RELATIONSHIPS BETWEEN THE CHEMICAL STRUCTURE AND CYTOTOXICITY OF 4-ALKYLMORPHOLINE N-OXIDES

Milan Miko[1] and Ferdinand Devínsky[2]

[1]*Department of Microbiology, Biochemistry and Biology*
Slovak Technical University, Kollarovo nam. 9
812 37 Bratislava
[2]*Faculty of Pharmacy*
Komensky University, Kalinciakova 8
832 32 Bratislava
Czechoslovakia

SUMMARY

The main objective of the present investigation was to screen a series of 4-alkylmorpholine N-oxides for *in vitro* cytotoxicity and to find out whether there is a quantitative structure-activity correlation (QSAR) between cytotoxic effect represented here by inhibition of incorporation of [^{14}C]adenine into nucleic acid or [^{14}C]valine into proteins in Ehrlich ascites carcinoma (EAC) cells and structure (as a structural parameter the number of carbon atoms *m* in the alkyl chain was used). On the basis of primary screening, one of the most active compounds, namely 4-dodecylmorpholine N-oxide, was chosen for further biochemical study. The drug inhibited the incorporation rate of [^{14}C] precursors (adenine, thymidine, uridine, valine) into appropriate macromolecules of Ehrlich cells, the extent of inhibition being dependent on both time and concentration of the compound in the incubation medium. The lengthening of the alkyl chain in 4-alkylmorpholine N-oxides positively affected their cytotoxic activity in Ehrlich cells. For these compounds the optimal *m*-value is 15-16.

KEY WORDS

4-alkylmorpholine N-oxides, Ehrlich ascites cells, screening, biosynthesis of macromolecules, QSAR

INTRODUCTION

Amine oxides represent a large group of compounds derived from tertiary amines containing a strongly polarized N→O bond /1, 2/. A great number of amine oxides occurring in nature, or prepared synthetically, are biologically active compounds (antimetabolites and chemotherapeutics, psychotropic and cancerostatic compounds, etc.). Though some non-aromatic amine oxides have found wide industrial utilization due to their good surface active properties /3/, relatively little attention has been paid to their biological activity, in contrast to aromatic amine oxides /4, 5/.

In addition to the interesting chemical and biological activities shown by these compounds /6-9/, Ferencik *et al.* /10/ have recently opened new perspectives in the field of immunomodulation due to their concentration dependent influence on the immune system. With selected compounds of this type, immunosuppression was found to be as high as that of cyclosporin A /11, 12/, used today as one of the most powerful known immunosuppressants.

In the present study cytotoxic activities and mode of action of 4-alkylmorpholine-N-oxides were investigated. One of the goals of this study was to find out whether there is a quantitative structure-activity correlation (QSAR) between cytotoxic effect, represented here by inhibition of incorporation of [^{14}C]adenine into nucleic acids or [^{14}C]valine into proteins in EAC cells, and structure (as a structural parameter the number of carbon atoms m in the alkyl chain was used). For quantification the bilinear approach was used which is superior to the classical Hansch's parabolical model as already shown /13-16/. The chemical structure of the substances studied is shown in Figure 1. Synthesis, properties and antimicrobial activity of the compounds have been described by Devínsky /7-9/. The compounds of this type belong to the so-called "soft" antimicrobially active compounds /17/.

Ehrlich ascites tumor cells have been extensively used as an experimental model for biochemical investigation /18, 19/. We have used Ehrlich cells also for the study of the mechanism of action of some antibiotics /20/, ethidium bromide /21/, isothiocyanates /22/ and other known cancerostatics /23/.

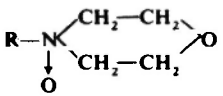
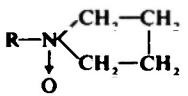
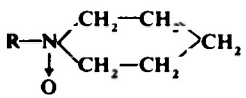
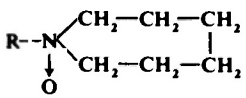
Group	Structural formula	Derivatives of	R	Number of compounds
A		morpholine	C_1 to C_{18}	18
B		pyrrolidine	$C_{12,14,16,18}$	4
C		piperidine	C_8 to C_{15}	8
D		perhydroazepine	C_6 to C_{10}	8
	Total number			38

Fig. 1: Survey of N-oxides investigated.

