Combining Etoposide and Dexrazoxane Synergizes with Radiotherapy and Improves Survival in Mice with Central Nervous System Tumors

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Abstract

Purpose: The treatment of patients with brain metastases is presently ineffective, but cerebral chemoradiotherapy using radiosensitizing agents seems promising. Etoposide targets topoisomerase II, resulting in lethal DNA breaks; such lesions may increase the effect of irradiation, which also depends on DNA damage. Coadministration of the topoisomerase II catalytic inhibitor dexrazoxane in mice allows for more than 3-fold higher dosing of etoposide. We hypothesized that dexrazoxane combined with escalated etoposide doses might improve the efficacy of cerebral radiotherapy.

Experimental Design: Mice with cerebrally inoculated Ehrlich ascites tumor (EHR2) cells were treated with combinations of etoposide + dexrazoxane + cerebral radiotherapy. Similar chemotherapy and radiation combinations were investigated by clonogenic assays using EHR2 cells, and by DNA double-strand break assay through quantification of phosphorylated histone H2AX (γH2AX).

Results: Escalated etoposide dosing (90 mg/kg) combined with dexrazoxane (125 mg/kg) and cerebral radiotherapy (10 Gy × 1) increased the median survival by 60% (P = 0.001) without increased toxicity, suggesting that escalated etoposide levels may indeed represent a new strategy for improving radiotherapy. Interestingly, 125 mg/kg dexrazoxane combined with normal etoposide doses (34 mg/kg) also increased survival from radiotherapy, but only by 27% (P = 0.002). This indicates a direct dexrazoxane modulation of the combined effects of etoposide and irradiation in brain tumors. Further, in vitro, concurrent dexrazoxane, etoposide, and irradiation significantly increased DNA double-strand breaks.

Conclusion: Combining etoposide (high or normal doses) and dexrazoxane synergizes with cerebral radiotherapy and significantly improves survival in mice with central nervous system tumors. This regimen may thus improve radiation therapy of central nervous system tumors.

Brain metastases are diagnosed in at least 10% of cancer patients during the course of their disease (1–3). This proportion is expected to increase due to both improved overall survival and the use of new high molecular weight biologicals, such as Herceptin, which poorly penetrate the blood-brain barrier (4). The majority of patients with brain metastases are currently treated with whole brain radiotherapy. However, survival is poor, and disease progression in the brain is the cause of death in 30% to 40% of these patients (5, 6). Attempts to improve the clinical outcome from whole brain radiotherapy by varying radiation doses and fractionation schedules have failed, and today 20 to 30 Gy in 5 or 10 fractions is a standard regimen (7, 8).

The DNA-alkylating agent temozolomide and the radiosensitizers motexafin gadolinium and RSR13 have recently been shown to improve the efficacy of radiotherapy in patients with brain metastases (9–11) and glioblastoma (12). Another potential drug target for combination therapy with irradiation is topoisomerase II. This nuclear enzyme catalyzes the formation of a transient DNA double-strand break through which another DNA molecule is transported (13), hereby solving topological constraints generated by various DNA metabolic processes (14). A number of clinically important compounds, exemplified by the epipodophyllotoxins etoposide and teniposide, the aminoacridines, and the anthracyclines, interfere with the topoisomerase II catalytic cycle at a stage where a DNA double strand is cleaved. This increases the level of lethal DNA double-strand breaks in cells (15). Because these compounds convert the enzyme into a cellular poison, they are collectively referred to as topoisomerase II poisons (16).

Thus, etoposide, which is widely used clinically, and radiation both cause DNA damage. Indeed, these two modalities...
have been shown to result in synergistic cell kill in vitro (17–21). Furthermore, in these studies, the level of cell kill seemed to correlate positively with the etoposide concentration. In clinical settings, the combination of whole brain radiotherapy and teniposide has been tested in patients with brain metastases. This regimen was shown to improve treatment efficacy, apparently without increasing toxicity (22).

