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# Membrane-active drugs potentiate the killing of tumor cells by D-glucosamine

(lidocaine/anesthetics/sterols/glial tumor cells)

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**ABSTRACT** D-Glucosamine is toxic to several malignant cell lines and *in vivo* tumors at concentrations that have little effect upon normal host tissues. Evidence is presented to support the hypothesis that cellular membranes may be the primary targets of glucosamine's tumoricidal activity. Treatment of rat C6 glioma cells with a cytotoxic concentration of glucosamine (20 mM) caused fragmentation of rough endoplasmic reticulum, proliferation of Golgi complexes, evagination of outer nuclear and mitochondrial membranes, and the accumulation of membranous vacuoles and lipid droplets in the cytoplasm. These changes were detected within the first 3 hr after treatment of cultures with glucosamine and became increasingly severe until cell lysis occurred between 24 and 48 hr of treatment. The cytotoxicity of glucosamine was potentiated by the local anesthetic lidocaine, and by other membrane-active drugs, at concentrations that were growth inhibitory but nonlytic. Most of these drugs possessed local anesthetic activity and inhibited glioma sterol synthesis. Within the same period of time required for ultrastructural changes in cellular membranes, glucosamine inhibited the incorporation of [2-$^{14}$C]acetate into sterols and into an unidentified 400-dalton lipid that migrated close to sterols on thin-layer chromatograms. This inhibition was potentiated by lidocaine and increased over the same range of D-glucosamine concentrations that led to increased cell toxicity after a 48-hr treatment. These findings suggest that the effects of glucosamine upon cellular membranes may be central to its tumoricidal activity and that glucosamine, in combination with membrane-active drugs, may be useful in the treatment of certain types of tumors, particularly those of the central nervous system.

The drugs available for clinical treatment of cancer are believed to act as antiproliferative agents. They have little selectivity (1) and attack rapidly growing tissues by interfering with cell division, inducing unbalanced growth, disrupting nucleotide metabolism, intercalating with or modifying DNA, or altering chromosome structure (2). Because of the diversity of proliferating host tissues (3), such agents are limited in clinical utility by their toxic side effects on normal host tissues. Although the antiproliferative drugs have a major role in the clinical treatment of cancer, an alternative basis for tumor chemotherapy would be valuable.

D-Glucosamine, a naturally occurring amino sugar, is an important carbohydrate component of many cellular glycoproteins, glycolipids, and glycosaminoglycans (4–7). It exhibits little toxicity toward normal host tissues, but is an effective lytic agent for several types of transplantable animal tumors and human tumor xenografts grown in athymic mice (8–18).

The mechanisms of glucosamine cytotoxicity are complex and appear to involve the concatenation of several different effects (19). The most prominent and rapidly occurring changes are produced in cellular membranes. Within the first 3 hr after its addition to cell cultures, D-glucosamine alters the ultrastructure of the plasma and intracellular membranes (17–19), inhibits membrane transport of nucleosides (20), increases nucleoside leakage to the extracellular space (20), shifts the distribution of tritiated glucosamine incorporation from glycoproteins to glycolipids (19), inhibits the flow of [$^{14}$C]acetate into sterols and lipids (19, 21), potentiates the inhibition of cholesterol synthesis by local anesthetics (21), and inhibits cell aggregation, homotypic cell sorting, and lectin agglutination (22–24).

These observations led us to postulate that the selective toxicity of D-glucosamine towards tumor cells might arise primarily from its effects upon cellular membranes (19). To explore this possibility, we have examined the effects upon cultured tumor cells of D-glucosamine in combination with lidocaine, a membrane-active drug that by itself has no known tumoricidal activity. Lidocaine potentiated the cytotoxicity of glucosamine toward the tumor cells, as did various other membrane-active drugs. Although the effective potentiators possessed different chemical structures, most had in common the ability to act as local anesthetics.

## METHODS AND MATERIALS

Rat C6 glioma cells (American Type Culture Collection) were cultured in Eagle's minimal essential medium plus 5% fetal calf serum and passaged at weekly intervals after dissociation with 0.25% trypsin and 1 mM EDTA. Experimental cultures were plated at 50% confluency ($7.5 \times 10^4$ cells per cm$^2$) in T75 flasks or 35-mm tissue-culture dishes 20 hr before labeling and drug treatment. The protein content of cultures was determined by the Lowry method (25), with bovine serum albumin as standard. DNA content was measured by the Burton diphenylamine assay (26).