MATERIALS AND METHODS

Ehrlich ascites carcinoma cells were maintained and propagated in strain H Swiss albino mice (Institute of Experimental Pharmacology, Slovak Academy of Sciences, Dobrá Voda, Czechoslovakia), about 10 weeks old and 20 to 25 g body weight, as described previously /24, 25/. Ascitic plasma was poured off and an incidental layer of erythrocytes was removed /26/. The cells were suspended in Krebs-Ringer phosphate buffer, pH 7.4, without calcium but with ascitic serum (2.5%, v/v) and glucose (final concentration, 3.0 mmol/l). The number of cells was adjusted to 5 x 10^6/ml of medium /25/. All operations were performed at 0-4°C. The cells were suspended in Krebs-Ringer phosphate buffer, pH 7.4, without calcium but with ascitic serum (2.5%, v/v) and glucose (final concentration, 3.0 mmol/l).

Materials

Chromatographically pure amine oxides were from Dept. of Inorganic and Organic Chemistry, Faculty of Pharmacy, Komensky University, Bratislava. Substances were dissolved in Krebs-Ringer

phosphate medium and/or in water shortly before experiments. [8-^{14}C]Adenine sulfate (specific activity, 44 mCi/mmol), [U-^{14}C]valine (specific activity, 175 mCi/mmol), [2-^{14}C]thymidine (specific activity, 53 mCi/mmol), and [2-^{14}C] uridine, (specific activity, 53 mCi/mmol) came from the Institute for Research, Production and Applications of Radioisotopes, Prague, Czechoslovakia. Other chemicals were supplied by Boehringer, Mannheim, Germany. All other reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Primary biochemical screening (cytotoxicity assays)

In our laboratory, a new system has been developed and is being used routinely for mass screening of candidate compounds for antineoplastic activity /27-29/. The procedure used in evaluating the cytotoxic effect of the compounds was similar to that used when testing other metabolic inhibitors /28, 30/. In short, cells were incubated 1 h in the presence of at least four selected concentrations of the substance, under defined conditions *in vitro*, and the active synthesis of nucleic acids and proteins was followed. After 1 h of drug exposure, the test-tubes were transferred into an ice bath. [8-^{14}C]Adenine was added to the first series to a final concentration of 0.187 μCi per 1.02 μg and L[U-^{14}C]valine was added to the second series to a final concentration of 0.165 μCi per 2.64 μg. Both series were again incubated for 1 h at 37°C. In control experiments only Krebs-Ringer phosphate medium or ethanol were used. Incorporation was terminated by adding 1 ml of 5% TCA to each test-tube in an ice bath. The samples were filtered through synpor membrane filters, pore size 4 μm (Synthesia, Prague), the precipitate washed with 10 ml of cold 2.5% TCA and 10 ml water and dried at 105°C. The radioactivity was measured on a methane flow counter (Frieseke und Hoepfner, Erlangen, Germany).

Kinetics of DNA, RNA and protein synthesis

To define further the mechanism of action of selected drugs, the kinetics of DNA, RNA and protein synthesis inhibition were examined using isotope incorporation. This method has been described in detail /30/. The cells were incubated in a water bath at 37°C without shaking. At the indicated time intervals, samples of suspensions (1 ml) were analyzed for radioactivity in acid-insoluble material. Ra-

dioactivity was measured on a methane flow counter as in primary biochemical screening. In some cases, the nature of the labeled material was checked by alkaline-acid hydrolysis. In the case of adenine incorporation, 60.6% of the incorporated radioactivity corresponds to the RNA fraction and 39.4% corresponds to that of DNA. In the case of thymidine, 90% of its incorporation was found in DNA. In the case of uridine, 87.5% of the radioactivity was found in the RNA fraction /25/. All the data points are from duplicate determinations. The precision of these measurements is ±5%.

