RADIOSENSITIZING HYPOXIC CELLS WITH NEW 3-NITRO-1,2,4-TRIAZOLE DERIVATIVES *IN VITRO* AND *IN VIVO*

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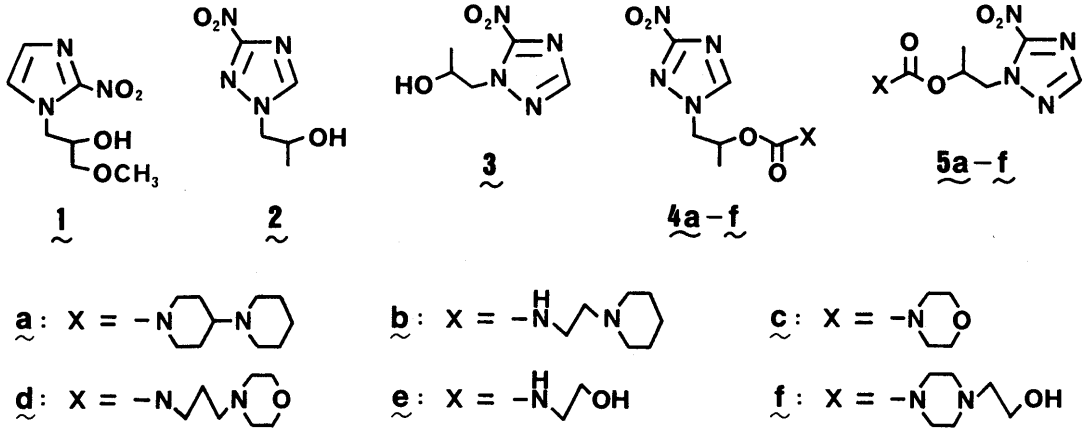
The new regioisomer derivatives 4a-f and 5a-f of 3-nitro-1,2,4-triazole (3-NTR) were synthesized for the development of new radiosensitizers of hypoxic cancer cells for radiotherapy. N(2)-Substituted 3-NTR derivatives 5a-f were stronger radiosensitizers of hypoxic cells *in vitro* (Chinese hamster V79 cells) than N(1)-substituted 3-NTR derivatives 4a-f, but *in vivo* they were weaker (SCCVII carcinoma cells inoculated into C3H/He mouse).

KEYWORDS nitrotriazole; radiosensitizer; hypoxic cell; regioisomer; one-electron reduction potential

2-Nitroimidazoles including misonidazole (1) have been investigated as promising radiosensitizers of hypoxic cancer cells for radiotherapy.[1-11] However, in spite of their high radiosensitizing ability, the clinical utility of 2-nitroimidazole (2-NIM) derivatives is limited because of their serious neurotoxicity.[12] We are especially interested in the development of new radiosensitizers with a 3-nitro-1,2,4-triazole (3-NTR) moiety. Such compounds could be less neurotoxic than 2-nitroimidazoles. Although there have been a few reports on the radiosensitization by N(1)-substituted 3-NTR derivatives,[13-15] no systematic experiment concerning N(2)-substituted ones has been documented. It is essential in designing new 3-NTR derivatives to know the radiosensitizing potential of the N(2)-substituted 3-NTR derivatives, which are structurally similar to the 2-NIM derivatives. We describe herein the radiosensitization effect of N(1)- and N(2)-substituted 3-NTR derivatives on hypoxic cells *in vitro* and *in vivo*.

New regioisomer derivatives 4a-f[16] and 5a-f[16] of 3-NTR were synthesized from the compounds 2[16] and 3,[16] respectively, which were obtained by treating 3-NTR with propylene oxide under heating. Thus, their sensitizing effects on hypoxic cells (Chinese hamster V79 cells *in vitro* or SCCVII carcinoma cells inoculated into C3H/He mouse) were investigated under irradiation with ^{60}Co γ-rays *in vitro* or with the



10-MV X-rays of a linear accelerator *in vivo* as described previously.[14,15,17] All results are given in terms of enhancement ratio (E.R.) which can be obtained from the survival curves of irradiated Chinese hamster V79 cells or the SCCVII carcinoma cells of tumors excised from mice (Table I).