Tissue culture media and sera were purchased from GIBCO. Glucosamine·HCl and papaverine were purchased from Sigma, ketamine from Bristol Laboratories (Syracuse, NY), lidocaine from Elkins-Sinn (Cherry Hill, NJ), haloperidol from McNeil Laboratories (Ft. Washington, PA), and lipid standards from Supelco (Bellefonte, PA) and Steraloids (Wilton, NH). Adiphenine was donated by Ciba-Geigy (Summit, NJ), thioridazine by Sandoz Pharmaceuticals (Hanover, NJ), and chlorprothixene by Hoffman-La Roche. [2-$^{14}$C]Acetate (55 Ci/mol) was purchased from Amersham and thin-layer chromatographic supplies were from Merck.

Cultures were incubated with growth medium and [2-

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[$^{14}$C]acetate (0.5 μCi/ml) for 6 hr, then washed three times with 137 mM NaCl/5.4 mM KCl/1 mM Tris, pH 7.4, scraped with a rubber policeman, and pelleted at low speed in a desk-top centrifuge. Pellets were stored frozen at −20°C until use, then extracted with 2:1 (vol/vol) chloroform/methanol. The extracts were dried under reduced pressure, redissolved in a small volume of chloroform/methanol, and cochromatographed with authentic neutral lipid standards on silica gel G thin-layer plates in benzene/diethyl ether/ethanol/water, 50:40:2:0.2 (vol/vol), followed by diethyl ether/hexane, 6:94 (vol/vol) (27). Recovery of [$^{3}$H]cholesterol added to the pellets was greater than 90%. Approximately equal amounts of radioactivity were applied to thin-layer plates for each sample analyzed. After development, plates were exposed to iodine vapor to localize lipid standards, then to Kodak Blue Brand x-ray film at −20°C to localize radioactive bands. The proportion of total radioactivity present in each of the separated neutral lipid fractions was determined by measuring areas under peaks obtained from quantitative densitometric scans of autoradiograms. These results were confirmed by direct measurement of thin-layer radioactive bands in Triton X-100/(toluene/2,5-diphenyloxazole/1,4-bis[2(5-phenyloxazolylyl)]benzene) scintillation fluid, 1:4 (vol/vol), in a Beckman liquid scintillation counter. The sterol fraction was composed of labeled desmosterol and cholesterol, as determined by argentation chromatography (28).

Cells in plastic tissue-culture dishes were prepared for electron microscopy by washing in Puck's saline G, fixing with cacodylate-buffered 3% glutaraldehyde for 1 hr, postfixing with 1% osmium for 1 hr, staining with uranyl acetate for 1 hr, then dehydrating in ethanol and embedding in Epon–Araldite. Samples were silver-sectioned on a Porter–Blum ultramicrotome, poststained with uranyl acetate and lead citrate, and examined with a Zeiss EM 9S-2 electron microscope.

