STUDIES ON THE MODE OF ACTION OF THE ANTITUMOUR ACRIDINE 4'-(9-ACRIDINYLAMINO)METHANESULPHON-*m*-ANISIDIDE (*m*-AMSA)

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by

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"Too much trust should not be put in an experiment done with the object of getting information."

W.D. Bancroft (1928)

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SUMMARY

(1) The mode of action of a novel antitumour acridine 4'-(9-acridinylamino)-methansulphon-*m*-anisidide (*m*-AMSA) has been investigated. Two congeners of *m*-AMSA, 4'-(9-acridinylamino)methanesulphonanilide (AMSA) and 4'-(9-acridinylamino)methanesulphon-*o*-anisidide (*o*-AMSA) were also studied for comparative purposes. *m*-AMSA is being evaluated clinically at present under the auspices of the National Cancer Institute, U.S.A.

(2) Treatment of mice bearing the mast cell tumour, mastocytoma P815, with *m*-AMSA provided some complete cures. AMSA was almost as effective as *m*-AMSA but its dose potency was 6-fold lower. *o*-AMSA was inactive.

(3) *m*-AMSA was found to have a short half-life in mice. Loss of *m*-AMSA from plasma was due, in part, to the formation of highly fluorescent covalent protein adducts. The rate of this reaction was similar for all three AMSA congeners, and is probably not required for antitumour activity.

(4) The reaction of AMSA drugs with proteins was shown to occur by nucleophilic displacement of the methanesulphonanilide moiety. Evidence is presented that thiols are the predominant reaction centres in proteins.

(5) A tissue culture model for the antitumour action of *m*-AMSA was developed using mastocytoma P815 cells. Profound growth inhibition and eventual killing was demonstrated using drug concentrations and durations of exposure attainable in mice. The potencies of the three AMSA congeners paralleled their antitumour potencies *in vivo*, except at very high drug concentrations.

(6) The rates of biotransformation of AMSA drugs, and their extent of uptake by cells in culture, could not account for the differing potencies of the three AMSA congeners.

(7) *m*-AMSA prevented the progression of mastocytoma cells through the cell division cycle under conditions where net cell growth was unaffected.

Physiologically attainable drug concentrations inhibited chromosome condensation in cells which were less than 10 minutes from the G_2-phase/mitosis boundary at the time of drug addition. The sedimentation rate on alkaline sucrose gradients of DNA from cells treated with *m*-AMSA was lower than that from untreated cells, suggesting that this agent may cause fragmentation of DNA.

(8) A new method for the investigation of the cell cycle stage selectivity of antitumour drugs was developed. This technique demonstrated that *m*-AMSA and AMSA have significant cycle stage selectivity, the growth of cells late in cycle being most affected.

(9) Spectrophotometric determinations of binding parameters for the interaction of AMSA derivatives with native DNA indicated that *m*-AMSA bound with lower affinity that did *o*-AMSA or AMSA. This conclusion was supported by the helix stabilization caused by these drugs. However, the association constants were sufficiently high for each derivative to ensure that essentially all intracellular drug available for binding to DNA would be bound.

(10) The ratio of the association constant for native DNA to that for a single-stranded viral RNA was higher for *m*-AMSA than for AMSA. This selectivity, if operative *in vivo*, could account for the high dose potency of *m*-AMSA.

