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UPTAKE AND CYTOTOXICITY OF 9-METHOXY-N^2-METHYLELLIPTICINIUM ACETATE IN HUMAN BRAIN AND NON-BRAIN TUMOR CELL LINES

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Abstract—9-Methoxy-N^2-methylellipticinium acetate (MMEA) was preferentially cytotoxic to human brain tumor cell lines in the *in vitro* primary screen of the U.S. National Cancer Institute. In the present study, the average intracellular accumulation of radioactivity derived from [^{14}C]MMEA concentrations that were selectively cytotoxic to sensitive brain tumor cell lines was nearly 4-fold greater than in human tumor cell lines derived from the lung, kidney, ovary and colon. The extent of peak cellular accumulation of [^{14}C]MMEA-derived radioactivity, achieved after 10–15 hr of drug exposure, was correlated positively with relative MMEA cytotoxicity in brain tumor cell lines ($r^2 = 0.963$). A similar correlation ($r^2 = 0.967$) was observed in selected non-brain tumor cell lines but required substantially higher (18-fold) concentrations of MMEA. [^{14}C]MMEA radioactivity accumulation by a selected glioblastoma cell line occurred via an energy-requiring system that was predominantly sodium and pH independent.

Key words: selective cytotoxicity; brain tumor; drug transport; quaternized ellipticines

MMEA† is representative of a series of quaternized ellipticine derivatives that have been found selectively cytotoxic to the human brain tumor cell line subpanel of the U.S. National Cancer Institute (NCI) *in vitro* primary screen [1]. The relative extent of MMEA cytotoxicity to the brain tumor cell lines was correlated positively with peak intracellular accumulation of the drug and/or metabolite(s) following exposure to selectively cytotoxic concentrations of MMEA [2]. The intracellular accumulation of radioactivity derived from [^{14}C]MMEA uptake occurred via a high-affinity uptake system [2]. Moreover, the accumulation was inhibited competitively by the plant alkaloid reserpine, resulting in chemoprotection of brain tumor cell lines from MMEA [2]. MMEA uptake was also inhibited, albeit with much lower affinity, by catecholamines (e.g. norepinephrine, dopamine and epinephrine), an indoleamine (serotonin), and by imipramine and related tricyclic antidepressant drugs [2].

These observations prompted the present investigation, which was designed to explore further the relationship between intracellular accumulation and cytotoxicity of MMEA in brain versus non-brain tumor cell lines, and to further characterize the MMEA transport mechanism.

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† Abbreviations: MMEA, 9-methoxy-N^2-methylellipticinium acetate; CCCP, carbonylcyanide-*m*-chlorophenyl hydrazone; IC_{50}, MMEA concentration that results in a 50% reduction in protein content in drug-treated cultures relative to control; and TCA, trichloroacetic acid.

MATERIALS AND METHODS

Materials. RPMI 1640 medium was obtained from Quality Biological, Inc. (Gaithersburg, MD). Fetal bovine serum was purchased from Hyclone, Inc. (Ogden, UT). D-Glucose, CCCP, bovine serum albumin (Fraction V), and HEPES were obtained from the Sigma Chemical Co. (St. Louis, MO). Radiochemically pure 9-methoxy[2-^{14}C]methylellipticinium acetate ([^{14}C]MMEA; 52 mCi/mmol) and unlabeled MMEA, provided by the NCI Drug Synthesis and Chemistry Branch, were used at the concentrations indicated in the text. The cytotoxic potencies of [^{14}C]MMEA and the unlabeled drug were verified to be equivalent. Stock solutions of both radiolabeled and unlabeled MMEA were prepared in 100% ethyl alcohol and utilized at the concentrations indicated in the text. The final ethanol concentration in the assay medium never exceeded 0.1%.

Growth of human tumor cell lines. All human tumor cell lines were obtained from the repository at the National Cancer Institute-Frederick Cancer Research and Development Center (NCI-FCRDC) and routinely grown in 75 cm^2 T-flasks in RPMI 1640 medium supplemented with 5% fetal bovine serum in a humidified atmosphere containing 5% CO_2. Cultures were routinely passaged weekly when cell density approached 80–90% of confluency. The morphology, immunocytochemical characteristics and clinical histories of cell lines utilized for these studies have been characterized extensively [3].

Cytotoxicity of MMEA to brain and non-brain tumor cell lines. Cell lines, grown in 75 cm^2 T-flasks as described above, were removed by trypsinization and seeded into 24-well culture dishes at the following densities (cells/well): U-251 glioblastoma, SNB-19

gliosarcoma, SF-268 glioblastoma, SF-295 glioblastoma, COLO-205 colon adenocarcinoma, HCT-15 colon adenocarcinoma and the OVCAR-8 undifferentiated ovarian carcinoma (2×10^4); A-549 non-small cell lung adenocarcinoma (3×10^4); SNB-75 astrocytoma, SNB-78 undifferentiated neoplasm, SN12-C renal cell carcinoma and the OVCAR-5 ovarian carcinoma (4×10^4); SF-539 undifferentiated neoplasm, A-498 renal cell carcinoma and the HOP-18 non-small cell lung adenocarcinoma (5×10^4); and the XF-498 gliosarcoma (6×10^4).

Cells were allowed to grow for 48 hr at 37° in a humidified atmosphere containing 5% carbon dioxide prior to the individual experiments. The cytotoxic potency of MMEA was evaluated following exposure of cells to the drug for varying periods of time as described for individual experiments. Experiments were terminated by the addition of 50% TCA to a final concentration of 10%, and protein was quantitated with sulforhodamine B [4].