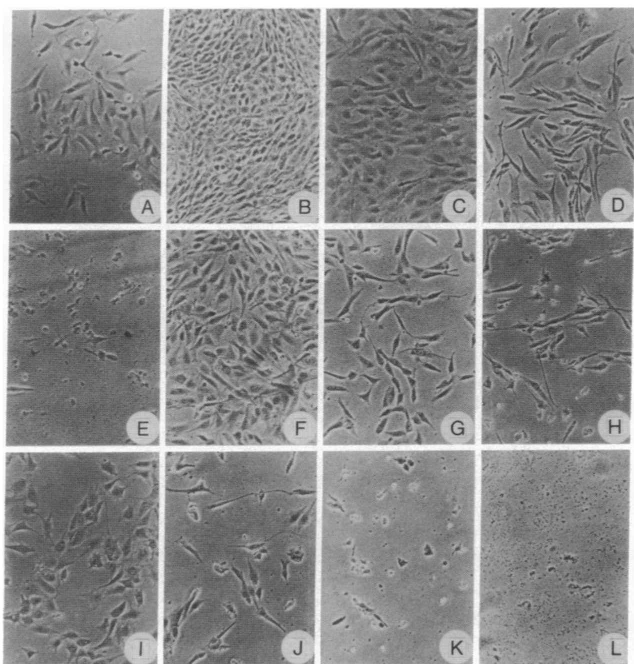


FIG. 1. Effects of glucosamine and lidocaine upon the growth and viability of rat C6 glioma cell cultures. Cells were plated out at a half-confluent density at time 0 (*A*), then incubated for 48 hr with control medium (*B*), 1 mM glucosamine (*C*), 10 mM glucosamine (*D*), 20 mM glucosamine (*E*), 1.25 mM lidocaine (*F*), 2.5 mM lidocaine (*G*), 3.75 mM lidocaine (*H*), 1 mM glucosamine plus 1.25 mM lidocaine (*I*), 1 mM glucosamine plus 1.88 mM lidocaine (*J*), 3 mM glucosamine plus 1.25 mM lidocaine (*K*), or 20 mM glucosamine plus 3.75 mM lidocaine (*L*). (×130.)

## RESULTS

**Glucosamine/Lidocaine Cytotoxicity.** Rat C6 glioma cells plated at moderate density (6 × 10$^{5}$ cells per 35-mm dish) grew rapidly and reached confluency within 48 hr (Fig. 1 *A* and *B*). Over 48 hr of incubation, D-glucosamine concentrations as low as 1 mM detectably inhibited growth; complete growth arrest occurred at about 10 mM (Fig. 1 *C* and *D*). With higher concentrations, cells were irreversibly injured and eventually lysed (Fig. 1*E*). Addition of uridine, hypoxanthine, or adenosine (0.01–1 mM) did not prevent or enhance glucosamine toxicity. Adenosine (1 mM) inhibited culture growth by 50% at 48 hr of treatment. The effects of lidocaine were similar to those of glucosamine. Within 48 hr of incubation, some growth inhibition was evident with 1.25 mM lidocaine (Fig. 1*F*); growth arrest occurred at about 2.5 mM (Fig. 1*G*) and cell lysis at about 3.75 mM (Fig. 1*H*).

When cultures were simultaneously incubated with both drugs, considerable potentiation of growth inhibition and irreversible cell injury occurred. A combination of 1.25 mM lidocaine and 1 mM glucosamine, for example, inhibited growth almost completely (Fig. 1*I*). Elevating either drug even slightly induced some degree of irreversible cell injury, shown by the formation of highly refractile cells, ghosts, and cell debris (Fig. 1 *J* and *K*). Higher concentrations of the two drugs produced total lysis between 24 and 48 hr of treatment (Fig. 1*L*). To determine whether the potentiation of glucosamine growth inhibition and cell lysis by lidocaine was additive or synergistic, we performed an isobologram analysis (29); the interaction between the two drugs was synergistic (Fig. 2).

**Membrane Effects of D-Glucosamine.** As a preliminary step in identifying the mechanisms responsible for its cytotoxicity, we examined the ultrastructural effects of glucosamine upon rat C6 glioma cells. Cultures were incubated with a cytotoxic concentration of glucosamine (20 mM), but were collected for fixation at early times prior to the onset of actual lysis. By comparison with controls, cells treated with glucosamine displayed fragmentation of their rough endoplasmic reticulum, proliferation of the Golgi apparatus, budding of the outer

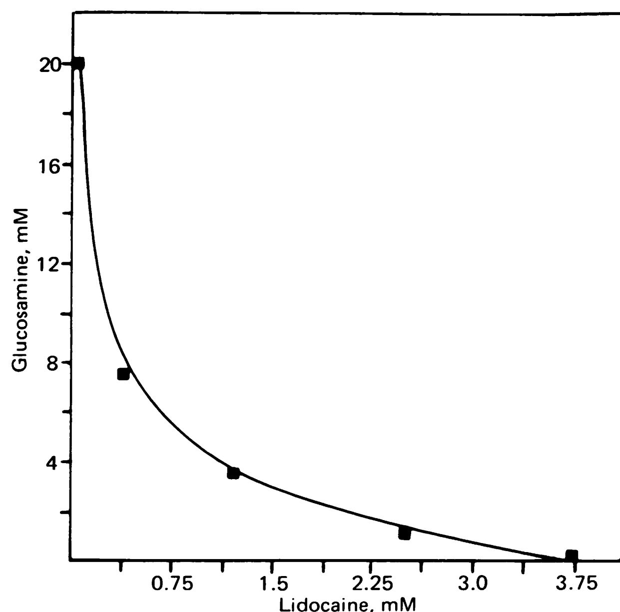


FIG. 2. Isobologram plot of glucosamine/lidocaine combinations that produced 60% cell lysis after 48 hr of treatment. Cell lysis was observed by phase-contrast microscopy and measured as the loss of adherent cell protein in comparison with control (untreated) cultures.

