Hydroxyurea for treatment of unresectable and recurrent meningiomas. I. Inhibition of primary human meningioma cells in culture and in meningioma transplants by induction of the apoptotic pathway

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Meningiomas, which invade intracranial bone structures and the adjacent connective tissue, are frequently unresectable because of their aggressive and recalcitrant growth behavior. They have a high recurrence rate, and in approximately 10% of these tumors there is an increased risk of malignancy. Significant morbidity and mortality rates associated with recurrent meningiomas demand nonsurgical approaches. To date, adjuvant hormonal treatment has not proven beneficial. The anticancer drug hydroxyurea was therefore tested for its potential use in the treatment of meningiomas.

Early-passaged cell cultures were established from 20 different meningiomas. The addition of 5×10^{-4} and 10^{-3} M hydroxyurea over a period of 5 to 9 days resulted in a remarkable decrease in cell proliferation and even blocked tumor cell growth when compared with untreated cells. A significant arrest of meningioma cell growth in the S phase of the cell cycle was revealed on DNA flow cytometry.

Electron micrographs of hydroxyurea-treated tumor cells showed ultrastructural features consistent with apoptosis, and light microscopy demonstrated DNA fragmentation by in situ DNA strand break labeling. Short-term treatment of meningioma cell cultures with hydroxyurea for 24 to 48 hours resulted in discrete oligonucleosomal fragments (DNA ladder), another characteristic sign of apoptosis. In addition to the in vitro studies, tissue from five different meningiomas was transplanted into nude mice followed by treatment with 0.5 mg/g body weight hydroxyurea over 15 days. In situ DNA strand break labeling demonstrated DNA fragmentation in distinct regions with different tumor cell densities in all hydroxyurea-treated meningioma transplants.

These data provide evidence that hydroxyurea is a powerful inhibitor of meningioma cell growth, most likely by causing apoptosis in the tumor cells. Thus, hydroxyurea may be a suitable chemotherapeutic agent for the long-term treatment of unresectable or semi- to malignant meningiomas, or for preventing recurrent growth of meningiomas after resection.

Key Words * hydroxyurea * meningioma * apoptosis * growth inhibition * cell culture * cell cycle analysis * nude mice

Meningiomas are mostly benign intracranial tumors (World Health Organization [WHO] Grade I) of mesodermal origin.[75] Among primary intracranial neoplasms, meningiomas occur at a frequency of approximately 20% [27,67] and have an incidence rate varying between 4.9 and 15 per 100,000.[37,46] They invade the dura mater, the skull, and skull base most consistently, resulting in a hyperplastic bony overgrowth.[75] These benign tumors may also affect the pia mater, which conforms closely to each fissure and sulcus of the brain, as well as the connective tissue of the blood channeling structures such as the sagittal and cavernous sinuses. A tumor invasion into the sagittal sinus may narrow or close its lumen. Growth into the cavernous sinus may lead to a palsy of the optic and motor nerves simply by compression.[17,18,32] Meningiomas of the cavernous sinus also infiltrate into the cranial nerves or into the wall of the internal carotid artery, suggesting that a total resection of this type of meningioma may be futile.[38] The growing tumor mass also may compress or shift brain regions. The resulting lesions in eloquent regions like the central motor cortex, the midbrain, or brainstem will substantially reduce patients' quality of life.

Surgical treatment is the primary therapy,[6,54] but the recurrence rate even in totally resected benign tumors (Simpson Grades 1-3) is approximately 10 to 20% after 5 years, 20 to 30% after 10 years, and approximately 50% after 20 years of follow-up review.[1,42,59] Moreover, approximately 10% of meningiomas have an increased risk of becoming a semimalignant or malignant tumor (WHO Grades II and III).[65,76]

The idea of treating a benign tumor with chemotherapeutic agents may not make sense intuitively; however, the risks and limitations of surgery and fractionated radiotherapy are well known, and despite years of investigation, hormonal chemotherapy has not proven particularly beneficial. Higher morbidity and mortality rates demand a more successful primary or adjuvant therapy. Each treatment--surgery, radiotherapy, and chemotherapy--has beneficial effects and side effects. It made sense to us to study a chemotherapeutic treatment for unresectable and recurrent meningiomas because established therapies have failed. The use of chemotherapeutic agents is limited by their side effects. Hydroxyurea, however, belongs to a small group of chemotherapeutic agents that have acceptable and reversible side effects even after years of application. A chemotherapeutic long-term treatment of proliferating meningioma remnants would be compatible with the biological character of this tumor.

