Factors responsible for the retention of fluid in human tumor edema and the effect of dexamethasone

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The components of vasogenic edema associated with brain tumors were investigated in human biopsy material sampled from tumor and peritumoral tissue during neurosurgical operations. Tissue from 60 patients with glioblastomas, gliomas, meningiomas, and metastases who had been treated with dexamethasone prior to surgery was used for measurement of water, electrolyte, hemoglobin, serum protein, and dexamethasone concentrations. In all samples except metastases, positive correlations were obtained between water content and both serum protein levels and sodium content in tumors and peritumoral edema, suggesting that these components simultaneously determine forces for extravasation of plasma-derived edema fluid. However, the mean serum protein content varied considerably, being high in glioblastomas (16 mg/ml) and low in peritumoral edema surrounding metastases (4 mg/ml). The mean cerebral blood volume in all samples, as calculated from the tissue hemoglobin content, was 2.5 ml/100 gm wet weight in tumor tissue and 1.6 to 2.0 ml/100 gm wet weight in peritumoral tissue. Sodium concentrations were not significantly different among the tumor types. Both water and serum protein content decreased with increasing dexamethasone concentrations in glioblastomas, while this effect was virtually absent in gliomas and meningiomas. A therapeutic threshold of dexamethasone at 500 mg/gm wet weight was obtained for tumoral and peritumoral tissue of glioblastomas and was effective in a dose-dependent manner as long as the water content and the serum protein concentration remained below 6 ml/gm dry weight and 30 mg/gm dry weight, respectively. These results suggest a previously unknown selectivity among tumor types for the reduction of both water content and serum proteins in corticosteroid-treated edematous tissue.

Key Words - brain neoplasm - dexamethasone - edema - glioblastoma - meningioma
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This raises the question of whether protein extravasation is an accompaniment rather than the primary pathogenic factor in the development of the vasogenic type of edema.

In the present study, the relationship between extravasated serum proteins, electrolyte content, and water accumulation was investigated in human brain-tumor biopsy material obtained during neurosurgical operations. Most of the patients had been treated with corticosteroids prior to surgery. By measuring the dexamethasone content of brain-tissue samples, it was also possible to investigate the influence of this treatment on both water and protein content. The results indicated a relationship between protein and water content in some but not all tumors, suggesting that this relationship is more complex than anticipated from previous experimental studies.

Clinical Material and Methods

Biopsy material from unselected patients with brain tumors was sampled during surgery and was immediately frozen in liquid nitrogen. Tumor and peritumoral brain tissue were identified and processed separately. Each sample was divided into two aliquots: one was investigated for the concentrations of water, electrolytes, and extravasated serum proteins and the other was used to determine the content of dexamethasone.

Measurement of Water and Electrolytes

Samples were dried at 110°C for 2 days to constant weight, and the water content (ml/gm dry weight) was calculated from the wet/dry weight relationship. Dried material was homogenized in water using a sonifier, and then passed through an affinity column as described below. The sodium and potassium content of the effluent was measured by atomic absorption spectroscopy and expressed as μEq/mg dry weight. Blanks and standard solutions were prepared for each electrolyte using the same final concentration of solvents as for serum protein extractions. For prevention of external ion contamination, all solutions, samples, and standards were prepared with de-ionized water and stored in plastic vials.

Measurement of Extravasated Serum Protein

The diluted homogenate of tissue samples was passed through immunosorbent columns containing antibodies directed against human serum proteins and hemoglobin, respectively. These sorbents were prepared by loading separate columns with antibodies as described before. Elution of specific-bound serum proteins or hemoglobin was carried out with 0.1 N acetic acid and 0.145 N NaCl (pH 2.7). Protein concentration was determined in the homogenate and the column eluates by the method of Lowry, et al.18

The amount of extravasated serum protein was calculated by subtracting the intravascular from the total brain serum protein content. Intravascular protein content was calculated from the serum protein concentration and the cerebral blood volume (CBV) which, in turn, were estimated from brain and blood hemoglobin levels, using a correction factor for the difference between peripheral and brain vessel hematocrit. Details of the methods and calculations have been published previously.3

Measurement of Dexamethasone Content

In a subgroup of 56 patients with glioblastomas, gliomas, and meningiomas, the dexamethasone content was measured in tumor and peritumoral brain tissue. For this purpose, aliquots of the frozen tissue samples were extracted in butanol and phosphate buffer and analyzed by high-performance liquid chromatography according to the method of Dolphin and Pergande.6

Classification of Brain Tumors

Biopsy material from all brain tumors was fixed by immersion in 4% formalin and processed for routine histology. Classification of brain tumors was carried out in the Laboratory of Neuropathology, Hospital Köln-Merheim (by courtesy of Dr. G. Ebhardt).