RESULTS

Biochemical screening of cytotoxic activity

The results from primary biochemical screening of the cytotoxic activity on Ehrlich ascites cells are summarized in Table 1. For the chemical structures of the substances studied, see Figure 1. The numbers represent cpm, with percentage of inhibition (or stimulation) in parentheses. The inhibitory effect was characterized by IC_{50} values (molar concentration of compound required for 50% reduction of the incorporation rate). As seen from the results in Table 1, derivatives I-X show little effect on incorporation of both precursors. On the other hand, derivatives with longer side-chains significantly depress the incorporation of both precursors investigated (substances XI-XVII). This has been confirmed not only by percentage inhibition (given in parentheses) but also by IC_{50} values. The lengthening of the alkyl chain in 4-alkylmorpholine-N-oxides positively affected their cytotoxic activity in Ehrlich cells. IC_{50} values are much higher for the first 10 substances than for substances XI-XVII, according to the length of the side-chain. In order to calculate IC_{50} values, however, much lower concentrations were needed and therefore we repeated the experiments as indicated in the lower portion of Table 1. IC_{50} values for adenine as well as for valine are very similar. Maximum activity was achieved with the compounds Nos. XIII-XVII (Table 1, lower part). Further lengthening led to decrease in activity.

Cytotoxic activity, expressed as IC_{50} values for adenine and valine, increased with increasing alkyl chain length, reaching a maximum with C_{13} to C_{15} (Fig. 2). The 4-alkylmorpholine-N-oxides containing

TABLE 1

Primary biochemical screening of 4-alkylmorpholine-N-oxides. The measure of the cytotoxic effect was the degree of inhibition of [^{14}C]adenine (a) and [^{14}C]valine (b) incorporation into TCA-insoluble fraction of Ehrlich ascites cells after 2 h incubation *in vitro*

No	R	Formula	M.W.		μmol/l					IC_{50} μmol/l	R
					0	75	150	300	600		
					Inhibition of incorporation in cpm or percent (in brackets)						
I	methyl	$C_5H_{11}NO_2$	118.15	(a)	1532(0)	1693(+10.51)	1415(7.64)	1714(+11.88)	1660(+8.35)	>600	?
				(b)	1822(0)	1638(10.1)	1727(5.22)	1639(10.4)	1813(0.49)	>600	
II	ethyl	$C_6H_{13}NO_2$	131.18		1532(0)	1631(+6.46)	1700(+10.97)	1431(6.59)	1066(30.42)	>600	?
					1822(0)	1776(2.53)	1705(6.42)	1237(32.11)	999(45.17)	>600	
III	propyl	$C_7H_{15}NO_2$	145.20		1532(0)	1573(+2.68)	1757(+14.69)	1106(27.81)	1844(+20.36)	>600	?
					1822(0)	1468(19.43)	1924(+5.59)	1558(14.49)	1530(16.05)	>600	
IV	butyl	$C_8H_{17}NO_2$	159.23		1532(0)	1710(+11.62)	1627(+6.20)	1401(8.55)	1371(10.51	>600	?
					1822(0)	1662(8.78)	1538(15.59)	1846(+1.32)	1284(29.53)	>600	
V	pentyl	$C_9H_{19}NO_2$	173.24		1532(0)	1781(+16.25)	1695(+10.64)	803(47.59)	724(52.74)	450	0.9
					1822(0)	1344(26.24)	1142(37.32)	1060(41.82)	820(55.0)	500	
VI	hexyl	$C_{10}H_{21}NO_2$	187.28		2366(0)	2126(9.7)	2140(9.6)	2140(9.6)	2079(12.1)	>600	?
					1766(0)	1956(+10.8)	1724(+2.4)	1843(+4.3)	1637(7.3)	>600	
VII	heptyl	$C_{11}H_{23}NO_2$	201.30		1453(0)	1540(+5.98)	1338(7.92)	1340(7.78)	1553(+7.23)	>600	?
					1452(0)	1272(12.40)	1448(0.28)	1376(5.24)	1256(13.5)	>600	
VIII	octyl	$C_{12}H_{25}NO_2$	215.33		1483(0)	1482(+1.99)	1354(6.81)	1314(9.57)	1366(5.99)	>600	?
					1452(0)	1396(3.86)	1396(3.86)	1456(+0.27)	1192(17.91)	>600	
IX	nonyl	$C_{13}H_{27}NO_2$	229.37		1483(0)	1446(0.48)	1310(9.84)	1392(4.2)	1372(5.58)	>600	?
					1452(0)	1394(4.0)	1378(5.10)	1362(6.20)	1142(21.35)	>600	

cont.