Hydroxyurea (M_r 79), has been known since 1869 and began to be applied clinically in the treatment of malignant tumors in the early- to mid-1960s.[2,9,20,29,36,61,64] To explain the antiproliferative effect of hydroxyurea, different mechanisms of action have been described, such as the inhibition of ribonucleotide reductase,[19,35] the inhibition of DNA synthesis without any effect on RNA or protein biosynthesis,[72,73] and ultimately the induction of cell death in the S phase of the cell cycle.[43] Today, hydroxyurea is a powerful drug in long-term treatment of chronic myelogenous leukemia.[29] However, experiments have not been conducted using hydroxyurea for the treatment of meningioma cells in vitro, and hydroxyurea has not been used in patients with intracranial meningiomas. The potential of hydroxyurea to induce cell death and the possibility of a long-term treatment over years encouraged us to test this chemotherapeutic agent on meningioma tissue.

MATERIALS AND METHODS

Tissue Preparation

Freshly resected meningioma tissue was obtained during surgery. Tumor tissue was divided and used for

tissue cultures, transplants in nude mice, or routine pathological studies. Twenty different primary early-passage cell cultures derived from tumors in 20 patients were used for these experiments. The 20 tumors were classified histologically using the WHO[75] grading system.

Cell Culture

For cell culture experiments, tissue was washed with phosphate-buffered saline (PBS) containing 200 U/ml penicillin and 200 µg/ml streptomycin. Subsequently, the tissue was chopped into small cubes (<1 mm³) and transferred to 75-cm² tissue culture flasks, with RPMI 1640 as the culture medium, substituted with 5% vol/vol fetal calf serum (FCS), nonessential amino acids, 100 U/ml penicillin, and 100 µg/ml streptomycin. The cells were grown in a nonhumidified incubator at 36.7°C with 6% CO₂ for several days, with periodic medium changes. Once the cells reached confluence, they were removed by adding trypsin/ethylenediamine tetraacetic acid (4 ml, 0.2% wt/vol in 0.02% ethylenediamine tetraacetic acid), washed, and resuspended in fresh culture medium. Aliquots (10⁵ cells/flask) were seeded into 25-cm² tissue culture flasks and grown for 48 hours. These cells were used for all subsequent experiments.

Cell Count

The culture medium was completely removed from the cells and fresh growth medium containing varying doses of hydroxyurea (10⁻⁵ to 10⁻³ M) was added. Fresh culture medium including the drug in the above concentrations was added every 3 days, and cells were grown for 5 to 9 days. The experiments were stopped when cells in the untreated (control) cultures were almost confluent. On the final day of culture, the number of cells per flask was determined by lysing the cells and counting the nuclei with a cell counter.[12]

Flow Cytometric Analysis of DNA

For the DNA flow cytometric analysis, cultures were treated with hydroxyurea (10⁻⁵-10⁻³ M) under the same conditions as described in Cell Culture. At the end of the incubation period, treated and untreated cell suspensions were washed twice with PBS and enzymatically generated by adding 3 ml 0.5% pepsin-HCl (pH 1.5) and continuously vortexed for 15 minutes at 37°C. Stoichiometric DNA fluorochroming of the nuclei was achieved by transferring the cells into a staining solution of ethidium bromide (12.5 µg/ml) and mithramycin (25µg/ml) dissolved in Tris buffer (pH 7.6), mixed with 250 µl of 0.1% ribonuclease.[74] The number of stained cells corresponded to the number of cells estimated in the cell counter, approximately 10⁵ to 10⁶ cells per flask. After 10 minutes the fluorochromed single-strand nuclear DNA was measured with a flow cytometer adapted with a mercury arc lamp and the fluorescence signals emitted were sorted via analogous digital conversion and stored in a multichannel cytoanalyzer according to their intensity. The cell cycle-stage distribution (percentage of cells in G₀/G₁, S, and G₂/M phases) was calculated using the planimetric method. The assumptions of this method are as follows: 1) the cell cycle model reflects mainly four phases; 2) a possible error factor of measurement is normally distributed; 3) the coefficient of variation is linear with respect to the DNA content measured; and 4) the S phase density can be described by a polynomial of fixed degree.[3]