Cellular accumulation of [^{14}C]*MMEA-derived radioactivity.* Cell lines were grown for 72–96 hr in 6-well plates in RPMI 1640 medium as described above to yield monolayers of approximately 80% confluency at the time of the uptake study. Seeding densities employed were: U-251, SF-268, SF-295, SNB-19, SF-539, COLO-205, HCT-15 and OVCAR-8 (6×10^4 cells/well); A-549 (9×10^4 cells/well); SNB-75, SNB-78, SN12-C and OVCAR-5 (1.2×10^5 cells/well); XF-498, A-498 and HOP-18 (1.5×10^5 cells/well). Growth medium was removed prior to the uptake study, and cells were rinsed twice with 37° transport medium (see individual experiments for composition) and maintained at 37° for the duration of the experiment, except where indicated. To assess the possible relationship between accumulation of [^{14}C]MMEA-derived radioactivity and cytotoxicity in non-brain tumor cell lines, a series of experiments was performed using drug exposure times up to 24 hr; for these experiments, cells were seeded at concentrations identical to those described above for cytotoxicity experiments, and drug exposure was initiated after 48 hr of growth. All uptake experiments were terminated by removal of the medium containing [^{14}C]MMEA. Plates were immediately placed on ice and rinsed three times with 4° phosphate-buffered saline; the cell monolayers were solubilized at room temperature for 16–24 hr with 1.0 mL of 0.2 N NaOH. Aliquots were removed for quantitation of radioactivity by liquid scintillation spectrometry and protein by the Coomassie Blue method [5].

RESULTS

Accumulation of [^{14}C]*MMEA-derived radioactivity by brain and non-brain tumor cell lines: correlation with MMEA cytotoxicity.* The current NCI *in vitro* primary screen [6, 7] employs a panel of sixty diverse human tumor cell lines [3] arrayed in various subpanels representing particular tumor types. In the present study, we selected, based upon primary screening data [1], some of the most MMEA-sensitive and -resistant cell lines from each of four non-brain subpanels (renal, ovarian, colon and lung) and compared both MMEA cytotoxicity and [^{14}C]-MMEA-derived radioactivity accumulation in these cell lines versus the eight cell lines comprising the brain tumor subpanel. The latter subpanel consists of cell lines derived from tumors of the supporting elements of the brain, and comprises three glioblastomas (U-251, SF-268, SF-295), two gliosarcomas (XF-498, SNB-19), one astrocytoma (SNB-75), and two undifferentiated neoplasms (SNB-78, SF-539).

The eight brain tumor cell lines were clearly separable into two subgroups, based upon relative sensitivity to MMEA; the more sensitive subgroup consisted of six lines (U-251, SF-268, SF-295, SNB-19, SF-539, SNB-75), whereas the less sensitive subgroup consisted of two lines (SNB-78 and XF-498) (Fig. 1, top panel). Examination of the cytotoxicity profile of MMEA to the non-brain tumor cell lines (Fig. 1, bottom panel) indicated that even the most sensitive non-brain tumor cell lines were more resistant to the drug than the six most MMEA-sensitive brain tumor cell lines. Only the SN12-C renal cell carcinoma ($IC_{50} = 200$ nM) exhibited sensitivity to MMEA which approached that observed with the more MMEA-sensitive brain tumor cell lines (IC_{50} range 37 to 210 nM).

The relative extent of accumulation of radioactivity from [^{14}C]MMEA in the individual lines within the brain tumor subpanel paralleled the relative sensitivity of the respective lines to MMEA. As evident in Fig. 2 (top panel), the average accumulation of [^{14}C]MMEA-derived radioactivity by the six most MMEA-sensitive cell lines in the brain tumor subpanel (0.95 ± 0.21 pmol/hr/μg protein) was nearly 4-fold greater than the accumulation of MMEA in the more resistant brain tumor lines (0.26 ± 0.11 pmol/hr/μg protein).

The average [^{14}C]MMEA radioactivity accumulation in the non-brain tumor cell lines in the present study was markedly less than that of the MMEA-sensitive brain tumor cell lines (Fig. 2, bottom panel). Only the SN12-C renal cell carcinoma ($IC_{50} = 200$ nM), the most sensitive non-brain tumor cell line in the sixty cell line panel, accumulated [^{14}C]-MMEA radioactivity to levels similar to those observed with the sensitive brain tumor cell lines.

Since previous studies [2] demonstrated that MMEA cytotoxicity to brain tumor cell lines is highly correlated with peak cellular accumulation of [^{14}C]-MMEA-derived radioactivity attained after 10–15 hr of drug exposure, similar experiments were undertaken with non-brain tumor cell lines. MMEA was utilized at a concentration (2 μM) that produced a range of cytotoxicity to the non-brain tumor cell lines (20–80% inhibition of cell growth) comparable to that produced by 0.11 μM MMEA in the brain tumor subpanel. The time course of accumulation of [^{14}C]MMEA-derived radioactivity in these non-brain tumor cell lines paralleled that observed with brain tumor cell lines in that peak values occurred following 10–15 hr of drug exposure (data not shown). It was noteworthy that the relative rate of accumulation (pmol/hr) of [^{14}C]MMEA-derived radioactivity by the most MMEA-resistant non-brain tumor cell lines (OVCAR-5, HCT-15, HOP-18, A-498) was much less than that observed by both the MMEA-sensitive brain tumor cell lines or by the