TABLE 1 (Continued)

No	R	Formula	MW	μmol/l					IC_{50} μmol/l	R
				0	75	150	300	600		
X	decyl	$C_{14}H_{29}NO_2$	243.40	1453(0)	1296(10.81)	1128(22.37)	1336(8.05)	1230(15.35)	>600	?
				1452(0)	1222(15.84)	1290(11.16)	1176(19.1)	1088(25.07)	>600	
XI	undecyl	$C_{15}H_{31}NO_2$	257.42	1453(0)	1468(+1.03)	1062(26.91)	1094(24.71)	256(82.38)	400	1.5
				1452(0)	1036(28.65)	892(38.57)	598(58.32)	221(84.85)	260	
XII	dodecyl	$C_{16}H_{33}NO_2$	271.45	4928.0)	1851(62.24)	974(80.24)	117(97.63)	133(97.32)	<75	?
				1653(0)	1056(36.51)	624(62.25)	84(94.92)	117(92.92)	120	
XIII	tridecyl	$C_{17}H_{35}NO_2$	285.47	4928.0)	508(89.69)	172(96.51)	141(97.14)	121(97.55)	<100	?
				1653(0)	341(79.37)	59(96.43)	230(86.09)	91(94.5)	<100	
XIV	tetradecyl	$C_{18}H_{37}NO_2$	299.50	4923(0)	286(94.20)	121(97.55)	113(97.71)	180(96.35)	<100	?
				1653(0)	85(94.86)	67(95.95)	81(95.10)	73(95.58)	<100	
XV	pentadecyl	$C_{19}H_{39}NO_2$	313.53	4928(0)	375(92.31)	210(98.07)	95(94.93)	92(98.13)	<100	?
				1653(0)	836(49.43)	91(94.19)	68(95.89)	71(95.71)	<100	
XVI	hexadecyl	$C_{20}H_{41}NO_2$	327.56	1887(0)	246(86.96)	159(91.57)	106(94.38)	–	<100	?
				1473(0)	110(92.53)	81(95.21)	66(95.52)	–	<100	
XVII	heptadecyl	$C_{21}H_{43}NO_2$	341.57	2366(0)	1332(27.6)	517(78.2)	453(80.9)	282(88.1)	<150	?
				1766(0)	1351(+7.4)	619(63.8)	200(88.7)	235(86.7)	<150	
XVIII	octadecyl	$C_{22}H_{45}NO_2$	355.61	2366(0)	2302(15.4)	2179(7.9)	1845(22.0)	1794(24.2)	>600	?
				1766(0)	1468(16.9)	1586(10.2)	1422(19.5)	1203(31.9)	>600	

cont.

TABLE 1 (Continued)

No	R	Formula	M.W.		μmol/l					IC_{50} μmol/l	R
					0	12.5	25	50	100		
					Inhibition of incorporation in cpm or percent (in brackets)						
XIII	tridecyl	$C_{17}H_{35}NO_2$	285.47	(a)	2400 (0)	2425 (+1.04)	2425 (+1.04)	2166 (9.75)	899 (62.54)	88	1.07
				(b)	3683 (0)	3606 (2.09)	3448 (6.38)	2528 (31.36)	1443 (60.69)	82	
XIV	tetradecyl	$C_{18}H_{37}NO_2$	299.50		2400 (0)	2154 (10.2)	3439 (+43.3)	2222 (7.4)	349 (85.45)	77	1.18
					3683 (0)	2416 (34.4)	2979 (19.12)	2491 (32.37)	337 (90.85)	65	
XV	pentadecyl	$C_{19}H_{39}NO_2$	313.53		2400 (0)	2377 (0.95)	1991 (17.04)	2151 (10.38)	353 (85.29)	75	0.98
					3683 (0)	3321 (9.83)	2610 (29.13)	3161 (14.17)	207 (94.38)	76	
XVI	hexadecyl	$C_{20}H_{41}NO_2$	327.56		2400 (0)	2397 (0.13)	2148 (10.5)	1805 (21.79)	282 (88.25)	70	1.02
					3683 (0)	3482 (5.46)	3364 (8.66)	2701 (26.6)	222 (93.97)	68	
XVII	heptadecyl	$C_{21}H_{43}NO_2$	341.57		2400 (0)	2182 (9.08)	2740 (+14.17)	2267 (5.54)	1350 (43.75)	108	1.2
					3683 (0)	3313 (10.05)	2634 (28.48)	2804 (23.87)	1510 (59.01)	88	

+stimulation over 100% against control sample. Substances Nos. I - XVI were dissolved in Krebs-Ringer phosphate medium and substances XVII - XVIII in dimethylsulfoxide shortly before experiments. R = IC_{50}adenine:IC_{50}valine