Cell Preparation for Transmission Electron Microscopy

Hydroxyurea-treated (10⁻⁵-10⁻³ M) and control monolayer cultures grown in 25-cm² flasks were scraped off and centrifuged at 800 G for 10 minutes to form cell pellets (100,000-500,000 cells/pellet). The pelleted cells were fixed with Ito's solution[24] and prepared for electron microscopic examination

according to standard methods.[22] Briefly, the specimens were postfixed with reduced osmium, transferred into 2% agar, stained en bloc with uranyl acetate, dehydrated in a graded series of ethanol solutions completed by pure acetone, and embedded in resin Epon 812. Ultrathin sections were cut, stained with uranyl acetate and lead citrate, and examined with transmission electron microscopes.

Chamber Slide Preparation for Light Microscopy

The chamber slides containing the monolayer were washed twice in PBS (pH 7.4), fixed in ice-cold 4% PBS-buffered formaldehyde for at least 4 hours, and processed by terminal deoxynucleotidyl transferase (TdT) labeling.

Treatment of Transplant Tissue and Preparation for Light Microscopy

The tumor tissue used for implantation into nude mice was washed in sterile 0.9% NaCl, cut into pieces 0.5 to 1 mm³ in size, and drawn into a sterile Hamilton syringe. Male or female (depending on the sex of donor) athymic nu/nu Balb C mice weighing between 31 g and 41 g were anesthetized with an ether/air atmosphere in a glass jar for approximately 10 to 20 seconds. After sterilization of the scalp area, 0.5 ml of the prepared tissue was injected subcutaneously onto the galea. Three weeks after implantation, we started treatment with hydroxyurea, which was injected intraperitoneally in a daily dosage of 0.5 mg hydroxyurea per gram of body weight for 15 days. The treated and control animals were housed separately in sterile cages and given free access to autoclaved food and water. At the end of the treatment period the animals were killed, the nodules removed, and the tissue fixed in 10% buffered formaldehyde.

Control and hydroxyurea-treated specimens of nude mouse meningioma transplants were fixed in buffered 10% formaldehyde for at least 16 hours and embedded in paraffin according to routine procedures. Sections 2 to 4 µm thick were mounted on precoated slides, fixed at 42°C, and stored at room temperature until used for further processing.

Sections of the tissue implanted in the nude mice were compared with histological sections from the original tumors (H & E staining) and were found to be the same type of tissue as diagnosed previously.

Terminal Deoxynucleotidyl Transferase Labeling

After deparaffinization and rehydration, chamber slides containing hydroxyurea-treated and control monolayer cells as well as tissue sections from nude mice were prepared for DNA strand break labeling. Light microscopic visualization of DNA fragmentation was performed using a commercial in situ hybridization kit based on the method introduced by Gavrieli, et al.[21] Thereby, digoxigenin-labeled oligonucleotides were coupled enzymatically to the 3'-OH ends of DNA strands; oligonucleotide binding was visualized by means of a peroxidase-conjugated antidigoxigenin antibody with diaminobenzidine as the chromogenic substrate.

Fragmentation Assay of DNA

Meningioma cell cultures (5 X 10⁵-10⁶ cells/flask) were treated for 24 and 48 hours with hydroxyurea. Following treatment, cells were washed and lysed on ice with reticulocyte standard buffer (10 mM Tris/HCl [pH 7.4], 10 mM NaCl, and 3 mM MgCl) plus 0.5% Triton X-100. Cell nuclei were pelleted, washed in reticulocyte standard buffer including DNase-free RNase, and incubated for 1 hour at 37°C, followed by another incubation with proteinase K for 12 hours at 37°C. The DNA was extracted with phenol-chloroform/isoamyl alcohol and precipitated with isopropanol containing 3 M sodium acetate.

Samples were resuspended and the DNA concentration was determined spectrophotometrically. Equal amounts of DNA were electrophoresed on a 1.5% agarose gel (including ethidium bromide) and visualized by ultraviolet fluorescence.

