.9

[a] Drug was removed after a 4- or 24-hour incubation with the cells. For growth-inhibition studies, cell numbers were determined at the end of 72 hours (total incubation time). Average of triplicate samples. For macromolecular synthesis, a pulse labeling (40 min) with radioactive thymidine, uridine, or leucine was immediately followed by the removal of drug.

thesis was measured immediately after the removal of drug. The results (Table 4) indicate that neither macromolecular synthesis nor growth of RPMI 6410 cells was inhibited after a 24-hour incubation with 0.23 μmole/ml clindamycin, but both synthesis and growth remained inhibited after a 4-hour incubation with higher drug concentration (1.08 μmole/ml). The inhibitory effects of tetracycline were apparent when cells were exposed to 0.023 μmole/ml drug for 24 hours. At 0.12 μmole/ml, the drug effects were irreversible after a 4-hour incubation. Clearly, the inhibitory effects exhibited by both drugs were dose and time dependent, and their effects on macromolecular synthesis closely correlated with the growth inhibition as illustrated in Table 4.

Discussion

In 1959, SMITH *et al*[4] described the cytotoxic effect of several antibiotics including chloramphenicol and tetracycline on human KB cells in culture. Recently, DONTA[7] reported that clindamycin at 10 μg/ml (0.022 μmole/ml) or higher concentrations markedly impaired the DNA synthesis of various functional mammalian tumor cells, and suggested that clindamycin might have potential use as an anticancer agent. We decided to evaluate this possibility using four mammalian cell lines; namely, mouse leukemia L1210, human KB, human lymphocyte RPMI 1788, and human acute myelogenous leukemia RPMI 6410. Several marketed antibiotics and metabolites of clindamycin[6,8] were included in this study. Cytotoxic effects were measured both by inhibition of cell growth and cell-kill and biochemical effects were determined by the inhibition of DNA, RNA, and protein synthesis. Several clinically useful anticancer drugs were also included in this study for comparison purposes.

Our studies showed that tetracycline, erythromycin, clindamycin, and cephaloglycin were cytotoxic ($ID_{50} < 0.4$ μmole/ml). In contrast, ampicillin, penicillin G, and lincomycin were not. Of the clindamycin metabolites, only N-demethyl clindamycin was as cytotoxic as clindamycin (Tables 1 and 3). The metabolic transformations at the anomeric center (R) of α-D-galactopyranoside (Fig. 1), such

as in clindamycin sulfoxide ($R=-\overset{O}{\overset{\uparrow}{S}}-CH_3$) or clindamycose ($R=-OH$), resulted in the complete loss of their cytotoxic effects (Tables 1 and 3). These results clearly indicate that the substitution ($R=-SCH$) of anomeric center of α-D-galatopyranoside moiety of clindamycin was necessary for its cytotoxicity towards these mammalian cells. A similar association with antimicrobial activity was reported elsewhere[9].

By and large, none of the antibiotics tested approached the cytotoxic effect of several classes of clinically useful anticancer drugs on three mammalian cell lines (Table 2). Although the cytotoxic effects of tetracycline approached those of hydroxyurea, tetracycline has not been known to be an effective anticancer drug. Therefore, the potential for developing these antibiotics, including clindamycin as suggested by DONTA, as anticancer agents is not very promising unless some other merits could be demonstrated.

It was of interest that both L1210 cells and RPMI 6410 were strikingly more sensitive to 5-fluorodeoxyuridine (850~2,500 times) than RPMI 1788 cells. Also, both cell lines were more sensitive (3~6 times) to 5-fluorouracil than RPMI 1788. Whether this difference reflects a difference in metabolism of these two drugs by the three cell lines is not known.