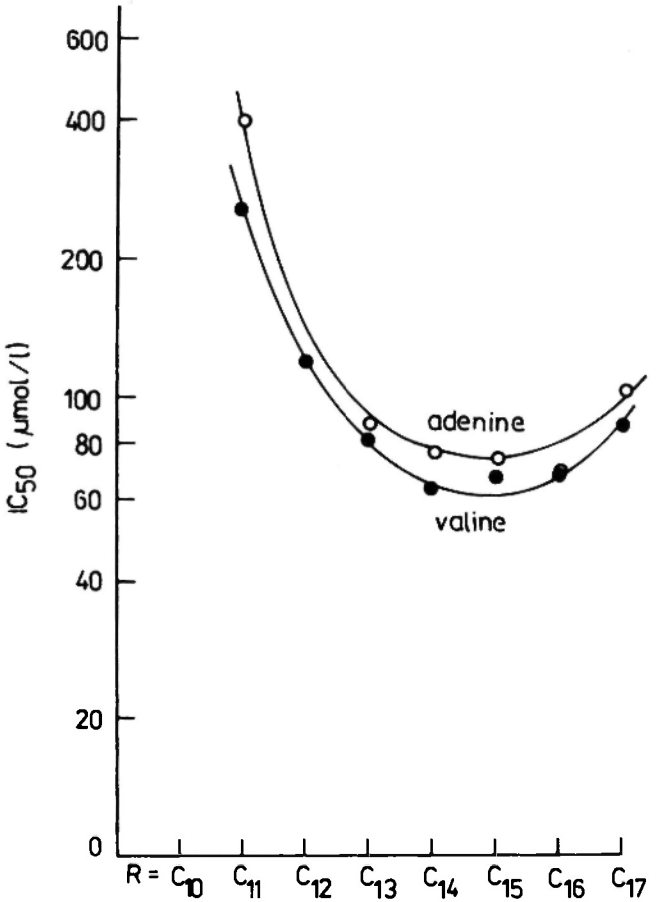


Fig. 2: Relationships between IC_{50} values (adenine, valine) and the length of side-chain in 4-alkylmorpholine-N-oxides.

an alkyl chain shorter than C_{10} were found to be less effective (IC_{50} $>600\ \mu$mol/l). Qualitatively, the same results were obtained for a homologous series of 1-alkylpyrrolidine-N-oxides (results not shown).

With the membrane active monoamine N-oxide amphiphiles investigated in this study, we focused our interest on the effect of alkyl chain length upon biological activity, manifested as inhibition of

incorporation of radioactive labeled adenine and valine in cancer cells.

The relationships $\log (1/IC_{50}) = f(m)$ (m is the number of carbon atoms in the alkyl chain C_mH_{2m+1}) showed a non-linear course (Table 2) which was quantified using the bilinear approach

$$\log (1/IC_{50}) = Am + B\log (\beta 10^m + 1) + C$$

According to the present state of knowledge of the biological activities of non-aromatic amine oxides, one can see that on lengthening the alkyl chain the activity increases and after having reached a maximum decreases again (Table 2). The heterocycle size variation

TABLE 2

Effect of 4-alkylmorpholine N-oxides on [^{14}C]adenine and [^{14}C]valine incorporation into whole Ehrlich ascites carcinoma cells [at 300 μmol/dm^3; log (I/IC$_{50}$)]

m	[^{14}C]adenine	[^{14}C]valine
8	2.8563	2.8368
9	2.8814	2.8658
10	2.8741	2.9295
11	2.9609	3.2232
12	3.9318	4.0757
13	3.8507	3.6382
14	3.9469	4.0915
15	4.0222	4.1674
16	3.9776	4.1804
17	3.3439	3.6989
18	2.7340	2.8471

Respective regression equations calculated from above data:

$$\log (I/IC_{50})_{AD} = (0.218\pm0.032)m - (0.881\pm0.132)\log(\beta 10^m + 1) + (0.925\pm0.378) \quad [1]$$

$n = 11$ $r = 0.932$ $s_D = 0.221$ $F = 26.45$ $\log\beta = -15.592$

$m_{opt} = 15.1$ $\log(I/IC_{50})_{AD,max} = 4.1092$

$$\log(I/IC_{50})_{VAL} = (0.222\pm0.028)m - (1.087\pm0.156)\log(\beta 10^m + 1) + (0.931\pm0.347) \quad [2]$$