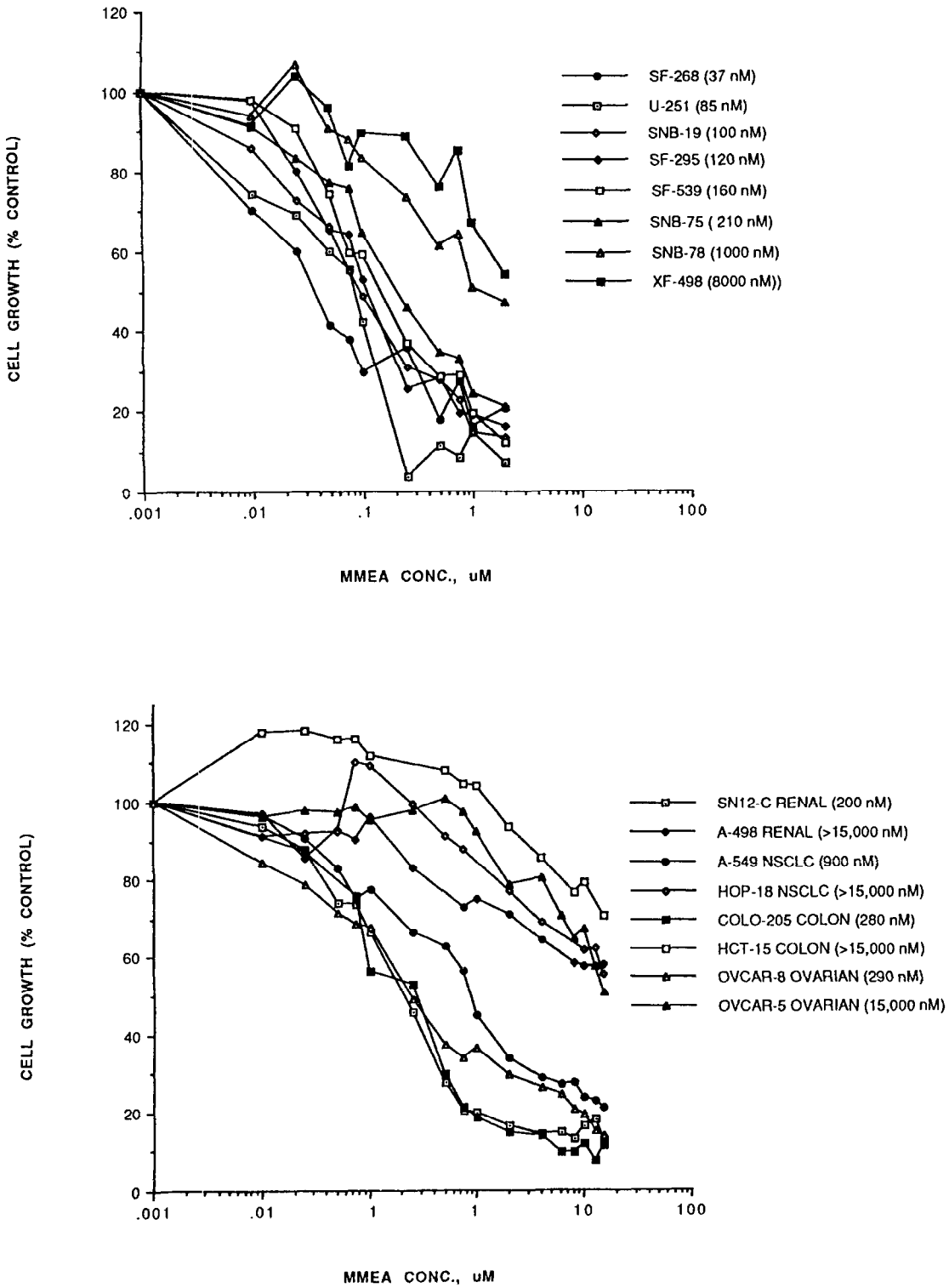

Fig. 1. Cytotoxicity of MMEA to human brain and non-brain tumor cell lines. Human brain (top panel) and non-brain (bottom panel) tumor cell lines were seeded into 24-well plates, as described in Materials and Methods, and allowed to grow for 48 hr. MMEA (0.01 to 20 μM) was added to RPMI 1640 growth medium and the incubation continued for 72 hr at 37° in a humidified atmosphere of 5% carbon dioxide. Cells were fixed *in situ* by the addition of 500 μL of 50% TCA. Cellular protein was quantitated using sulforhodamine B [4]. Values are the means of 10 separate determinations. Standard deviation values did not exceed 5% and were omitted for clarity. The MMEA concentration that reduced the cellular protein content to 50% of control (IC_{50}) is indicated in parentheses next to each cell line.