Statistical Analysis, Specificity, and Toxicity Control

Cell culture experiments were performed in triplicate and analyzed by Student's t-test. Each bar with its standard deviation represented three cell culture flasks.

To confirm that the cultured meningioma cells retained the histopathological characteristics of the primary tumor tissue, three cell samples were grown on plastic in chamber slides in parallel with the triplicate experiments.

For toxicity control a toluidine blue exclusion test was performed with a second series of meningioma cells grown in triplicate in 24-multiwell plates.

Sources of Supplies and Equipment

The cell counter was purchased from Coulter Corp., Bedfordshire, England. The flow cytometer (model ICP-22) was acquired from Phywe, Göttingen, Germany, and the cytoanalyzer (model IN-96) from Intertechnique, Mainz, Germany. Model 109 and 906 transmission electron microscopes were purchased from Zeiss, Oberkochen, Germany. The ApoTag hybridization kit was obtained from Oncor, Gaithersburg, MD, and the Lab-Tek chamber slides from Nunc Inc., Naperville, IL.

RESULTS

Tumor Classification

Routine histological classification revealed 13 meningotheliomatous (11 Grade I and two Grade II), four fibromatous (three Grade I and one Grade II), one angiomatous (Grade I), and two anaplastic (Grade III) meningiomas (20 tumors). At the end of each cell culture experiment, control cultures grown on chamber slides were classified for each case as meningiomas by conventional histopathological staining.

Fig. 1. Graphs showing cell density measurements in cultures derived from a Grade II meningiotheliomatous meningioma. Hydroxyurea (HU) decreased growth (cell density) of human meningioma cultures in a dose-dependent manner and blocked the proliferation at 10^{-3} M. Line graph and bar graph (inset) show the inhibitory effect of HU in concentrations of 10^{-4} M (* $p < 0.05$ vs. control), 5×10^{-4} M, and 10^{-3} M (*** $p < 0.001$ vs. control), whereas inhibition at concentrations of 10^{-5} M and 5×10^{-5} M was not significant. The maximum decrease was seen at HU concentrations of 10^{-3} M in 14 of 20 cases below the number of seeded cells, that is, below 0 and below the baseline (bar B, inset). Inset: Black bars represent HU-treated cultures. A = seeded cells; B = baseline after 48 hours of lag phase but before treatment; C = control. Each bar represents the mean $\pm$ standard deviation (SD) of triplicate experiments.

Dose-Dependent Decreases in the Growth Rate of Cells in Culture

After a period of 6 to 9 days, hydroxyurea administration (10^{-4} - 10^{-3} M) led to a dose-dependent decrease ($p < 0.001$) in cell proliferation, whereas dosage levels of 10^{-5} M had no effect (Figs. 1 and 2). Hydroxyurea in concentrations of 10^{-3} M not only blocked cell growth (Fig. 2) but lowered the number of meningioma cells in 14 of 20 tumors (number of cells after the lag phase) below the baseline established before hydroxyurea treatment (Fig. 1). Cultures of all types of meningiomas listed in Tumor Classification showed similar results. Figures 1 and 2 show the results in a Grade II and a Grade I meningiotheliomatous meningioma, respectively. The viability ($> 99\%$) of the adherent cells in the monolayer did not differ between controls and the highest concentration of hydroxyurea (10^{-3} M). After withdrawal of hydroxyurea, the treated cultures showed the same doubling rates as the control cultures.

Fig. 2. Graphs showing cell density measurements in cultures derived from a Grade I meningiotheliomatous meningioma. Hydroxyurea decreased growth (cell density) of human meningioma cultures in a dose-dependent manner and blocked the proliferation at 10^{-3} M. Line graph and bar graph (inset) show the inhibitory effect of HU in concentrations of 10^{-4} M (** $p < 0.01$ vs. control), 5×10^{-4} M, and 10^{-3} M (*** $p < 0.001$ vs. control). The maximum decrease was seen at HU concentrations of 10^{-3} M in six of 20 cases at the baseline (bar B). Compared to Fig. 1 the decrease in cell numbers is more pronounced, even at lower levels of HU (10^{-4}). Inset: Black bars represent HU-treated cultures. A = seeded cells; B = baseline after 48 hours of lag phase but before treatment; C = control. Each bar represents the mean $\pm$ SD of triplicate experiments.