$n = 11$ $r = 0.944$ $s_D = 0.213$ $F = 32.54$ $\log\beta = -16.18$

$m_{opt} = 15.5$ $\log(I/IC_{50})_{VAL,max} = 4.2650$

(five, six and seven membered rings - results not shown, paper in preparation) in principle has no effect upon the activity. However, the optimal calculated values (m_{opt}, eqs. 1 and 2 and m_{opt} for other heterocycle sizes) are different for different heterocycles, and with 4-alkylmorpholine N-oxides were shifted to 15-16 (in comparison, e.g., with piperidine analogues where the m_{opt} was found to be 12-13).

On the basis of our previous results /27-30/ it is convenient to use the IC_{50} adenine : IC_{50} valine ratio (R) as a suitable parameter to indicate the possible primary mode of action of the substance under investigation. All ratios, as shown in Table 1, are in the range 0.90 to 1.5. Such ratios are typical also for other biologically active compounds which interfere with energy-generating systems of cells. Inhibition of energy metabolism may, for example, be due to direct interaction or through the disorganization of the membrane structure.

Effect on macromolecule biosynthesis

The values from biochemical screening represent the first fundamental information about cytotoxic activity of new derivatives. The data obtained in a relatively short time indicate whether the tested substance has cytotoxic activity at all, and perhaps also its possible mode of action (ratio). In a first approach to determine the mode of action of the cytotoxically active compounds, the kinetics of DNA, RNA and protein synthesis inhibition were examined using isotope incorporation. Only when the time course is known it is possible to state at what time and concentration the inhibitory effect appears.

On the basis of primary screening, one of the most active compounds, namely 4-dodecylmorpholine-N-oxide, was chosen for further biochemical study. Figure 3 shows the inhibitory effect of compound No. XII (Table 1) upon biosynthesis of macromolecules, indicated by incorporation of [^{14}C]adenine and [^{14}C]valine into TCA-insoluble material of Ehrlich ascites cells. As can be seen from Fig. 3, compound No. XII inhibited incorporation of both precursors into appropriate macromolecules of Ehrlich cells, the extent of inhibition being dependent on both time and concentration of the compound in the incubation medium. At the highest concentration tested nearly complete inhibition of incorporation of both precursors occurred in cancer cells. At the same time, the results appear to indicate that the inhibition takes place on addition of the drug to the cancer cell

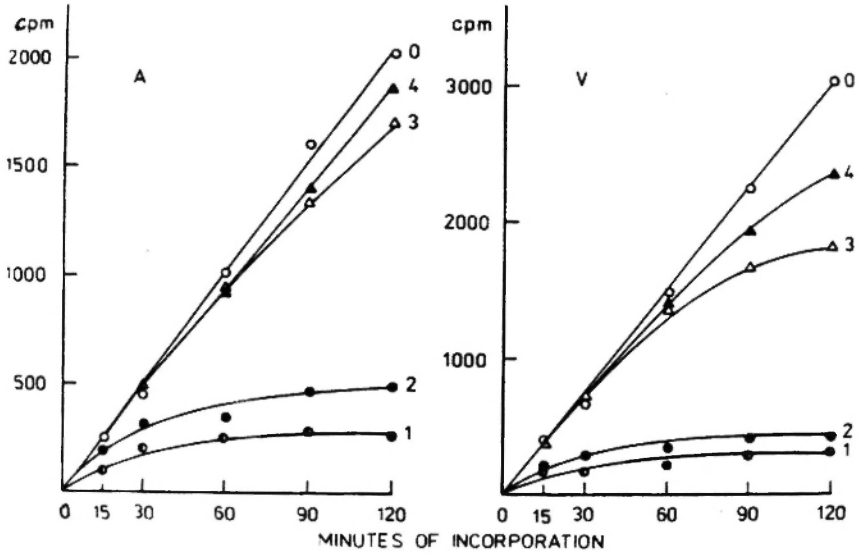


Fig. 3: The effect of 4-dodecylmorpholine-N-oxide on macromolecule synthesis of Ehrlich ascites cells. Incorporation of radioactive adenine (A) and valine (V) into acid-insoluble fractions was determined by incubating cells with appropriate ^{14}C-precursors. Radioactive precursors and amine oxide were added to the cells at the same time. The test-tubes were incubated at 37°C, and 1 ml samples of suspension were analyzed for radioactivity in acid-insoluble material. The results are expressed as cpm/5x10^6 cells. Concentrations: 0 - none, 1 = 600, 2 = 300, 3 = 150, 4 = 75 μmol/l.

suspension, i.e., without a lag phase. Our results confirmed the data obtained in the biochemical screening.