In contrast to the above-mentioned topoisomerase II poisons, the catalytic inhibitors act by arresting the enzyme at stages in its catalytic cycle where the DNA is not cleaved (23, 24). Thus, dexrazoxane functions by sequestering the enzyme when it is in its closed protein clamp conformation on DNA, rendering it unable to carry out DNA strand passage (25–27). It is hereby able to modulate the effect of etoposide in cells (28) as well as in vivo (29, 30). Dexrazoxane is highly hydrophilic and thus has poor blood-brain barrier penetration, which is in contrast to the lipophilic etoposide. Consequently, coadministration of dexrazoxane and etoposide allows for etoposide dose escalation by selectively protecting tissues outside the blood-brain barrier (29, 31), resulting in improved targeting of central nervous system (CNS) tumors in a mouse model (32).

The ability of dexrazoxane to allow for increased concentrations of etoposide in the CNS prompted us to investigate whether escalated doses of etoposide in combination with dexrazoxane may have the potential to improve the clinical outcome from cerebral radiotherapy in mice with CNS tumors, measured as survival. We here present data establishing that 125 mg/kg dexrazoxane in combination with an escalated etoposide dose of 90 mg/kg and cerebral radiotherapy leads to a 60% increase in survival compared with radiotherapy alone ($P = 0.001$). Interestingly, a normal dose of etoposide of 34 mg/kg in combination with 125 mg/kg dexrazoxane and cerebral radiotherapy also increased survival by 27% compared with radiotherapy alone ($P = 0.002$), whereas neither etoposide nor dexrazoxane increased the efficacy from radiotherapy when administered alone. The possible mechanism underlying this synergism is discussed.

### Materials and Methods

#### Animals, housing, and environmental enrichment.

B6D2F1 female mice, weighing 19 to 22 g, were obtained from Taconic M&B (Ry, Denmark), and were allowed to become acclimatized for at least 1 week before being included into experiments. They were kept in standard type III plastic cages (Scanbur B-K, Koege, Denmark) on wood chip bedding with nest material and plastic houses for environmental enrichment (Brogaard, Gentofte, Denmark). They were kept in a 12 hour light-12 hour darkness cycle with ad libitum access to tap water and standard laboratory diet (Altromin, Brogaard). Tumor inoculations and treatments were given during the light cycle period.

#### Drugs and materials.

Dexrazoxane (Zincard) was from Pharmacia (Kalamazoo, MI) and etoposide (Vepesid) from Pfizer A/S (Copenhagen, Denmark). A solution of 1 part Fentanyl-fluanisone (bypnorp) from Janssen-Cilag (Birkerød, Denmark), 1 part midazolam (dormicum) from Roche (Hvidovre, Denmark), and 2 parts isotonic sodium chloride was used for anesthesia. Vehicle corresponding to dosages with 34 and 90 mg/kg etoposide was made from 650 mg polyethylene glycol 300 (Macrogol), 2 mg citric acid, 80 mg polysorbat 80 (Tween 80), and ethanol to 1 mL. Evans blue dye and Lissamine Green were from Sigma-Aldrich (Vallensbaek, Denmark), and were prepared as 2% solutions.

Except for the dyes, drugs were administered by the i.p. route. All drugs were commercially obtained.

#### In vivo experimental procedure.

Cerebral inoculation was done essentially as described in (32). Briefly, Ehrlich ascites tumor cells (EH2R) were harvested from the peritoneal cavity of female B6D2F1 mice, and $1.5 \times 10^5$ cells in 30 μL isotonic saline supplemented with 10% penicillin were manually injected into the temporal hemisphere midways between the right eye and ear at a depth of 4 mm during $\mathrm{O}_2/\mathrm{CO}_2$-induced anesthesia using a 27-gauge needle from Becton Dickinson (Broendby, Denmark). The entire procedure takes about 10 seconds, after which the mice wake up and show normal behavior. The tumor cell inoculation results in single solid intracerebral tumors, with no extracranial spread of disease (32). The animals were then randomly allocated to treatment, which was administered on day 3. Dexrazoxane and etoposide were administered 50 and 30 minutes before radiation, respectively. Compliance was achieved by the administration of 50 μL of anesthetic solution per 20 g body weight, 5 minutes before radiotherapy. The percent median increase in life span was calculated as $\left(\text{MS chemoradiotherapy} - \text{MS saline}\right)/ \left(\text{MS radiotherapy} - \text{MS saline}\right) \times 100\%$; MS is median survival. The doses of etoposide used alone (34 mg/kg) and combined with dexrazoxane (90 and 34 mg/kg) were equaly safe, and 125 mg/kg dexrazoxane has previously been shown to be an effective protective dose against etoposide-induced toxicity (29). The animal experiments were conducted according to institutional and national guidelines for the care and use of laboratory animals, and approved by the Danish Experimental Animal Inspectorate, Department of Justice.