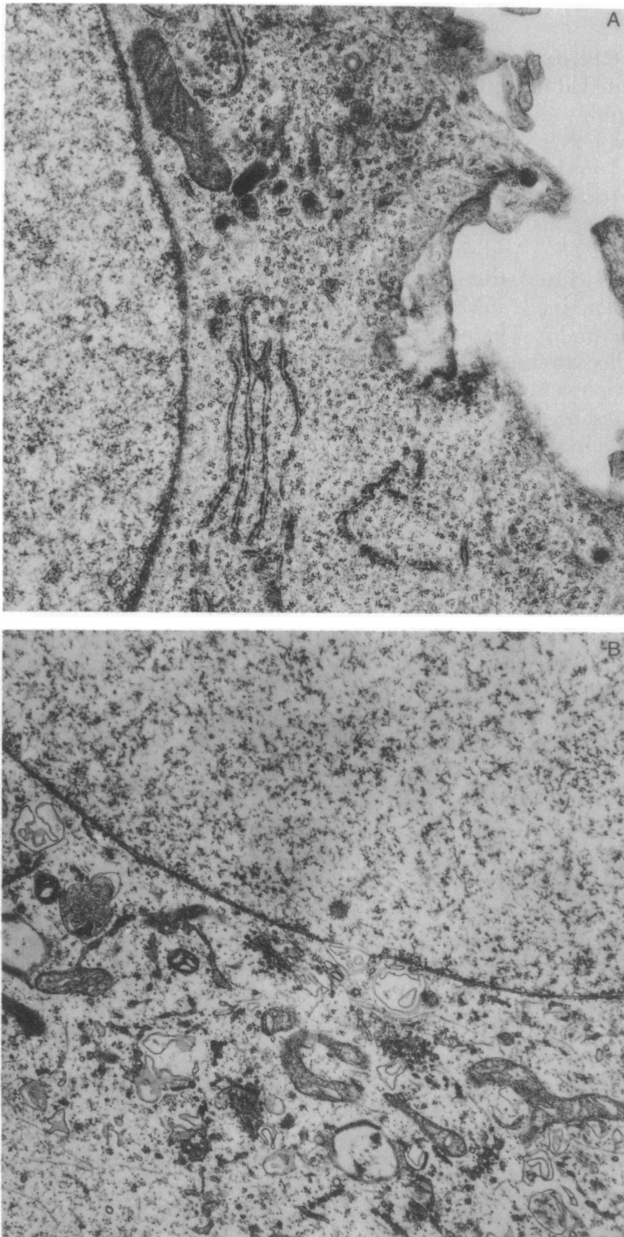


FIG. 3. Ultrastructure of C6 glioma cells before (*A*) and after (*B*) 3 hr of incubation with 20 mM glucosamine. By comparison with controls, treated cells show fragmentation of the rough endoplasmic reticulum, blebbing of the outer nuclear and mitochondrial membranes, increase in vesicles and Golgi apparatus, and decrease in polysome frequency. (×13,600.)

membranes of mitochondria and nuclei, formation of membrane-bounded vacuoles (Fig. 3), accumulation of lipid droplets, and vesiculation of the plasma membrane (not shown). These effects began within 3 hr of incubation with glucosamine and were greatly accentuated by 24 hr.

**Sterol and Lipid Metabolism.** D-Glucosamine inhibited [2-$^{14}$C]acetate incorporation into cellular sterols and into an unidentified lipid that migrated just below the sterol fraction on Silica Gel G thin-layer plates developed with a neutral lipid solvent system (Fig. 4). This effect reached a maximum of about 90% inhibition at 20 mM glucosamine. Preliminary mass spectroscopic studies suggest that the unidentified lipid is an oxygenated hydrocarbon with a mass of 400 daltons (S. J. Friedman and P. Fennessey, unpublished observation).

Lidocaine did not interfere with the incorporation of [2-$^{14}$C]acetate into this lipid, but did inhibit incorporation into the sterol fraction (Fig. 4), which in glioma cells consists of cholesterol and desmosterol (21, 30).