As [^{14}C]adenine is incorporated into both DNA and RNA, we determined which of these nucleic acids was more sensitive by the experiments presented in Fig. 4. These results show that the amine oxide inhibited incorporation of both precursors into appropriate macromolecules of Ehrlich cells in proportion to its concentration. The complete inhibition of uridine and thymidine incorporation was reached at the highest concentrations used of the drug. These concentrations almost fully depress the glycolysis of Ehrlich ascites cells

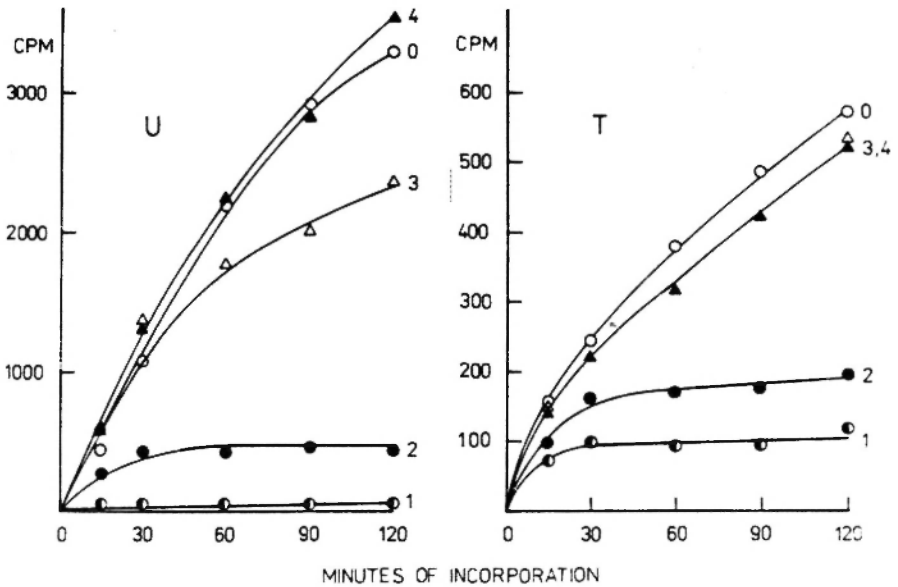


Fig. 4: The effect of 4-dodecylmorpholine-N-oxide on kinetics of [^{14}C]uridine (U) and [^{14}C]thymidine (T) incorporation into TCA-insoluble fractions of Ehrlich cells. Other experimental conditions and symbols are the same as for Fig. 3.

(results not shown). The lower concentrations of amine oxide inhibited incorporation of both precursors in proportion to the tested concentrations. The incorporation of all precursors was followed in the incubation medium containing glucose as a sole energy source.

DISCUSSION

We have previously reported a rapid radiometric *in vitro* technique of primary screening for anticancer substances /27-29/. This method, which measures the drug-induced inhibition of [^{14}C]adenine and [^{14}C]valine incorporation, is relatively simple, reliable and sensitive. In Ehrlich carcinoma cells, the degree of influence on metabolic activity is identified by uniformly selected concentrations of the substances, in definite conditions *in vitro*, ensuring the active synthesis of proteins and nucleic acids.

From the results presented in Table 1 it is evident that the amine oxides affected the incorporation of both precursors into appropriate macromolecules of Ehrlich cells, in a concentration dependent manner. Maximum activity was achieved with the compounds Nos. XIII-XVII (Table 1, lower part). On lengthening the alkyl chain, the activity increases and after having reached a maximum decreases again (Fig. 2). Similar results were obtained by Subik *et al.* /32/ and Devinsky *et al.* /31, 33/ in the study of antimicrobial activity. The ratios IC_{50} adenine:IC_{50}valine show the difference in the cytotoxicity of the substances, and they indicate primarily the similarity or diversity in the mode of action (in the initial changes). All ratios, as demonstrated in Table 1, are in the range 0.90 to 1.50. Such ratios are typical for other biologically active compounds which interfere with generation or utilization of energy in cancer cells /29, 30/. Inhibition of energy metabolism may be due to direct interaction or through the disorganization of the membrane structure. Volm /34/ found reasonably good correlations between a test based on the inhibition of radioactive nucleoside uptake and *in vivo* chemosensitivity of several rodent tumors.