**Radiotherapy.** A homogeneous field of irradiation was delivered to the whole brain only, without irradiation of any other parts of the animal. Radiation was generated by a Stabilipan (Siemens, Munich, Germany) using 300 kV and 12 mA, and the dose rate was 4.7 Gy/min.

**Humane end points.** To ensure the validity of data without compromising animal welfare, an activity scoring system was applied. The end point was defined as the number of days until the mice showed signs of disease according to activity scores as defined below. Hereafter, the animal was euthanized. This was then translated into survival. The mice were observed twice daily, weighted, and assigned an activity score from 1 to 5 as follows. Score 5: The mouse exhibits a normal active and curious behavior. It moves about and stands upright at the sides of the cage. Score 4: The mouse is not quite as active. It does not stand up so often, and prefers to stay in the corners of the cage. Score 3: The mouse is less active, and when it moves it often stops and sits. It stays in the nest corner. Score 2: The mouse moves only when touched, and only for a short distance. It preferably hides in the nest corner. Score 1: The mouse is moribund. Activity scores of 5 or 4 were accepted. Mice having an activity score of 3 were monitored intensively. Mice that had a score of 3 for more than 3 days and mice that had a score of ≤2 for only 1 day, as well as mice that suffered a weight loss of ≥25% regardless of an otherwise acceptable activity score, were euthanized. In addition, mice with sudden signs of cerebral affection such as paralysis were also euthanized. Mice with intracranial EH2R and an activity score of 3 usually eat and drink and show normal behavior such as fur grooming and interest in cage mates, and therefore the Danish Experimental Animal Inspectorate specifically allowed for the mice to show an activity score of 3 for up to 3 days. Around 10% of mice with intracranial EH2R had an activity score of 3 for 2 or 3 days. In efficacy experiments, the observation period was 60 days, and in toxicity experiments it was 90 days.

**Histologic evaluation of the blood-brain barrier.** Surgical anesthesia was obtained with 100 μL of the anesthetic solution per 20 g body weight; then 80 μL of Lissamine Green solution or 25 μL of the Evans blue dye were slowly infused in the tail vein and allowed to circulate. After trans-cardiac perfusion of formalin (4%), the brain was removed. Unambiguous coloration of extracerebral tissues was ensured before the analysis of the staining of brain and tumor. Adjacent sections of 200 μm were cut with a vibratome, and alternate sections were either
immediately stained with hematoxylin and loaded on coverslips, to avoid shrinkage before measurement, or left unstained to be used for image analysis software and its volume was calculated as area × section thickness × numbers of sections.

In vitro clonogenic assay. Subconfluent, asynchronous EHR2 cells in suspension were maintained at 37°C in RPMI 1640 + 10% FCS supplemented with 50 units/mL penicillin and 50 μg/mL streptomycin. Cells were incubated with etoposide for 30 minutes, then irradiated in the presence of the drug and then further incubated with drug for another 120 minutes. The clonogenic assay was done essentially as described (33). Briefly, following drug incubation, cells were washed twice in drug-free media, and 2 × 10^4 cells were plated in triplicate on soft agar on a feeder layer of sheep RBC. This resulted in an average colony count in untreated controls of 6,487 (SD: 2,114). Cells were kept in a humidified atmosphere containing 7.5% CO₂ + air at 37°C and colonies (>64 cells) were counted after 3 weeks. Drug-irradiation interaction was determined according to the principle of median effect analysis (34) using the Calcsyn software from Biosoft (Cambridge, United Kingdom). The combination index denotes synergy, additivity, or antagonism when it is <1, 1, or >1, respectively.