Effects of Hydroxyurea on Cell Cycle Stages

Because hydroxyurea blocks cells in the S phase, we evaluated the effect of hydroxyurea on the cell cycle stages by DNA flow cytometry (three Grade I and two Grade II meningiotheliomatous meningiomas). To confirm that the cells used for DNA flow cytometric analysis responded to hydroxyurea, the growth rates were determined in parallel with each individual stock of meningioma cells. Hydroxyurea in concentrations of 10^{-5} to 10^{-4} M did not change the cell cycle phases significantly.

However, hydroxyurea in concentrations of 5×10^{-4} M decreased the percentage of cells in the $G_{0/1}$ phase by as much as 80% (vs. control at 93%, $p < 0.001$), increased the percentage of cells in the S phase by as much as 12% (vs. control at 1.9%, $p < 0.001$), and increased the G_2/M phase up to 8.6% (vs.

control at 5%, $p < 0.05$) (Fig. 3a, b, and d).

Fig. 3. a-c: Flow cytometric histograms showing the effect of HU on cell cycle phases after a 7-day incubation period, using cell cultures derived from Grade II meningioma used in the experiments shown in Fig. 1. The horizontal axes represent fluorescence (in channel number), which is directly proportional to nuclear DNA content in different stages of the cell cycle. C = control culture; HU 5 X 10⁻⁴ M and HU 10⁻³ M = treated cultures. d: Chart showing percentage of DNA content in various cell cycle phases. Hydroxyurea at concentrations of 5 X 10⁻⁴ M and 10⁻³ M significantly increased the percentage of cells in the S phase up to 12% and 39%, respectively (vs. control at 1.9%, $p < 0.001$). Data represent mean $\pm$ SD of triplicate experiments. * $p < 0.05$ and *** $p < 0.001$ vs. control, respectively.

With hydroxyurea concentrations of 10⁻³ M this effect was more pronounced, because hydroxyurea decreased the percentage of cells in the G_{0/1} phase by as much as 53% (vs. control at 93%, $p < 0.001$), increased the percentage of cells in the S phase by as much as 39% (vs. control at 1.9%, $p < 0.001$), and increased the G₂/M phase up to 7% (vs. control at 5%, $p < 0.05$) (Fig 3a, c, and d). These data demonstrate a hydroxyurea-induced arrest in the S phase of the cell cycle.

Apoptosis in Cultured Meningioma Cells Treated With Hydroxyurea

Light Microscopy. Because cell culture experiments revealed disintegrated cell nuclei, we screened 10 hydroxyurea-treated cultures for apoptotic cells. At hydroxyurea concentrations of 10⁻³ M, the monolayer cultures of five tumors sporadically exhibited cells with fragmented DNA as confirmed by TdT labeling

(not shown).

Because hydroxyurea decreased the cell number below the number present prior to treatment, we verified that apoptotic cells may be detached from the tissue layer and thereby become lost for examination. Cytospin extracts from the supernatant of 10^{-3} M hydroxyurea-treated cells revealed many apoptotic cells, whereas only a few such cells could be detected in the supernatants of the control cultures.

Fig. 4. Electron micrographs showing the effect of hydroxyurea on cultured meningioma cells. Cells treated with 10^{-3} hydroxyurea were investigated ultrastructurally. In cells in the early stages of apoptosis, a small amount of heterochromatin is condensed along the nuclear membrane, and the nucleolus is clearly demarcated. The peripheral cytoplasm begins to disintegrate in membrane-limited blebs (a). Finally, apoptotic cells have completely fallen apart into apoptotic bodies (b). Original magnification X 30,000.

Electron Microscopy. Using electron microscopy, cells with rather dark cytoplasm were seen shedding numerous membrane-bound cytoplasmic bodies (Fig. 4a). Some cells had almost completely fallen apart into these bodies (Fig. 4b), which eventually contained nuclear fragments besides other cell organelles such as enlarged cisterns of rough cytoplasmic reticulum. In cells in the early stages of apoptosis, a small amount of heterochromatin was condensed along the nuclear membrane, and the nucleolus was clearly demarcated. Because the nuclei of the tumor cells consisted almost entirely of euchromatin, the usually clear demarcation between dark and light nuclear regions (typical of early apoptosis seen in nontumor cells) was not prominent.