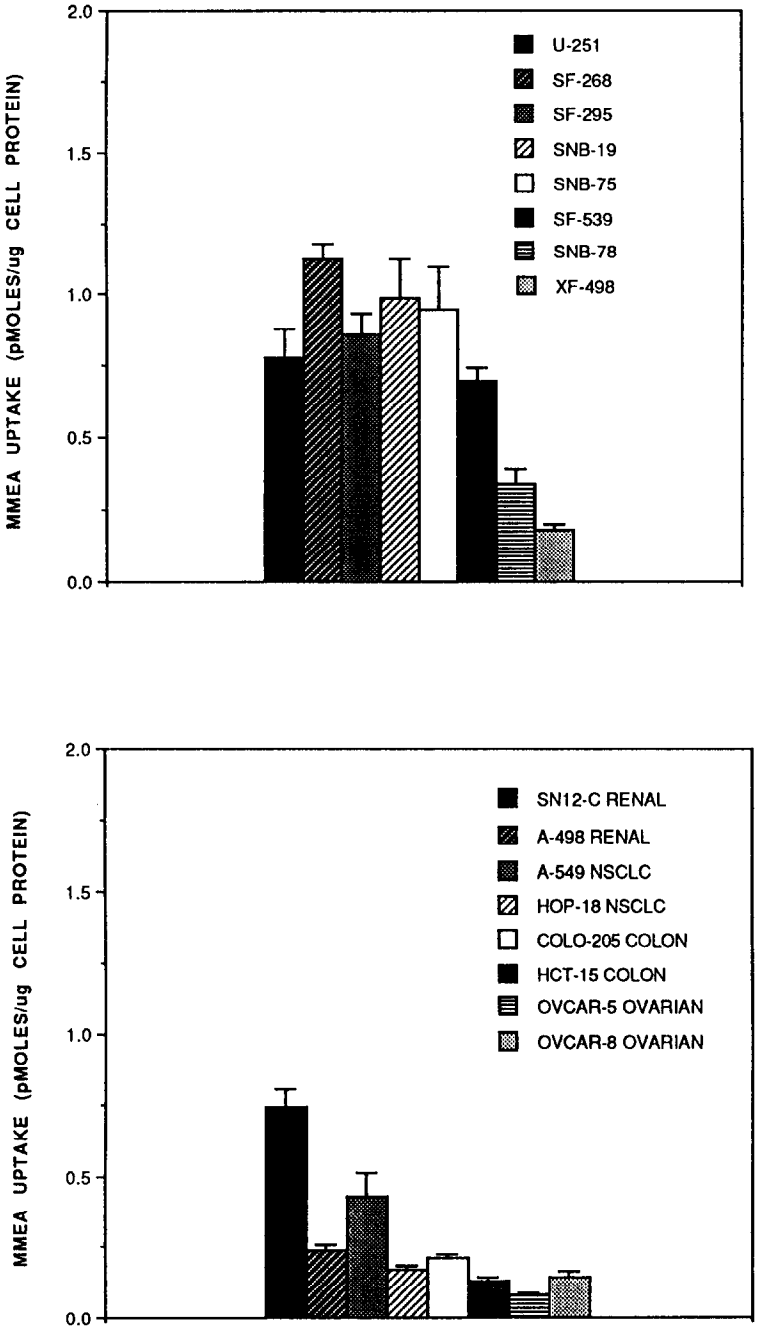

Fig. 2. Accumulation of [^{14}C]MMEA-derived radioactivity in human brain and non-brain tumor cell lines. Human brain (top panel) and non-brain (bottom panel) cell lines were seeded into 6-well plates, as described in Materials and Methods, and allowed to grow for 72–96 hr to approximately 80% confluency. Growth medium was removed, and the cells were rinsed twice with Dulbecco's phosphate-buffered saline containing 0.1 mM bovine serum albumin and 15 mM D-glucose. [^{14}C]MMEA was added to a final concentration of 0.5 μM, and the incubation was continued for 60 min at 37°. The experiment was terminated as described in Materials and Methods. Values are means ± SD (N = 6).

less MMEA-sensitive non-brain tumor cell lines (SN12-C, A-549, COLO-205, OVCAR-8). This resulted in small differences between accumulation values at early time points and the peak values observed at 10–15 hr in these cell lines. The results of these experiments (Fig. 3) indicate that peak cellular accumulation of MMEA and/or its reactive metabolite(s) correlates with cytotoxicity in both brain tumor and non-brain tumor cell lines.

Sodium independence of MMEA accumulation. The observation that the intracellular accumulation of [^{14}C]MMEA-derived radioactivity was inhibited