The in vitro γH2AX DNA double-strand break assay. After treatment, cells were pelleted and washed in cold PBS, and lysed in ice-cold lysis buffer [10 mmol/L Tris-HCl (pH 6.5), 50 mmol/L sodium bisulfate, 1% Triton X-100, 10 mmol/L MgCl₂, 8.6% sucrose] by applying 20 strokes in a tight-fitting Dounce homogenizer. Released nuclei were pelleted by centrifugation at 2,500 × g for 10 minutes at 4°C, and washed in lysis buffer followed by wash buffer (10 mmol/L Tris-HCl, 13 mmol/L EDTA (pH 7.4)]. The pellet was then resuspended in 100 μL of ice-cold 0.4 mol/L H₂SO₄ and incubated for 1 hour at 4°C before centrifugation. The supernatant was transferred to a clean tube, and 1 mL ice-cold acetone was added followed by incubation overnight for histone precipitation. After centrifugation, the pellet was air-dried and resuspended in 40 μL H₂O, and the protein concentration was determined by the Bradford protein assay (Bio-Rad Laboratories, Hercules, CA). Western blotting was done by loading 10 μg of total histones on a 4% to 12% gradient gel (NuPage Bis-Tris Gel, Invitrogen). Separated proteins were transferred to nitrocellulose membranes (Bio-Rad), which were blocked with 10% skimmed milk (Fluka) and incubated overnight with anti-γH2AX primary antibody diluted 1:500 (Upstate Technology), followed by detection with goat ant-mouse 1:2,000 for 1 hour (Amersham, Biosciences, Buckinghamshire, United Kingdom). Detection with ECL Plus (Amersham) was done by scanning on a STORM 840 (Molecular Dynamics, Inc., Chesham, United Kingdom), on which the image was optimized and bands quantified by Image Quant version 5.0 (Molecular Dynamics).

Results

Identifying the optimal efficacy of single-dose cerebral radiotherapy. First, we determined the dose of cerebral radiotherapy to be used in the following chemoradiation experiments: Mice with brain tumors were treated with increasing doses of cerebral radiotherapy, and the maximum survival was found to be obtained from a dose of 10 Gy (Fig. 1).

Toxicity from chemoradiotherapy. Following the acute chemotherapy-induced weight loss, all animals resumed growth. At 3 months, there were no differences in body weight between mice treated with chemotherapy compared with those treated with chemoradiotherapy, and all mice had a performance score of 5. Toxicity data are summarized in Table 1. Weight loss following even identical doses of chemotherapy alone (no radiotherapy) was obtained with a dose of 10 Gy whereas higher doses did not improve survival. O, saline-treated controls; △, 5 Gy × 1; □, 10 Gy × 1; ×, 15 Gy × 1; and ○, 20 Gy × 1.

| Table 1. Toxicity from chemotherapy + concomitant cerebral radiotherapy |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Saline 125 mg/kg| Dexrazoxane 125 mg/kg | Etoposide 34 mg/kg | Etoposide 34 mg/kg + dexrazoxane 125 mg/kg |
| Chemotherapy alone (no radiotherapy) Relative weight on nadir (Mean ± SD)           | 0.97 ± 0.03     | 0.96 ± 0.01               | 0.90 ± 0.05               | 0.90 ± 0.07               | 0.82 ± 0.05               |
| Chemotherapy plus concomitant cerebral radiotherapy Relative weight on nadir (Mean ± SD)  | 0.95 ± 0.02     | 0.93 ± 0.04               | 0.89 ± 0.04               | 0.88 ± 0.08               | 0.84 ± 0.06               |
| P                | 0.26            | 0.056                     | 0.80                        | 0.67                        | 0.37                        |

NOTE: Toxicity was evaluated after treatment with chemotherapy alone (top rows) or after treatment with chemotherapy and concomitant radiotherapy (bottom rows). There were nine mice in each group. The mean relative weight on nadir (the weight at nadir divided by the weight pretreatment) is shown. There was no difference in toxicity after treatment with chemotherapy alone compared with chemoradiotherapy. One mouse died on day 2 after treatment with etoposide 34 mg/kg and cerebral radiotherapy, probably from a procedure-related complication. On day 90 (end of observation period), the mean relative weight was 1.26, ranging from 1.22 to 1.30 (SD: 0.02), and all animals had a performance score of 5. There were no significant differences between saline alone (without radiotherapy) and others.
cytotoxics can differ substantially, and the weight loss from the higher dose of etoposide was greater than expected, based on our previous experience with these drugs in the same strain of mice (29, 31). Nevertheless, survival and long-term toxicity were as predicted. Thus, 10 Gy as a single dose was safe and did not increase the chemotherapy toxicity. This dose was therefore used in the following chemotherapy experiments.