Recently, Von Hoff *et al.* /35/ developed a radiometric system for the screening of antitumor agents. The index of cytotoxic effectiveness was based on the inhibition of transformation of [^{14}C]glucose into [$^{14}CO_2$]. This radiometric system (BAC-TEC 460) was optimalized with the aid of tumor cell lines of both human and animal origin. Scheithauser *et al.* /36/ used this new screening system for the selection of antitumor agents for the treatment of human colorectal tumors.

Our results show that 4-dodecylmorpholine-N-oxide inhibited incorporation of all 4 precursors (Figs. 3-4) into appropriate macromolecules of Ehrlich cells. This fact suggests that the effect of the amine oxide lies at an underlying level of energy generation or transfer rather than at specific reactions in the biosynthesis of DNA and protein. The process of DNA synthesis is actually the culmination of many synthetic pathways. In the intact cell, interference with any of these pathways, as well as alterations and variation in the pool size of precursors, can alter the apparent rate of DNA synthesis and obscure specific drug effects. The rate of DNA synthesis is rapidly affected by the lowering of the level of any of the four deoxyribonucleotide triphosphates. Interference with the generation of high-energy phosphate bonds is one of the mechanisms available for

induction of nucleotide deficiency. A depletion of nucleotide pools can serve as an efficient tool to inhibit cellular growth and to induce cell death under some circumstances.

Although in the case of many antineoplastic agents, attention has been focused upon their effects on DNA, RNA and protein synthesis, the data of Hill /37/ emphasize that this is an oversimplification. Most agents have multiple effective target sites within the cells and the primary cytotoxic events responsible for their clinical effectiveness remain to be elucidated. The work by Farber /38/ and others indicates that the inability to synthesize ATP in a cell leads to multiple secondary derangements in cellular metabolism.

The substance investigated showed a considerable inhibitory effect on all the metabolic processes examined, especially at the highest concentrations utilized. We assumed, therefore, that the cytotoxic effect could be the consequence of cytolytic activity of the amine oxide investigated. As recently found /39/, the antimicrobials (1-methyldodecyl)dimethylamine oxide and (1-methyldodecyl)trimethylammonium bromide affect the cytoplasmic membrane of *E. coli*. The interaction results in release of intracellular material (K^+ 260 nm-absorbing material), an effect on dehydrogenase enzyme activity and inhibition of respiration. The final effect of both substances is the same; they differ only in their dynamics. Kopecka-Leitmanova *et al.* /40/ summarized their results about the mode of action of quaternary ammonium salts and amine oxides upon bacteria determined *in vitro* in three stages. The first stage is characterized by the rate of onset of the action for which the polar interactions of molecules with the bacterial membrane are responsible. In the second stage, polar and hydrophobic interactions are involved; this stage involves destructive effects on the membrane. The third stage is represented by hydrophobic interactions which lead to cell death.

The results from this QSAR study show that there is no doubt that the mode of action for the incorporation of precursors of all the investigated compounds must be the same. However, additional work is required to clarify this point, even though it has already been shown /39, 41/ that non-aromatic amine oxides incorporate predominantly into the outer membrane of cells and disturb their architecture and fluidity and consequently all processes associated with the membrane.

Many types of agents have been found to have membrane action even though they were originally designed to inhibit the synthesis or

function of DNA /42/. New drugs have been synthesized which have lipophilic or membrane-selective structure and some of these are in early clinical trials /42/.

The surface membrane alterations which characterize neoplastic transformation offer the potential for cytotoxic selectivity. Modification of the lipid and consequent physical properties of membranes has been shown to enhance the sensitivity of neoplastic cells to certain anticancer drugs in tissue culture, and this approach should be investigated for its potential therapeutic value /42/.

Tumor cell membranes are potentially important targets for selective chemotherapeutic attack /43/. Further research is needed to elucidate the functional consequences of structural and conformational changes in cell membrane molecules, in order to permit the development of new classes of selectively toxic anti-tumor drugs (for a review see ref. /44/).

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