Whether DNA synthesis inhibition is important in the pathogenesis of clindamycin-associated colitis[10] as suggested by DONTA[7], remains an issue to be resolved. Although ampicillin and lincomycin have been reported to be associated with pseudomembranous colitis[11], the drugs, in our hand have not been found to be very cytotoxic towards mammalian cells (Tables 1 and 3). DONTA's study did not show whether the inhibition of DNA synthesis by clindamycin was reflected in the inhibition of cell growth. Furthermore, clindamycin was about 50-times more inhibitory towards the DNA synthesis of mammalian tumor cells used by DONTA than towards those of RPMI 6410 (Table 4) and RPMI 1788 (data not presented). These results clearly indicate that the sensitivity of mammalian cells to clindamycin varied significantly among cell lines, suggesting that one should proceed with caution in trying to extrapolate the results obtained from different biological systems. A proper model system is needed for the evaluation of etiology of pseudomembranous colitis and the pathological manifestation should be monitored closely with drug concentration and duration.

Recently, ALPERS and GRIMME[12] reported the effects of clindamycin and its metabolites on DNA synthesis of rabbit colon maintained in organ culture. Clindamycose was found to be inhibitory in their system but was inactive in our tests. Although N-demethyl clindamycin was most inhibitory, the effects were generally reversible. No conclusion could be drawn from their study whether the pathogenesis of pseudomembranous colitis correlated with the transient biochemical effects observed.

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References

1) LI, L. H. & C. H. COWIE: Biochemical effects of ellipticine on leukemia L1210 cells. Biochim. Biophys. Acta 353: 375~384, 1974
2) MINOWADA, J.; M. NONOYAMA, G. E. MOORE, A. M. RAUCH & J. S. PAGANO: The presence of the EPSTEIN-BARR viral genome in human lymphoblastoid B-cell lines and its absence in a myeloma cell line. Cancer Res. 34: 1898~1903, 1974
3) GLICK, J. L.; C. LOCKWOOD, J. WILLIAMS, B. W. PAPERMASTER, A. A. BURNS & A. C. DUTTON: Human serum or an economical substitute for fetal bovine serum in lymphoid cell cultures. Transplantation 18: 86~87, 1974
4) SMITH, C. G.; W. L. LUMMIS & J. E. GRADY: An improved tissue culture assay. II. Cytotoxicity studies with antibiotics, chemicals, and solvents. Cancer Res. 19: 847~852, 1959
5) BHUYAN, B. K.; T. J. FRASER, L. G. GRAY, S. L. KUENTZEL & G. L. NEIL: Cell-kill kinetics of several S-phase-specific drugs. Cancer Res. 33: 888~894, 1973
6) BRODASKY, T. F.; A. D. ARGOUDELIS & T. E. EBLE: The characterization and thin-layer chromatographic

quantitation of the human metabolite of 7-deoxy-7(S)chlorolincomycin (U-21,251F). J. Antibiotics 21: 327~333, 1968
7) DONTA, S. T.: Inhibition of mammalian cell DNA synthesis by clindamycin. 15th Interscience conference on Antimicrobial Agents and Chemotherapy. Paper 171, Washington, September 24~26, 1975
8) BRODASKY, T. F.; C. LEWIS & T. E. EBLE: Distribution and metabolism of antibiotics in the whole animal. I. Clindamycin in the rat. J. Chromatogr. 123: 33~44, 1976
9) MAGERLEIN, B. J.: Modification of lincomycin. *In* D. PERLMAN (ed), Advances in Applied Microbiology, Vol. 14, pp. 185~229. Academic Press, New York, 1971
10) STROEHLEIN, J. R.; R. E. SEDLACK, H. N. HOFFMAN, Jr. & A. D. NEWCOMER: Clindamycin-associated colitis. Clin. Proc. 48: 240~243, 1974
11) SCHAPIRO, R. L. & A. NEWMAN: Acute enterocolitis. Radiology 108: 263~268, 1973
12) ALPERS, D. & N. GRIMME: Clindamycin-HCl inhibits DNA synthesis in mammalian colonic mucosa. Gastroenterology 70: 859, 1976