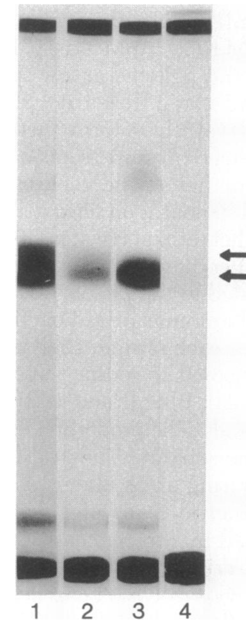


FIG. 4. Effects of glucosamine and lidocaine upon incorporation of [2-$^{14}$C]acetate into neutral lipids. Thin-layer chromatogram is shown. Lane 1, acetate incorporation by control cells into the sterol band (upper arrow) and an unidentified lipid (lower arrow). Lane 2, cultures treated with 10 mM glucosamine. Lane 3, cultures incubated with 2.5 mM lidocaine. Lane 4, cultures receiving 10 mM glucosamine plus 2.5 mM lidocaine. Cultures were incubated for 6 hr with isotope and drugs.

When glioma cultures were simultaneously incubated with glucosamine and lidocaine, there was a large increase in the inhibition of [2-$^{14}$C]acetate incorporation into the sterol and unidentified lipid fractions (Fig. 4 and Table 1). Replicate

Table 1. Cell toxicity and inhibition of lipid synthesis by glucosamine plus lidocaine

| | | [$^{14}$C]Acetate incorporation, cpm/mg cell protein | |
|---|---|---|---|
| Drug | DNA, μg/culture | Unidentified lipid | Sterols |
| None | 12.7 | 5720 | 4629 |
| Lidocaine (2.5 mM) | 11.6 | 4149 | 679 |
| Glucosamine (10 mM) | 6.1 | 793 | 673 |
| Glucosamine (3 mM) + lidocaine (2.5 mM) | 10.0 | 1401 | 651 |
| Glucosamine (6 mM) + lidocaine (2.5 mM) | 5.8 | 930 | 307 |
| Glucosamine (10 mM) + lidocaine (2.5 mM) | 4.7 | 392 | 0 |
| Glucosamine (10 mM) + lidocaine (1.5 mM) | 6.0 | 851 | 327 |

Cultures were treated with the indicated drugs for 6 hr and simultaneously labeled with [2-$^{14}$C]acetate (0.5 μCi/ml). Labeled neutral lipids and protein content of cell monolayers were then measured. Triplicate cultures were treated with the drug(s) for 48 hr, then assayed for DNA content. Average deviation from the mean DNA content shown above was ±7%. Duplicate samples of labeled lipids differed by ±10% or less from the average value shown. Approximately 10,000 cpm of $^{14}$C-labeled chloroform/methanol extract was chromatographed for each sample.

Table 2. Glioma cell toxicity produced by glucosamine in combination with membrane-active drugs

| Drug | % inhibition of protein content | Cell lysis |
|---|---|---|
| Glucosamine (3 mM) | 26 | − |
| Papaverine (100 $\mu$M) | 48 | − |
| Glucosamine + papaverine | 70 | + |
| Chlorprothixene (2.5 $\mu$M) | 12 | − |
| Glucosamine + chlorprothixene | 44 | + |
| Thioridazine (3 $\mu$M) | 24 | − |
| Glucosamine + thioridazine | 62 | + |
| Adiphenine (350 $\mu$M) | 30 | − |
| Glucosamine + adiphenine | 55 | + |
| Glucosamine (10 mM) | 45 | − |
| Lidocaine (2.5 mM) | 40 | − |
| Glucosamine + lidocaine | 97 | + |
| Haloperidol (50 $\mu$M) | 42 | − |
| Glucosamine + haloperidol | 92 | + |
| Ketamine (1 mM) | 12 | − |
| Glucosamine + ketamine | 68 | + |

Results are shown for two separate experiments. Percent inhibition was measured as 100 − % test/control protein content of cell cultures. Duplicate cultures were collected 48 hr after drug treatment was begun. Protein contents of duplicate cultures differed from the average by ±5% or less. All cultures were examined for cell lysis at the time of their collection for protein analysis. The percent inhibition for all drug combinations was significantly greater than the expected inhibition for summation of mutually exclusive inhibitors calculated from $(f_i/f_v)_{\mathrm{drug}\ 1} + (f_i/f_v)_{\mathrm{drug}\ 2}$, where $f_i = 1 - f_v$ and $f_v$ = drug-treated/control protein (31, 32). The significance of the difference between the summation calculated from results obtained with each drug alone and the results obtained with drugs in combination was tested by Wilcoxon's rank sum test. For all drugs, the means of the expression $(f_i/f_v)_1 + (f_i/f_v)_2$ were less than those observed for $(f_i/f_v)_{1,2}$ at the 99% confidence level. An exception was chlorprothixene, which was significant at the 90% confidence level.

cultures assayed at 48 hr showed a decline in DNA content over the same range of glucosamine concentrations that inhibited [2-$^{14}$C]acetate incorporation into the sterol and unidentified lipid fractions (Table 1).