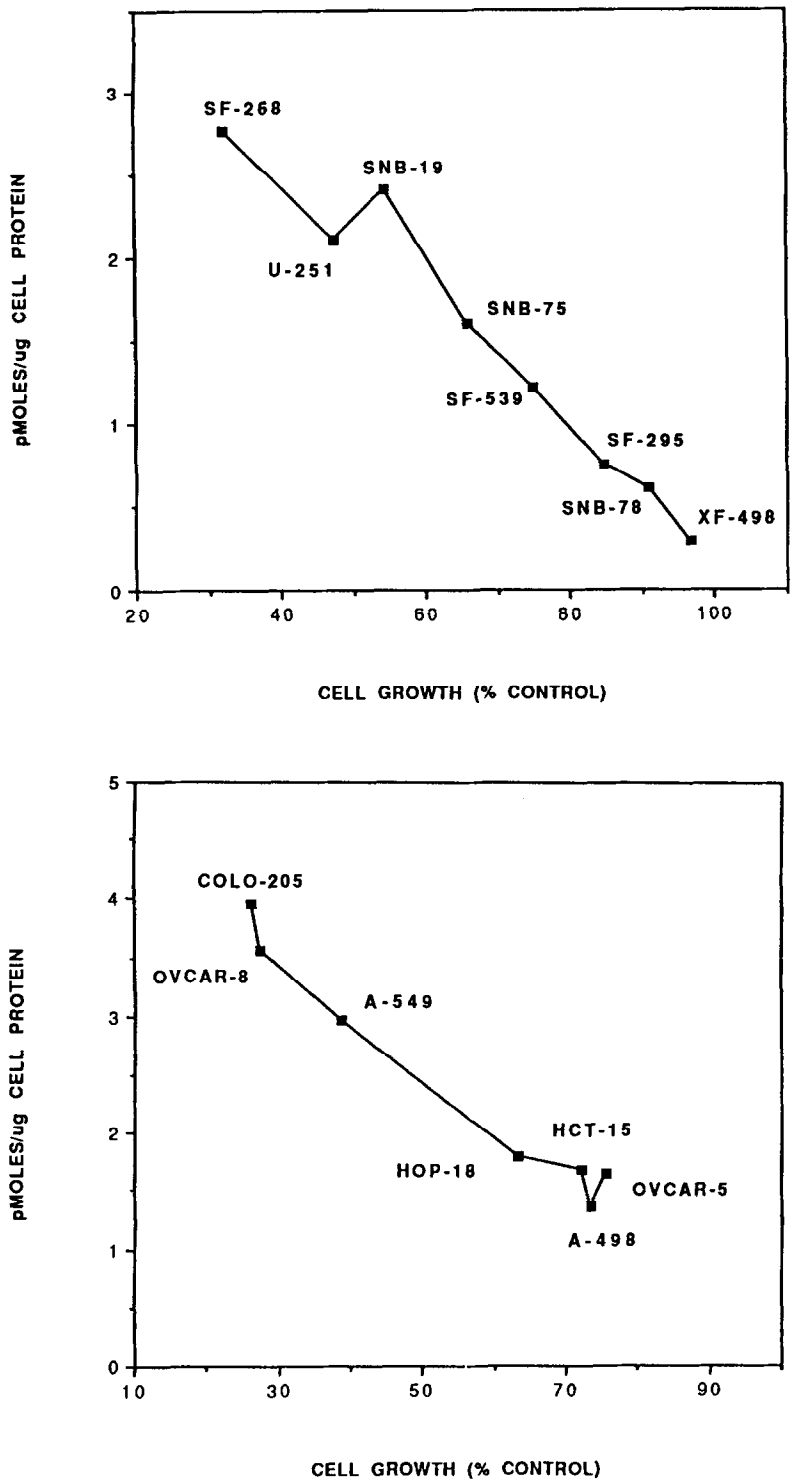

Fig. 3. Correlation of [^{14}C]MMEA radioactivity accumulation and cytotoxicity to human brain tumor and non-brain tumor cell lines. MMEA cytotoxicity to human brain tumor and non-brain tumor cell lines was evaluated following a 72-hr exposure to the drug, as described in Materials and Methods. Peak cellular accumulation of radioactivity derived from [^{14}C]MMEA was determined following exposure of cells to 0.11 μM (brain tumor cell lines) or 2.0 μM (non-brain tumor cell lines) [^{14}C]MMEA for 1–24 hr in RPMI 1640 medium containing 5% fetal bovine serum. Each data point represents the mean of 10 separate determinations for cytotoxicity data and 6 separate determinations for radioactivity accumulation. Linear regression analysis yielded the equation $y = -0.039 + 4.153$ ($r^2 = 0.963$) for the brain tumor cell lines (top panel) and $y = -0.0463 + 4.908$ ($r^2 = 0.967$) for the non-brain tumor cell lines (bottom panel). Standard deviation values for all radioactivity accumulation and cytotoxicity data were less than 5% of the indicated mean values.

Table 1. Effect of sodium ions on [^{14}C]MMEA radioactivity accumulation by U-251 glioblastoma cells*

	Radioactivity accumulated (pmol/μg cell protein)															
	MMEA concentration (μM)															
	0.23		0.50		0.95		1.8		3.5		7.2					
	Sodium															
Minutes	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
10	0.11 ± 0.01	0.10 ± 0.01	0.20 ± 0.02	0.22 ± 0.02	0.38 ± 0.02	0.37 ± 0.03	0.64 ± 0.03	0.56 ± 0.04	0.85 ± 0.05	1.06 ± 0.05	1.07 ± 0.06	2.00 ± 0.36				
25	0.20 ± 0.01	0.22 ± 0.02	0.41 ± 0.03	0.36 ± 0.02	0.79 ± 0.04	0.63 ± 0.03	1.05 ± 0.08	1.00 ± 0.05	1.46 ± 0.11	1.98 ± 0.20	2.32 ± 0.28	3.41 ± 0.32				
40	0.28 ± 0.04	0.30 ± 0.02	0.64 ± 0.07	0.51 ± 0.04	1.07 ± 0.03	0.81 ± 0.04	1.64 ± 0.16	1.35 ± 0.07	2.12 ± 0.15	2.36 ± 0.17	2.66 ± 0.44	3.91 ± 0.29				
60	0.36 ± 0.03	0.34 ± 0.02	0.74 ± 0.05	0.61 ± 0.06	1.34 ± 0.22	1.08 ± 0.09	2.22 ± 0.17	1.80 ± 0.09	2.51 ± 0.26	3.32 ± 0.28	3.89 ± 0.47	5.04 ± 0.41				

* U-251 glioblastoma cells, grown in 6-well plates as described in Materials and Methods were rinsed three times with 37° transport medium containing the following constituents [mmol/L]: D-glucose [10], $CaCl_2{\cdot}2H_2O$ [0.68], KCl [2.68], KH_2PO_4 [1.5], $MgCl_2{\cdot}6H_2O$ [0.5], HEPES [25] and either NaCl [125] or choline [125]. [^{14}C]MMEA was added to the final indicated concentration, and the plates were incubated at 37° for 10, 25, 40 or 60 min. The incubation was terminated by the removal of the transport medium; the plates were placed on ice, washed three times with 4° transport medium, and the monolayers were solubilized overnight with 0.2 N NaOH. Aliquots were utilized for liquid scintillation counting and protein determinations. Values are means ± SD, N = 6.

competitively, both by the catecholamines norepinephrine and dopamine and by the indoleamine serotonin [2], and the recollection that the transport of these neurotransmitters at normal neuronal synaptic terminals is sodium dependent [8], prompted investigation into the requirement of sodium ions for MMEA transport. As apparent in Table 1, [^{14}C]MMEA radioactivity accumulation by U-251 glioblastoma cells was not substantially dependent on sodium ions; in the absence of sodium, there was no decrease in accumulation at the lowest MMEA concentration examined (0.23 μM). A 10–20% reduction in accumulation was observed at 0.50, 0.95 and 1.8 μM MMEA while, at higher MMEA concentrations, the presence of sodium produced some inhibition of MMEA accumulation.