Dexrazoxane and etoposide synergize with cerebral radiotherapy and increase the median survival time in mice with brain tumors. Dexrazoxane increases the maximal tolerable dose of etoposide in mice (29), and this allowed us to study the effects of combining both normal and supranormal etoposide doses with cerebral radiotherapy. First, the effect on survival following treatment with etoposide at 34 mg/kg in the presence and absence of radiotherapy was assessed. The chemotherapy alone increased the survival compared with controls ($P = 0.0001$), but the effect of radiotherapy was not further improved by coadministration with this dose of etoposide (Fig. 2A). When applied as a single agent, 125 mg/kg dexrazoxane only modestly increased survival time compared with saline-treated controls ($P = 0.025$) and, again, coadministration with radiotherapy did not improve survival time (Fig. 2B). We next tested the effect of coadministration of this dexrazoxane dose and the normal dose of etoposide of 34 mg/kg in the absence and presence of radiotherapy (Fig. 2C). In the absence of radiation, no synergism was obtained from the combination of 34 mg/kg etoposide and 125 mg/kg dexrazoxane, as compared with when each of these two drugs were used as monotherapy (compare Fig. 2A and B to Fig. 2C). However, when this two-drug regimen was combined with radiation therapy, the median survival time was increased by 27% as compared with radiotherapy alone ($P = 0.002$). We finally tested the effect of coadministering 125 mg/kg dexrazoxane and an escalated dose of etoposide of 90 mg/kg in the absence and presence of radiotherapy. In combination with dexrazoxane at 125 mg/kg, this escalated etoposide dose improved survival as compared with that obtained with etoposide 34 mg/kg alone (compare Fig. 2A and D). Most importantly, however, when this high-dose two-drug regimen was combined with radiotherapy, survival was significantly improved by 60% compared with radiotherapy alone ($P = 0.001$), thus confirming our initial hypothesis that elevated etoposide levels in the CNS represent a means of improving radiation therapy. Efficacy data are summarized in Table 2. In identical experiments, chemotherapy was
substituted with etoposide vehicle in amounts corresponding to what is administered when dosing with etoposide at 34 and 90 mg/kg (n = 18 in each experimental arm). Survival from vehicle (both doses) was identical to saline-treated controls and, similarly, survival after radiotherapy and vehicle was identical to that obtained from radiotherapy alone (data not shown).

The blood-brain barrier is intact in mice with Ehrlich ascites tumor cells in the brain. Tumor size and gross capillary permeability were assessed on day 3 in two groups of five mice by the use of Evans blue dye and Lissamine Green. The cerebrally inoculated EHR2 cells formed a single solid tumor nodule (mean tumor volume: 1.4 mm³; SD: 1.1). There was no staining of either tumor or the surrounding unaffected brain from the application of these dyes, confirming the notion that these small tumors possessed an intact blood-brain barrier.

Synergistic cell kill following high concentrations of etoposide and irradiation in clonogenic assays. The beneficial effect of high-dose etoposide on the outcome of radiotherapy in vivo described above indicates that the efficacy resulting from combining etoposide and radiation may depend on reaching a certain critical etoposide concentration in the tumor. To address this possibility directly, we tested the effects of different combinations of etoposide and dexrazoxane in combination with irradiation in clonogenic assays using the same EHR2 cell line as was used for tumor inoculation in vivo.

Irradiation with 7.5 Gy in the presence of etoposide resulted in supra-additive cell kill (Fig. 3A); computation of the combination indices revealed synergy at etoposide concentrations of 5 μmol/L or higher, whereas lower drug concentrations in fact were antagonistic (Table 3). This result is in line with our in vivo data suggesting that etoposide at 90 mg/kg represents an improvement, as compared with etoposide at 34 mg/kg, in augmenting the effect of cerebral irradiation. In vitro, however, the radioenhancing effect of adding dexrazoxane to etoposide was unaltered compared with irradiation in the presence of etoposide alone (compare Fig. 3A and C), and dexrazoxane alone did not act as a radiosensitizer (Fig. 3B; Table 3).