**Glucosamine in Combination with Other Membrane-Active Drugs.** The pronounced effects of glucosamine upon a variety of cellular membrane systems, together with its ability to synergize with the membrane-active drug lidocaine in producing cytotoxicity, suggested to us that the primary mechanism of glucosamine cytotoxicity might involve effects upon cellular membranes rather than upon cell proliferation. If this hypothesis were correct, then various membrane-active drugs might be expected to potentiate the lytic effects of glucosamine.

Glioma cultures were incubated with papaverine, chlorprothixene, thioridazine, adiphenine, lidocaine, haloperidol, or ketamine either singly or in combination with glucosamine (Table 2). Individually, each of these membrane-active drugs produced some degree of growth inhibition at pharmacological concentrations, but did not produce visible cell lysis. However, in combination with growth-inhibitory concentrations of glucosamine (3 or 10 mM), irreversible cell damage resulted. With each combination shown in Table 2, the reduction in culture protein content was greater than would be expected for summation of mutually exclusive inhibitors (31). Phase-contrast microscopic examination of cultures receiving the drug combinations revealed extensive cell lysis at 48 hr. The reduction in culture protein was therefore the result not only of cell growth inhibition, but of cell death as well. Thus, in several respects, the interaction between glucosamine and several different types of membrane-active drugs is similar to that already described for glucosamine plus lidocaine.

## DISCUSSION

A major difficulty in clinical cancer chemotherapy has been the inability to develop drug regimes that will selectively destroy neoplastic cells without harming normal host tissues. Although several drugs will preferentially accumulate in specific tissues, few display any useful degree of selective toxicity toward malignant neoplasms (1). Certain amino sugars are potentially useful exceptions. D-Glucosamine, particularly, is selectively toxic to several malignant cell lines and *in vivo* tumors at concentrations that have little effect upon normal host tissues (8–18, 33). Although the mechanisms by which glucosamine acts are uncertain, two hypotheses have been advanced to account for its toxicity toward tumor cells. The first theory postulates that glucosamine causes tumor cell lysis by depleting cellular nucleotide pools (16, 34–36); the second postulates that it disrupts the structure and function of cellular membrane systems (19).

The nucleotide-depletion hypothesis arises from the ability of glucosamine to deplete cellular pools of nucleotides and their precursors in at least four different ways: (*i*) by trapping uridylate as a UDP-hexosamine complex and by ATP consumption during glucosamine phosphorylation (34–39); (*ii*) by inhibition of nucleotide salvage pathways (20, 40); (*iii*) by inhibiting the uptake of exogenous thymidine (20); and (*iv*) by promoting the cellular leakage of nucleotides and their metabolites (20, 34). However, no firm correlation has yet been established between any of these effects and the development of cytotoxicity. The induction of severe ATP and UTP depletion by glucosamine does not appear to be a sufficient condition for causing tumor cell lethality (34). Glucosamine-induced uridylate trapping does not correlate with cytotoxicity (36, 41–43). Glucosamine cytotoxicity is not prevented by exogenous uridine or by the purines hypoxanthine and adenosine (ref. 16 and this report). These observations suggest that glucosamine's cytotoxicity mechanism may differ from that proposed for the hepatotoxic amino sugar, galactosamine (36).