The E.R. values of the N(1)-substituted 3-NTR derivatives in comparison with those of the corresponding N(2)-substituted 3-NTR ones proved to be dramatically different depending upon the testing system, *in vitro* or *in vivo*. In all cases with *in vitro* testing, the N(2)-substituted 3-NTR derivatives 5a-f clearly showed higher E.R. values than the corresponding N(1)-substituted 3-NTR ones 4a-f. But with

Table I. Radiosensitization and Reduction Potentials of Regioisomers of 3-NTR Derivatives

Compound	$E^{Red}_{1/2}$ [a] (V)	In vitro [b] Conc. (mM)	E.R.	In vivo [c] Dose (mg/kg)	E.R.
1	-1.040	0.1	1.13	100	1.35
		1	1.62	200	1.57
2	-1.120	0.5	1.54	200	1.37
		1	1.69		
		5	2.11		
3 [d]	-0.800	0.1	1.49	200	1.22
		0.5	1.80		
		1	2.13		
		2.5	2.42		
4a	-1.050	0.1	1.07	100 [e]	1.03
		1	1.60		
5a	-0.840	0.1	1.45	___ [f]	___ [f]
		1	2.64		
4b	-1.030	0.1	1.32	75 [e]	1.09
		0.5	1.55		
		1	1.77		
		5	2.34		
5b	-0.760	0.05	1.32	___ [f]	___ [f]
		0.1	1.55-1.61		
		0.5	2.22		
4c	-1.060	0.1	1.32	100 [e]	1.35
		1	1.78		
5c	-0.780	0.1	1.59	200	1.24
		1	2.60		
4d	-1.060	0.1	1.22	200	1.40
		0.5	1.45		
		1	1.69		
5d	-0.785	0.1	1.45	200	1.08
		0.5	1.98		
		1	2.34		
4e	-1.090	0.1	1.17	200	1.45
		1	1.61		
5e	-0.775	0.1	1.39	200	1.28
		1	2.18		
4f	-1.065	0.1	1.07	200	1.42
		1	1.55		
5f	-0.785	0.1	1.24	200	1.00
		1	1.97		

[a] The reduction potential of each compound was evaluated with a Ag/AgCl (saturated) / 3.5 M KCl electrode using cyclic voltammetry for an Ar-purged N,N-dimethylformamide solution (0.01 M) containing 0.1 M tetra-N-butylammonium perchlorate as a supporting electrode. [b] Test compound was dissolved in MEM. [c] Test compound was dissolved in sterile physiological saline. [d] An inseparable mixture of 5 and 1-(2'-hydroxy-1'-methylethyl)-3-nitro-1,2,4-triazole (5:1) was used.[16] [e] Testing for a higher dose was not carried out because it was less soluble. [f] Not examined.

in vivo testing, in some cases the E.R. values of N(2)-substituted compounds 5c-f were lower than those of the corresponding N(1)-substituted ones, 4c-f. Such an obvious difference in the E.R. values between the 5 series compounds and the 4 series ones *in vitro* can be rationalized in terms of the electron affinity expressed as the one-electron reduction potentials [See $E^{Red}_{1/2}$(V) value in Table I]. Namely, the 5 series compounds having higher electron affinity in comparison with the corresponding 4 series ones should exhibit higher E.R. values *in vitro*. However, we have no evidence to explain readily the *in vivo* testing results. It could be speculated that the 5 series compounds, having strong oxidizing ability, instantly disappear in the living system by reacting with reducing substances such as non-protein SH compounds and enzymes involving NADH or NADPH.[18] Molecular design based on the electron affinity seems to be insufficient for developing efficient radiosensitizers *in vivo*. We should consider the effective and selective transportation of the agents into the tumor without their being decomposed, quickly metabolized, or trapped by protein.

Thus, we have demonstrated that N(1)-substituted 3-NTR derivatives should be more promising radiosensitizers to hypoxic cells *in vivo* than N(2)-substituted 3-NTR ones.

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