The combination of irradiation, etoposide, and dexrazoxane results in increased levels of DNA double-strand breaks in vitro. Phosphorylation of histone H2AX on serine 139, also referred to as γH2AX induction, is an established marker of DNA double-strand breaks in cells (35, 36) and quantification of double-strand breaks in EHR2 cells could thus hint at possible mechanisms of action: Whereas the application of 187.5 μmol/L dexrazoxane or 7.5 μmol/L etoposide did not cause any significant increase in the level of γH2AX induction as assessed 120 minutes after irradiation compared with irradiation alone, the cotreatment with the exact same concentrations of these two drugs significantly increased the level of γH2AX induction observed at this time point (Fig. 4). This result suggested that the efficacy from the two-drug chemoradiation regimen may be caused by the induction of an increased number of cytotoxic double-strand breaks.

### Table 2. Increase in survival after chemoradiotherapy compared with radiotherapy alone

<table>
<thead>
<tr>
<th>Chemotherapy</th>
<th>Etoposide 90 mg/kg + dexrazoxane 125 mg/kg</th>
<th>Etoposide 34 mg/kg + dexrazoxane 125 mg/kg</th>
<th>Etoposide 34 mg/kg</th>
<th>Dexrazoxane 125 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of mice</td>
<td>25</td>
<td>18</td>
<td>26</td>
<td>16</td>
</tr>
<tr>
<td>% ILS</td>
<td>60</td>
<td>27</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P</td>
<td>0.001</td>
<td>0.002</td>
<td>0.13 (ns)</td>
<td>0.44 (ns)</td>
</tr>
</tbody>
</table>

NOTE: The total number of mice treated with each of the experimental treatments was compiled from two or three separate experiments. The percentage increase in lifespan (% ILS) was calculated as (MS chemoradiotherapy − MS saline) / (MS radiotherapy − MS saline) × 100%; MS is median survival. The P values were from comparisons of the survival after chemoradiotherapy and radiotherapy alone, using the log-rank test.
Discussion

The diagnosis of brain metastases represents an ultimate progressive state of malignancy: Treatment successes are virtually nonexistent, except for those few patients amenable to aggressive focal therapy. Median survival ranges from 3 to 4 months, and attempts to improve efficacy from radiotherapy by dose escalation and alterations of treatment schedules have largely been unsuccessful. However, corticosteroids remain the palliative drugs of choice for most patients.

The epipodophyllotoxins are good candidates for use with whole brain radiotherapy because they possess no intrinsic neurotoxicity (37, 38). Furthermore, in small-cell lung cancer patients with brain metastases, standard doses of teniposide and whole brain radiotherapy have shown encouraging responses, indicating improved anticancer activity without increased toxicity. However, survival was not significantly prolonged (22). In patients, the concentrations of epipodophyllotoxins in normal brain and cerebral tumors are ~5% and 20% of concurrent plasma values, respectively, which are both considerably lower than what is found in extracerebral normal tissues and tumors (39–41). Dexrazoxane has not been investigated in brain parenchyma, but penetration of the blood-brain barrier is low due to its high hydrophilicity (42).

Based on the findings that (i) the uptake of epipodophyllotoxin in the brain and in cerebral tumors is less than in extracerebral tissues, (ii) that improved cerebral response rates have been shown after chemoradiotherapy in small-cell lung cancer patients with brain metastases, and (iii) that this synergism depends on the drug concentration, we hypothesized that escalated doses of etoposide, as allowed by dexrazoxane coadministration, might prove beneficial in combination with radiation therapy. This hypothesis was tested by combining 125 mg/kg dexrazoxane with an escalated etoposide dose of 90 mg/kg and concurrent whole brain irradiation in mice inoculated with EHR2 cells in the brain. This two-drug chemoradiation regimen was found to increase the median survival time by 60% as compared with saline-treated controls receiving a similar dose of irradiation. In contrast, application of the 34 mg/kg etoposide dose had no beneficial effect on the outcome of radiotherapy, suggesting that in combination with radiation therapy, escalated levels of etoposide in the brain may represent a treatment advantage. This suggests that despite the fact that each of the two etoposide doses possessed significant anticancer effect by themselves, only the high dose reached a threshold concentration necessary for synergism with radiation. A direct comparison of survival from cerebral radiotherapy with and without etoposide 90 mg/kg and no dexrazoxane is not possible because this etoposide dose when administered alone is lethal (29).

To our surprise, the combination of 125 mg/kg dexrazoxane with an etoposide dose of 34 mg/kg also increased the efficacy of cerebral radiotherapy. Because the blood-brain barrier was found to be intact in our dye exclusion experiments, it suggests that dexrazoxane does in fact cross the blood-brain barrier, if only in small amounts, and thus is able to modulate the effect of etoposide and radiation.