The membrane theory of glucosamine cytotoxicity arises from the fact that the amino sugar induces a broad spectrum of changes in membrane ultrastructure and function and in the metabolic pathways by which membrane constituents are synthesized. Ultrastructurally, glucosamine causes the fragmentation or vesiculation of the plasma membrane and various intracellular membrane systems of rat C6 glioma cells in culture (Fig. 3). These effects upon cellular membranes are early events in the development of glucosamine cytotoxicity. *In vivo*, glucosamine causes nuclear shrinkage, cytoplasmic retraction and vacuolization, dilation and fragmentation of the endoplasmic reticulum, and loss of mitochondrial cristae by tumor cells (13). Glucosamine inhibits the membrane transport of thymidine (20), promotes the transmembrane leakage of several nucleotide metabolites (20, 34), and modifies the binding pattern of $^{125}$I-labeled fetal calf serum components to the cell surface (19). It inhibits the incorporation of acetate into sterols and an unidentified lipid (Fig. 4), but promotes the flow of tritiated glucosamine into ceramide-Glc-Gal(AcNeu-AcNeu)-Gal-NAc-Gal (GD1), ceramide-Glc-Gal(AcNeu)-GalNAc-Gal (GM1), ceramide-Glc-Gal(AcNeu)-GalNAc (GM2), and lactosyl ceramide (19).

To further explore the possible involvement of membrane events in glucosamine cytotoxicity, we investigated the effects of glucosamine in combination with various membrane-active drugs. Lidocaine synergistically potentiated rat glial tumor cell

lysis by glucosamine in culture (Figs. 1 and 2). Individually, glucosamine inhibited [$^{14}$C]acetate incorporation into sterols and an unidentified lipid, whereas lidocaine inhibited only the incorporation into sterols (Fig. 4 and Table 1). When cell cultures were simultaneously incubated with both drugs, there was an enhanced inhibition of acetate incorporation into both bands. This effect was rapid and was detectable within 1–3 hr after addition of the drugs. It preceded the onset of cell lysis by about 24 hr. We therefore suggest that the cytotoxicity of glucosamine and of the combination of glucosamine and lidocaine results from membrane alterations caused by inhibition of the synthesis of sterols and an unidentified lipid. The possibility that altered nucleotide pools might contribute to the production of these membrane lesions has not been investigated in the rat C6 glioma system. Early changes in plasma membranes of galactosamine-treated liver are prevented or reversed by treatments that prevent hepatotoxicity (e.g., provision of uridine) (44).

Other membrane-active drugs also potentiated the cytotoxicity of glucosamine (Table 2). These drugs differed considerably in their chemical structures and primary pharmacological activities. Among them were an aromatic amine (adiphenine), a benzylisoquinoline (papaverine), a butyrophenone (haloperidol), a cyclohexanone (ketamine), a phenothiazine (thioridazine), and a thioxanthine (chlorprothixene). Most of these drugs possess local anesthetic activity and inhibit acetate incorporation into sterols at concentrations similar to those used in the cytotoxicity experiments (21). This latter observation lends further support to our suggestion that the inhibition of sterol synthesis may be central to the development of cytotoxicity by glucosamine and glucosamine in combination with other membrane-active drugs.

These findings raise the possibility that chemotherapy using glucosamine in combination with local anesthetics and related membrane-active drugs may provide a clinically valuable supplement to the use of antiproliferative agents in the management of certain neoplasms. Preliminary experiments suggest that certain of these combinations may be selectively toxic to several animal tumors *in vivo* and to human neural and glial tumor xenografts in athymic mice (33). Because of their ability to cross the blood–brain barrier, such combinations may prove particularly useful in the treatment of central nervous system tumors.