Fragmentation of DNA on Agarose Gel Electrophoresis

After 24 to 48 hours of hydroxyurea treatment an intense degradation of DNA was seen on the agarose gel demonstrating the classic ladder of oligonucleosomal DNA, sized in a multiple of 180 bp (data not shown). No DNA degradation was visible in untreated control cells.

Apoptosis in Meningioma Transplants in Nude Mice

The transplanted meningioma cells retained the histological characteristics of the surgical specimen, as confirmed for untreated and treated tissue by microscopic analysis (Fig. 5a). The photomicrograph shows typical characteristics of meningiotheliomatous meningiomas (Grade I) with regularly spaced vesicular nuclei forming whorls in a syncytial background.

Fig. 5. Light photomicrographs demonstrating the effect of hydroxyurea (HU) on meningioma transplants in nude mice. a: Untreated transplants (controls) were removed on the same day as the hydroxyurea-treated transplants and stained to reveal the cell nuclei (in blue). Note the encapsulated meningioma within the tissue. The transplanted cells show the whorllike growth behavior typical of meningiomas. H & E, original magnification X 60. b: Untreated meningioma transplants (controls) were stained for DNA strand breaks using the TdT assay and apoptotic cells were absent in all tissue sections examined. TdT labeling, original magnification X 120. c and d: Transplants removed from HU-treated animals (0.5 mg HU/g body weight/day) stained positive for DNA strand breaks. Apoptotic cell nuclei appear in brown, whereas nonapoptotic cell nuclei are unstained. Both HU-treated transplants (two different mice) contain distinct areas with high numbers of apoptotic cells. TdT labeling, original magnification X 120.

To confirm that hydroxyurea induced apoptosis, 50 meningioma transplants in 3-week-old nude mice were treated with hydroxyurea for a period of 15 days (five meningiomas, 10 transplants each). The dose of 0.5 mg of intraperitoneally administered hydroxyurea per gram of body weight is a dosage commonly used in rats and mice.[23,50] Labeling with TdT revealed significant amounts of labeled cells in distinct regions with alternate density, demonstrating that hydroxyurea induced apoptotic cell death in tumor transplants in nude mice (Fig. 5c and d, meningotheliomatous meningiomas, Grades I and II; positive TdT labeling, brown cells).

DISCUSSION

Adjuvant Drug Therapy for Unresectable Meningiomas

Because of their intracranial location, 25 to 50% of all surgically treated meningiomas are incompletely resected or will recur.[1,14,25,59] The likelihood of death in patients who undergo subtotal tumor

removal is 4.2 times higher than in those who undergo total removal and is comparable to that in patients with malignant meningiomas (4.6 times).[26] Radiation therapy is a standard treatment for unresectable tumor remnants.[4,30,45,48,62] In past years, medical research for controlling meningioma proliferation has been focused on steroids,[8,13,47,49,53] growth factors,[7,33,44,56,68-70] aminergic agents,[55,58] or cytokines.[11,34,57,66] Although some of these concepts are promising, none of them has proven to be effective in a clinical setting. Therefore, there is still a need for an alternative adjuvant drug therapy in patients with unresectable meningiomas.

Effects of Hydroxyurea on Cell Growth and Cell Cycle Stages

Our results clearly demonstrate that hydroxyurea is a potent growth inhibitor of cultured meningioma cells. In cell culture experiments, hydroxyurea prevents growth of meningioma cells in a dose-dependent manner. The highest concentration (10^{-3} M) used in the present experiments not only blocks but decreases the cell count below the number of cells present after the lag phase prior to treatment. The decline in cell number indicated a toxic effect, but the toluidine blue exclusion test revealed excellent viability of the remaining adherent cells. Withdrawal of hydroxyurea resulted in normal doubling rates of treated cells held in check by the toxicological effects of the agent.