This raises the question on which possible mechanisms could explain this observation. The catalytic inhibitor bufalin decreased the repair of radiation-induced DNA breaks (43), and this could suggest a similar mechanism for dexrazoxane; however, this failed to explain why 125 mg/kg dexrazoxane did not improve the outcome of radiation therapy when used as a single agent.

In colony assays, it was shown that pretreatment with high concentrations of ICRF-193 (an analogue of dexrazoxane)

| Table 3. Combination indices of radiation and etoposide and/or dexrazoxane |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| **Etoposide (μmol/L)** | **Dexrazoxane (μmol/L)** | **Radiation (Gy)** | **Mutually nonexclusive combination indices** | **Classification of effect** |
| Radiation + etoposide | | | | |
| 1.25 | 0 | 1.25 | 1.53 | – – – | Antagonism |
| 2.5 | 0 | 1.25 | 1.89 | – – – | Antagonism |
| 7.5 | 0 | 1.25 | 1.22 | – – | Moderate antagonism |
| 12.5 | 0 | 7.5 | 1.11 | – | Slight antagonism |
| 2.5 | 0 | 7.5 | 0.92 | ± | Nearly additive |
| 5 | 0 | 7.5 | 0.62 | ++ + | Synergism |
| 7.5 | 0 | 7.5 | 0.51 | ++ + | Synergism |
| 10 | 0 | 7.5 | 0.17 | ++ + + | Strong Synergism |
| Radiation + etoposide + dexrazoxane | | | | |
| 2.5 | 62.5 | 7.5 | 0.93 | ± | Nearly additive |
| 5 | 125 | 7.5 | 0.69 | ++ + | Synergism |
| 7.5 | 187.5 | 7.5 | 0.49 | ++ + | Synergism |
| 10 | 250 | 7.5 | 0.44 | ++ + | Synergism |

NOTE: The interactions of radiation and etoposide (top rows) and of radiation and a fixed concentration ratio of etoposide and dexrazoxane (bottom rows) in colony assays were analyzed according to the median effect analysis. The mutually exclusive combination index (CI) was calculated as CI = CIX/BIX/A + CIX/BIX/B + CIX/BIX × CIX/BIX/A × CIX/BIX/B, where CIX and CIX/B are the concentrations of drugs A and B used in combination to achieve x% drug effect. ICICX/A and ICICX/B are the concentrations for single agents to achieve the same effect. A CI < 1 signifies synergy, meaning that less drug was needed for a specified effect when the drugs were combined than predicted from the potency of each drug alone and vice versa; a CI > 1 signals less potency of the drugs in combination compared with their activities when used alone. A CI ~ 1 means that the combined activity was additive. The classification of effects was done according to the Calcusyn manual.
assessed by Western blotting as described in Materials and Methods. Bars, SE of drug(s), total histones were extracted, and the level of dexrazoxane for 30 minutes, and then irradiated with 7.5 Gy. Cells were then dexrazoxane, or with the combination of 7.5 Gy after 120 minutes was significantly higher than the level induced by radiation alone, which is indeed in line with our in vivo data.

In conclusion, etoposide dose escalation, as allowed by coadministration with dexrazoxane, improved the outcome of radiotherapy in mice with CNS tumor, and elevated etoposide levels in the tumor are the most probable reason for this. The improved survival from radiotherapy following dexrazoxane and normal dose of etoposide is difficult to explain and may involve any of a number of possible mechanisms (e.g., pharmacokinetics, inhibition of DNA repair, or generation of additional DNA damage).

A pharmacokinetics based explanation is attractive because it offers insight into how these drugs may optimally be administered to patients: Possibly, the prolonged coexposure of the cerebral tumor to etoposide and low concentrations of dexrazoxane enhances the outcome from radiotherapy whereas, extracerebrally, the much higher dexrazoxane concentration counteracts unwanted toxic effects (e.g., myelosuppression; ref. 31).

By applying our current knowledge of the differential cellular effects from the DNA cleavage enhancers and the catalytic inhibitors, further insight into the biological effects of topoisomerase II and radiation may be achieved; hopefully, this and a rational pharmacokinetics based approach to the administration of drugs may prove to be of real clinical benefit to future patients with CNS tumors.

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References


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