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1. Clarysse, A., Kenis, Y. & Mathé, G. (1966) *Recent Results Cancer Res.* **53**, 130–163.
2. Sartorelli, A. C. & Johns, D. G. (1975) *Antineoplastic and Immunosuppressive Agents* (Springer, New York), Part 2.
3. Cameron, I. L. (1971) in *Cellular and Molecular Renewal in the Mammalian Body*, eds. Cameron, I. L. & Thrasher, J. D. (Academic, New York), pp. 45–85.
4. Kimura, A. (1961) *Exp. Cell Res.* **23**, 616–618.
5. Spiro, R. G. (1966) in *The Amino Sugars*, eds. Balazs, E. A. & Jeanloz, R. W. (Academic, New York), Vol. 2A, pp. 47–78.
6. Kornfeld, S. & Ginsburg, V. (1966) *Exp. Cell Res.* **41**, 592–600.
7. Plagemann, P. G. W. & Erbe, J. (1966) *Cancer Res.* **33**, 482–492.
8. Quastel, J. H. & Cantero, A. (1953) *Nature (London)* **171**, 252–254.
9. Rubin, A., Springer, G. F. & Hoque, M. J. (1954) *Cancer Res.* **14**, 456–458.
10. Fjelde, A., Sorkin, E. & Rhodes, J. (1956) *Exp. Cell Res.* **10**, 88–98.
11. Ball, H. A., Wick, A. N. & Sanders, C. (1957) *Cancer Res.* **17**, 235–239.
12. Lindner, J., Schweinitz, H. A. & Becker, K. (1960) *Klin. Wochenschr.* **38**, 763–768.
13. Bekesi, J. G., Molnar, Z. & Winzler, R. J. (1969) *Cancer Res.* **29**, 353–359.
14. Bekesi, J. G., Bekesi, E. & Winzler, R. J. (1969) *J. Biol. Chem.* **244**, 3766–3772.
15. Bekesi, J. G. & Winzler, R. J. (1970) *Cancer Res.* **30**, 2905–2912.
16. Bosmann, H. B. (1971) *Biochim. Biophys. Acta* **240**, 74–93.
17. Molnar, Z. & Bekesi, J. G. (1972) *Cancer Res.* **32**, 380–389.
18. Molnar, Z. & Bekesi, J. G. (1972) *Cancer Res.* **32**, 756–765.
19. Friedman, S. J. & Skehan, P. (1978) *In Vitro* **14**, 340 (abstr.).
20. Friedman, S. J., Trotter, C. D., Kimball, T. & Skehan, P. (1977) *Cancer Res.* **37**, 1141–1146.
21. Friedman, S. J. & Skehan, P. (1979) *FEBS Lett.* **102**, 235–240.
22. Kuroda, Y. (1974) *Cancer Res.* **32**, 403–409.
23. Garber, B. (1963) *Dev. Biol.* **7**, 630–641.
24. Kaluza, G., Scholtissek, C. & Rott, R. (1972) *J. Gen. Virol.* **14**, 251–259.
25. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
26. Burton, K. A. (1956) *Biochem. J.* **62**, 315–323.
27. Freeman, C. P. & West, D. (1966) *J. Lipid Res.* **7**, 324–327.
28. Copius-Peereboom, J. W. & Beekes, H. W. (1965) *J. Chromatogr.* **17**, 99–113.
29. Webb, J. L. (1965) *Enzymes and Metabolic Inhibitors* (Academic, New York), Vol. 1, pp. 183–191.
30. Volpe, J. J. & Hennessy, S. W. (1977) *Biochim. Biophys. Acta* **486**, 408–420.
31. Chou, T.-C. & Talalay, P. (1977) *J. Biol. Chem.* **252**, 6438–6442.
32. Chou, T. C. & Talalay, P. (1979) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 645 (abstr.).
33. Helson, L., Helson, C., Sordillo, P., Friedman, S. & Skehan, P. (1979) *Proc. Am. Assoc. Cancer Res.* **20**, 248 (abstr.).
34. Plagemann, P. G. W. & Erbe, J. (1973) *Cancer Res.* **33**, 482–492.
35. Sukeno, T., Kikuchi, H., Saeki, H. & Tsuiki, S. (1971) *Biochim. Biophys. Acta* **244**, 19–29.
36. Decker, K., Keppler, D., Rudigier, J. & Domschke, W. (1971) *Hoppe-Seyler's Z. Physiol. Chem.* **352**, 412–418.
37. Bekesi, J. G. & Winzler, R. J. (1969) *J. Biol. Chem.* **244**, 5663–5668.
38. Harpur, R. P. & Quastel, J. H. (1949) *Nature (London)* **164**, 693–694.
39. Tesoriere, G., Vento, R. & Calvaruso, G. (1975) *Biochim. Biophys. Acta* **385**, 58–67.
40. Friedman, S. J., Kimball, T., Totter, C. D. & Skehan, P. (1977) *Cancer Res.* **37**, 1068–1074.
41. Bernacki, R., Porter, C., Korytnyk, W. & Mihich, E. (1978) *Adv. Enzyme Regul.* **16**, 217–237.
42. Scholtissek, C. (1972) *Biochim. Biophys. Acta* **277**, 459–465.
43. Scholtissek, C., Rott, R. & Klenk, H. D. (1975) *Virology* **63**, 191–200.
44. El-Mofty, S. K., Scrutton, M. C., Serooni, A., Nicolini, C. & Farber, J. L. (1975) *Am. J. Pathol.* **79**, 579–595.