SYMBOLS AND ABBREVIATIONS

A	amplification factor (Coulter Counter)
9-AA	9-aminoacridine
[*acridinyl*-G-^{3}H]AMSA	AMSA randomly labelled (^{3}H) in the acridine ring
[*acridinyl*-G-^{3}H]*m*-AMSA	*m*-AMSA randomly labelled (^{3}H) in acridine ring
[*acridinyl*-G-^{3}H]*o*-AMSA	*o*-AMSA randomly labelled (^{3}H) in acridine ring
AMSA	4'-(9-acridinylamino)methanesulphonanilide
Ara-C	1-β-D-arabinofuranosylcytosine
A_x	absorbance at wavelength x(nm)
BCNU	1,3-bis(2-chloroethyl)-1-nitrosourea
BSA	bovine serum albumin
C_b	concentration of bound ligand
C_f	concentration of free ligand
Ci	curie
CM-protein	carboxymethylated protein
cpm	counts per minute
[^{14}C]TdR	[2-^{14}C]thymidine
CT DNA	deoxyribonucleic acid from calf thymus
DNA	deoxyribonucleic acid
dpm	disintegrations per minute
DTNB	5,5'-dithiobis(2-nitrobenzoic acid)
DTT	dithiothreitol
F	aperture current factor (Coulter Counter)
3'-F-AMSA	4'-(9-acridinylamino)methanesulphon-*m*-fluoro-anilide
fl	femtolitre
fu	unit of fluorescence intensity
g	gram, or gravitational acceleration
h	hour
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid
HU	hydroxyurea
i.c	intracerebral
i.p.	intraperitoneal

i.v.	intravenous
k	calibration factor (Coulter Counter)
K_e	association constant for a single lattice residue
K_s	association constant for a site defined by the saturation binding ratio
kg	kilogram
LD_{10}	dose of drug which is lethal to 10% of the test organisms
LDS	lithium dodecyl sulphate
LFER	linear free energy relationship
m	metre (unless otherwise defined)
M	molar
m-AMSA	4'-(9-acridinylamino)methanesulphon-*m*-anisidide
3'-Me-AMSA	4'-(9-acridinylamino)methanesulphon-*m*-toluidide
MeOH	methanol
mCi	millicurie
mg	milligram
min	minute
min.	minimum
ml	millilitre
mol	mole
mmol	millimole
n	nucleic acid binding site size, in nucleotides
n'	nucleic acid binding site size, in base pairs
N	nucleic acid concentration in nucleotide residues (unless otherwise defined)
N_i	number of pulses in the ith channel
nm	nanometre
n*M*	nanomolar
nmol	nanomole
NNCS	neonatal calf serum
o-AMSA	4'-(9-acridinylamino)methanesulphon-*o*-anisidide
P	partition coefficient
PBS	phosphate-buffered saline
PF	proflavine
pg	picogram

[*phenyl*-G-^{3}H]AMSA	AMSA randomly labelled (^{3}H) in the phenyl ring
pmol	picomole
POPOP	1,4-bis[2-(5-phenyloxazolyl)]-benzene;phenyl-oxazolylphenyl-oxazolylphenyl
PPO	2,5-diphenyloxazole
q.d.	daily administration
QSAR	quantitative structure-activity relationship
r'	radius
r	binding ratio (unless otherwise defined)
R_f	chromatographic mobility
RNA	ribonucleic acid
rpm	revolutions per minute
s	second
SBSS	Shortman's balanced salt solution
s.c.	subcutaneous
S.D.	standard deviation
S_f	concentration of free (unoccupied) ligand binding sites
SHE	buffer (saline-HEPES-EDTA)
t	elapsed time
T	sedimentation time
$T_{\frac{1}{2}}$	half-life (unless otherwise defined)
T_g	cell generation time
TCA	Trichloroacetic acid
TdR	thymidine
TLC	thin layer chromatography
tris	tris(hydroxymethyl)aminomethane
TYMV	turnip yellow mosaic virus
uv	ultraviolet
$\overline{V}$	mean cell volume
V_m	volume of medium in wet pellet
V_c	volume of cells in wet pellet
w/v	weight per volume
v/v	volume per volume
α	number of ligand binding sites per lattice residue
δ_i	channel number
ε	molar extinction coefficient

$\Delta\varepsilon$	differential molar extinction coefficient
η	absolute viscosity
λ	wavelength
λ_i	wavelength of an isobestic point
λ_m	wavelength at maximum of difference spectrum
μ	ionic strength
μCi	microcurie
μg	microgram
μl	microlitre
μM	micromolar
μmol	micromole
ν	binding ratio (mol ligand/mol base pairs)
ν_L	binding ratio (mol ligand/mol lattice residues)
ρ	absolute density of cells
ρ_o	absolute density of solution
ρ_w	absolute density of water
σ_m	Hammett substituent constant (meta)
σ_p	Hammett substituent constant (para)
Φ	cell volume fraction
ω	mitotic index