Energy dependence of MMEA accumulation. Accumulation of radioactivity derived from [^{14}C]-MMEA by U-251 glioblastoma cells was decreased when the uptake assay was performed in culture medium devoid of D-glucose and containing CCCP, an inhibitor of mitochondrial oxidative phosphorylation (Fig. 4, top panel); moreover, the decrease in accumulation was accompanied by an apparent decrease in the cytotoxicity of MMEA to glioblastoma cells (Fig. 4, bottom panel).

Effect of temperature and pH on MMEA accumulation. The accumulation of [^{14}C]MMEA radioactivity by U-251 glioblastoma cells was markedly affected by temperature (Fig. 5, top panel). The rate of accumulation at 37° (15.5 fmol/min/μg cell protein) and 20° (3.8 fmol/min/μg cell protein) was 15.5-fold and 3.8-fold greater than that observed at 4° (1 fmol/min/μg cell protein).

The initial velocity of intracellular accumulation of [^{14}C]MMEA radioactivity by U-251 glioblastoma cells was relatively insensitive to changes in the pH of the extracellular environment between 7.0 and 9.0, although there was a general trend toward increased accumulation at higher pH values (Fig. 5, bottom panel). A slight reduction in MMEA accumulation was apparent below pH 7.0.

DISCUSSION

The results described herein indicate that the relative extent of accumulation of [^{14}C]MMEA-derived radioactivity [presumably reflecting MMEA itself and/or metabolite(s) thereof] by sensitive brain tumor cell lines very substantially exceeded that of the majority of non-brain (lung, ovarian, colon and renal) human tumor cell lines examined (Fig. 2). Moreover, within the brain tumor cell line subpanel, sensitivity to MMEA was closely correlated with peak intracellular accumulation of [^{14}C]MMEA-derived radioactivity achieved after 10–15 hr of drug exposure (Fig. 3).

A similar correlation between accumulation of [^{14}C]MMEA derived radioactivity and cytotoxicity was also observed in non-brain tumor cell lines (Fig. 3). Importantly, because the non-brain tumor cell lines are inherently less sensitive to MMEA, this required the use of higher MMEA concentrations to produce a level of cytotoxicity roughly equivalent to that observed in brain tumor cell lines. Thus, it would appear that brain tumor cells possess a