The data obtained by flow cytometry demonstrated that hydroxyurea in concentrations between 5×10^{-4} and 10^{-3} M led to a proportional decrease of cells in the $G_{0/1}$ phase and a strong proportional increase of cells in the S phase in meningioma cell cultures. This profile is well known in other tumor cells and is interpreted as a block in the S phase leading to cell death.[31,43,60]

Hydroxyurea and Apoptosis in Cultured Meningioma Cells and Meningioma Transplants

The light-microscopic screening of hydroxyurea-treated meningioma cell cultures revealed cell nuclei that tended to disintegrate, a feature that may indicate apoptosis. This assumption was confirmed by light and electron microscopy. The effects were only present in a portion of cultured cells, because apoptotic cells detach and are lost for examination. The supernatant from hydroxyurea-treated cultures exhibited a significant number of cells stained positively for DNA strand breaks. The induction of apoptosis was confirmed by the presence of discrete oligonucleosomal DNA fragments (DNA ladder).

To verify that hydroxyurea induces apoptosis in meningioma transplants in nude mice, meningioma xenografts were treated with hydroxyurea for 15 days. The histological staining of these transplants revealed a significant number of apoptotic cells as demonstrated by the TdT reaction in distinct regions with different densities in the well-vascularized tissue layer.

We have shown that hydroxyurea in concentrations of 10^{-4} and 10^{-3} M induces apoptosis and not simply toxicity in meningioma tissue. Toxicity would lead to necrosis of the meningioma cells, characterized by a loss of cellular membrane and cell compartment integrity. However, apoptosis, or programmed cell death, is a highly organized process of morphologically defined events and synchronized activation and deactivation of specific genes dependent on de novo protein synthesis.[63,71] Hydroxyurea induced alterations in meningioma tissue, such as cytoplasmic shrinking and surface blebbing as seen with electron microscopy. The formation of discrete oligonucleosomal DNA fragments and the TdT reaction shown histochemically in slices of hydroxyurea-treated meningioma xenografts are consistent with apoptosis-related responses. The evidence that a chemotherapeutic drug controls meningioma cell growth by inducing apoptosis may lead to a new medical concept in meningioma treatment. The molecular basis of hydroxyurea-induced apoptosis in meningioma cells is currently being investigated.

Potential Clinical Applications of Hydroxyurea

Hydroxyurea alone is no longer considered a reliable and adequate therapy for malignant metastatic tumors such as melanomas, renal cell carcinoma, prostate carcinoma, and breast cancer.[41] Attempts to treat intracranial tumors such as medulloblastomas, astrocytomas, and glioblastomas multiforme with hydroxyurea combined with external radiotherapy have met with minor success.[39,40,51] However, meningiomas are nonglial brain tumors that have nothing in common with glial brain tumors such as astrocytomas and glioblastomas. Hydroxyurea has never been used to treat cerebral meningiomas in vitro or in vivo. At present, hydroxyurea is used in solid tumors as an accompanying therapy prior to radiation therapy or chemotherapy for the purpose of tumor cell synchronization. With respect to hematopoietic disorders, hydroxyurea is the treatment of choice for patients with chronic myelogenous leukemia.[29] Based on these data, the compatibility and side effects of hydroxyurea are well known.[28,29] Intravenous or oral application yields high plasma concentrations of hydroxyurea that do not differ significantly with mode of application.[5,10] Within the first 2 hours after an oral dose of 40 to 80 mg/kg/day, hydroxyurea reached a maximum plasma concentration of between 0.5 and 2 mmol/L, equal to 5×10^{-4} M and 2×10^{-3} M respectively, with a half-life of 3.5 to 5 hours.[5,10,15,16,52] Side effects that limit therapeutic use of hydroxyurea are declines in red blood cell counts to 2×10^7 mm³, thrombocyte levels to 60,000 mm³, and white blood cell counts to 2500 mm³.

CONCLUSIONS

We have clearly demonstrated that hydroxyurea induces apoptosis in meningotheiomatous tissue in vitro and in vivo. The growth of cultured meningioma cells can be controlled effectively by this drug. Effective hydroxyurea concentrations of 5×10^{-4} M and 10^{-3} M used in meningioma cell culture experiments are in the range for practical oral doses. The potent antiproliferative effect, caused most likely by inducing the apoptotic pathway, as well as the easy therapeutic adaptation combined with tolerable and reversible side effects, justify a chemotherapeutic treatment of unresectable and rapidly proliferating meningiomas with hydroxyurea in selected cases.

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