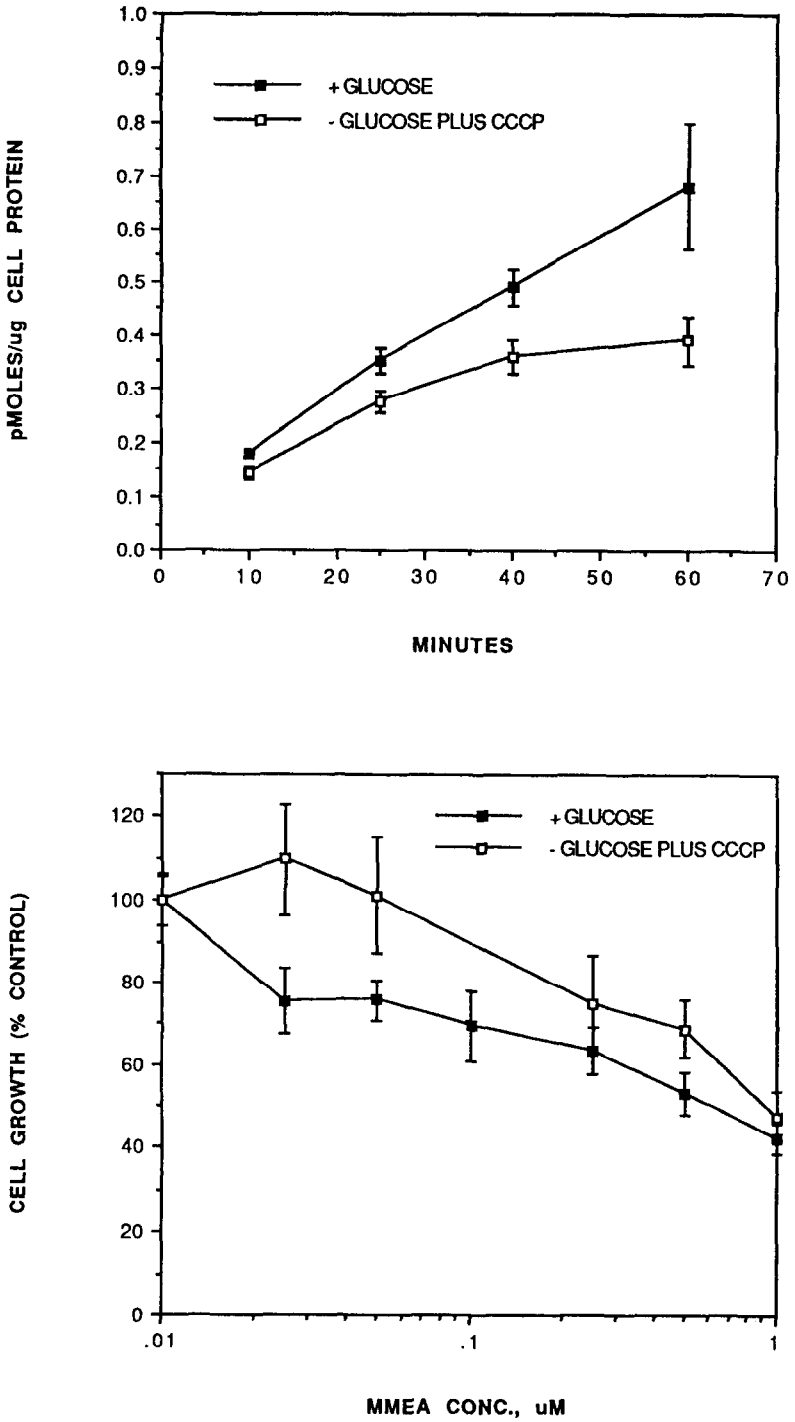

Fig. 4. Energy dependence of [^{14}C]MMEA radioactivity accumulation and MMEA cytotoxicity. For accumulation studies (top panel), U-251 glioblastoma cells were seeded into 6-well plates, as described in Materials and Methods, and were allowed to grow for 72–96 hr. Growth medium was removed, and the cells were rinsed twice with Dulbecco's phosphate-buffered saline containing 0.1 mM bovine serum albumin with or without 15 mM D-glucose and 50 μM CCCP; then the incubation was continued for 30 min at 37°. Medium was removed and [^{14}C]MMEA, in the appropriate medium, was added to a final concentration of 0.5 μM. Plates were harvested at 10, 25, 40 and 60 min and processed, as described in Materials and Methods. Values are means ± SD ($N = 6$). For cytotoxicity studies (bottom panel), U-251 glioblastoma cells were seeded into 24-well plates and allowed to grow for 24–48 hr. RPMI 1640 growth medium was removed, and the cells were rinsed twice with transport medium with or without D-glucose and 50 μM CCCP as described above for the uptake studies; then the incubation was continued for 60 min at 37°. Experimental medium was removed, and the cells were rinsed twice with RPMI 1640 growth medium and incubated in growth medium for 72 hr. The experiment was terminated as described in the legend to Fig. 1.

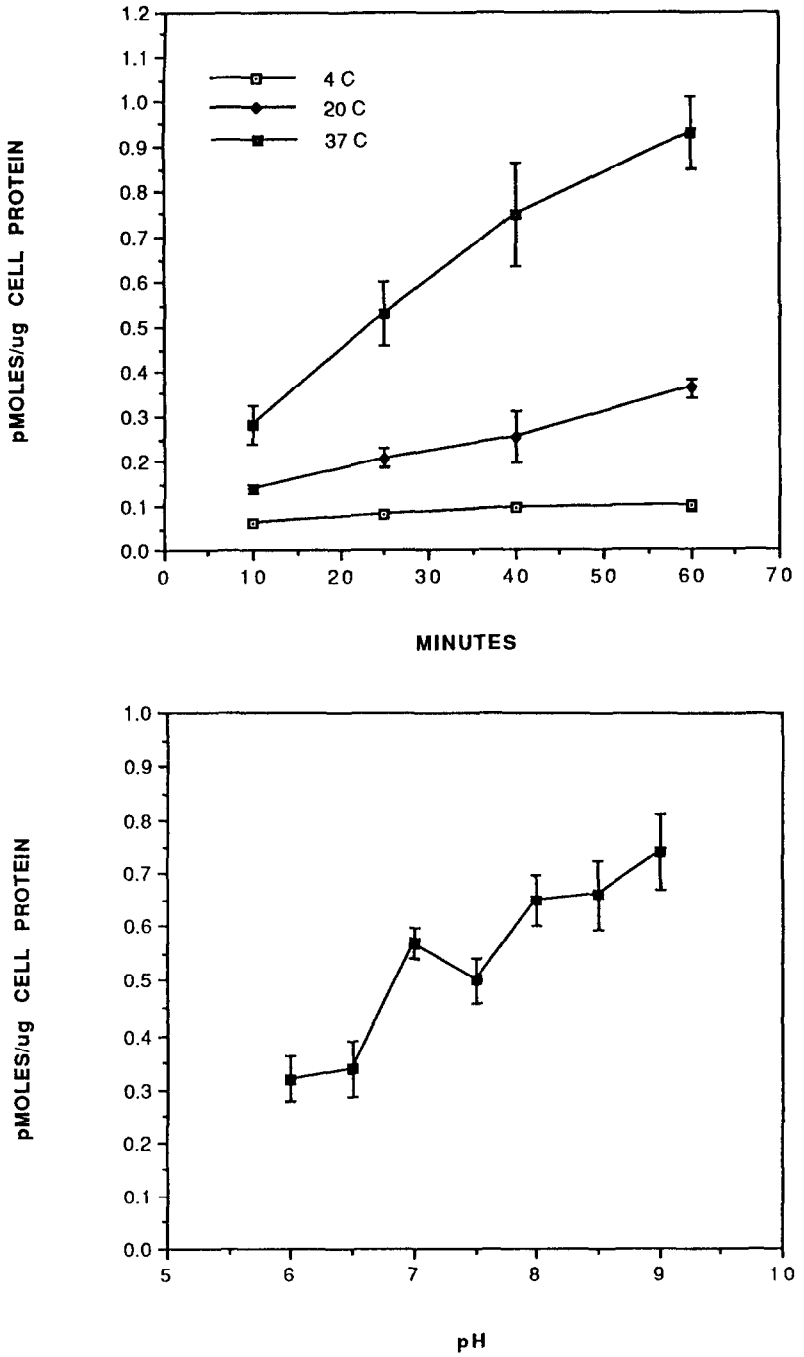

Fig. 5. Effects of temperature and pH on the accumulation of [^{14}C]MMEA-derived radioactivity. For temperature studies (top panel), U-251 glioblastoma cells were seeded into 6-well plates, as described in Materials and Methods, and allowed to grow for 72–96 hr. Growth medium was removed and the cells were rinsed twice with Dulbecco's phosphate-buffered saline containing 0.1 mM bovine serum albumin and 15 mM D-glucose (pH 7.4) at either 4°, 20° or 37° (top panel). Cells were allowed to equilibrate for 15 min, and [^{14}C]MMEA was then added in the medium at the appropriate temperature to a final concentration of 0.5 μM. Plates were harvested at 10, 25, 40 and 60 min and processed as described in Materials and Methods. Values are means ± SD (N = 6). The rate of accumulation of MMEA at each temperature was calculated as follows:

$$\frac{\text{MMEA (40 min} - \text{10 min)}}{30} \times 1000 = \text{fmol MMEA/min/}\mu\text{g cell protein.}$$

For pH studies (bottom panel), cells were equilibrated for 15 min in Dulbecco's phosphate-buffered saline containing 0.1 mM bovine serum albumin and 15 mM D-glucose prepared at pH 6.0–9.0. [^{14}C]-MMEA was added to a final concentration of 0.5 μM; the plates were harvested after 20 min and processed as described in Materials and Methods. Values are means ± SD (N = 6).

mechanism that facilitates selective intracellular accumulation of low concentrations of the drug and/or reactive metabolite(s). Resistance to MMEA may be conferred by virtue of either reduced affinity (apparent K_m) and/or capacity (apparent V_{max}) for MMEA; alternatively, resistant cells may lack altogether the mechanism by which sensitive brain tumor cells accumulate and/or convert MMEA to reactive metabolite(s).

The correlation between accumulation and sensitivity to MMEA was further strengthened by the finding that compromising the ability of the cell to produce energy resulted in both decreased accumulation of [^{14}C]MMEA radioactivity and decreased cytotoxicity of MMEA (Fig. 4). This observation, as well as the decrease in accumulation at reduced temperature (Fig. 5), suggested the involvement of a cellular transport system in the intracellular accumulation of [^{14}C]MMEA-derived radioactivity. Previous results [2] indicated that the intracellular accumulation of [^{14}C]MMEA radioactivity was inhibited competitively by the plant alkaloid reserpine [9]. Since MMEA and reserpine have a similar A-B indole nucleus, and the depletion of brain serotonin [10], noradrenaline [11] and dopamine [12] by this alkaloid has been attributed to blockage of uptake of these amines [13], it seemed plausible that MMEA transport in brain tumor cells might be mediated, at least in part, by a system regulating the accumulation of these biogenic amines in normal brain cells. However, results presented here and elsewhere [2] indicate that there are marked differences in several features of the [^{14}C]MMEA-derived radioactivity accumulation by these sensitive glial-derived brain tumor cell lines, compared with the uptake of catecholamines and serotonin at neuronal synaptic terminals. First, the affinity of serotonin, norepinephrine and dopamine for the presumptive MMEA uptake transporter in brain tumor cells was very low (apparent K_i values ranging from 4 mM for serotonin to 7 mM for the catecholamines) relative to MMEA (apparent K_m = 1–2 μM). The low affinity of these neurotransmitters for uptake in these cells contrasts markedly with the high affinity (apparent K_m values of 0.2 to 1.0 μM) these neurotransmitters exhibit for their neuronal uptake sites [8]. Second, the results presented here indicate that [^{14}C]MMEA radioactivity accumulation was influenced minimally by sodium (Table 1). This is in striking contrast to the strict sodium dependency of the classical neurotransmitter uptake systems at synaptic terminals [8]. Finally, the equivalent competitive inhibition of [^{14}C]MMEA radioactivity uptake by a series of tricyclic antidepressant drugs [2] contrasts with their reported selectivity for inhibition of specific sodium-dependent neurotransmitter uptake systems. For example, imipramine, a tertiary amine that inhibits the neuronal transport of serotonin and norepinephrine, was an equally effective competitive inhibitor of [^{14}C]MMEA radioactivity uptake as was the secondary amine desipramine, which primarily inhibits the uptake of norepinephrine [14]. These differences in selected characteristics of [^{14}C]MMEA radioactivity accumulation, in comparison to biogenic amine transport at neuronal synaptic terminals, suggested that MMEA transport into brain tumor cells may be mediated by a system that is distinct from the classical neurotransmitter uptake sites located at the synaptic terminals [8]. It will be of further interest to determine if an MMEA transporter in these glial-derived tumor cells is related in any way to a binding site for the tricyclic antidepressant imipramine, which has been described on intact astrocytes [15].

Regardless of what might be the normal physiological counterpart, a particular transport process is implicated in the present and previous studies [2] as a determinant, at least in part, of the relative sensitivity [1] of human brain tumor cell lines to certain cytotoxic ellipticiniums, such as MMEA. Quaternization of the pyridine nitrogen appears to be essential for both high-affinity interaction with the uptake system and for brain tumor specificity, since apparent K_i values for ellipticine and other non-quaternized analogs, which do not exhibit such selectivity [1], are >20-fold higher than that of MMEA [2]. Additionally, within the quaternized ellipticine series, several substituted ellipticiniums (e.g. the 9-methyl and 9-chloro analogs) that exhibit comparable selective cytotoxicity toward brain tumor cell lines [1], were equally as effective as MMEA in competitively inhibiting [^{14}C]MMEA radioactivity accumulation [2]. This contrasts with the observation that the affinity of glioblastomas for 9-hydroxy-N^2-methylellipticinium (Celiptium), which does not exhibit selective cytotoxicity [1], is 30-fold less than that observed for MMEA.

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