Results

General Clinical Data

The tumor material investigated in this study comprised 30 glioblastomas, 12 gliomas, 17 meningiomas, and nine metastases obtained from 60 patients. The clinical data of tumor patients are summarized in Table 1. The patients with gliomas were the youngest age group (44.7 ± 13.8 years, mean ± standard deviation), followed by the patients with metastases (51.1 ± 16.9 years), glioblastomas (60.0 ± 9.4 years), and meningiomas (62.1 ± 22.2 years). In three tumor groups sex differences were noted: about 70% of patients with glioblastomas and metastases were male, whereas 62% of patients with meningiomas were female. These observations agree with previous brain-tumor statistics and confirm that patient groups are representative.30

Average body weight and the serum content of protein, sodium, and potassium did not vary markedly among the four tumor groups. However, the amount of dexamethasone given to individual patients before surgery varied from 0 to 64 mg/day; the average dose was about 15 mg/day in patients with gliomas and meningiomas, and about 20 mg/day in patients with metastases and glioblastomas.

Edema Constituents

The analytical data for water, electrolytes, and serum proteins in tumors and peritumoral brain tissue are summarized in Table 2. A considerable variation of the measured parameters was observed within each group. However, several consistent observations could be
TABLE 1
Clinical data in 60 patients with brain tumors*

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>No. of Tumors</th>
<th>Patients' Age (yrs)</th>
<th>Sex (%)</th>
<th>Body Weight (kg)</th>
<th>Protein (gm/100 ml)</th>
<th>Sodium (µEq/ml)</th>
<th>Potassium (µEq/ml)</th>
<th>Dexamethasone Treatment (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>glioblastoma</td>
<td>30</td>
<td>60 ± 9.4</td>
<td>70</td>
<td>30</td>
<td>67.1 ± 9.3</td>
<td>5.9 ± 0.8</td>
<td>139 ± 4.8</td>
<td>4.1 ± 0.4</td>
</tr>
<tr>
<td>glioma</td>
<td>12</td>
<td>44.7 ± 13.8</td>
<td>56</td>
<td>44</td>
<td>66.4 ± 14.7</td>
<td>6.4 ± 0.9</td>
<td>142 ± 3.2</td>
<td>4.5 ± 4.6</td>
</tr>
<tr>
<td>meningioma</td>
<td>17</td>
<td>62.1 ± 22.2</td>
<td>38</td>
<td>62</td>
<td>61.7 ± 22.2</td>
<td>6.5 ± 0.7</td>
<td>143 ± 3.3</td>
<td>4.2 ± 0.4</td>
</tr>
<tr>
<td>metastasis</td>
<td>9</td>
<td>51.1 ± 16.9</td>
<td>73</td>
<td>27</td>
<td>67.8 ± 11.5</td>
<td>6.4 ± 0.6</td>
<td>139 ± 3.0</td>
<td>4.3 ± 4.4</td>
</tr>
</tbody>
</table>

* Values are means ± standard deviations.

TABLE 2
Analytical data of biopsy material obtained from tumor and peritumoral edema*

<table>
<thead>
<tr>
<th>Tissue Sampled</th>
<th>No. of Samples</th>
<th>Water Content (ml/100 gm)</th>
<th>Extravasated Protein Content (mg/gm)</th>
<th>Sodium Content (µEq/gm dry wt)</th>
<th>Potassium Content (µEq/gm dry wt)</th>
<th>Dexamethasone Content (ng/gm wet wt)</th>
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</thead>
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<tr>
<td>glioblastoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tumor</td>
<td>30</td>
<td>84.9 ± 3.3</td>
<td>30.9 ± 2.02</td>
<td>646 ± 335</td>
<td>350 ± 133</td>
<td>208.7 ± 169.8</td>
</tr>
<tr>
<td>edema</td>
<td>16</td>
<td>84.6 ± 4.1</td>
<td>49.6 ± 17.1</td>
<td>624 ± 304</td>
<td>382 ± 107</td>
<td>214.4 ± 148.2</td>
</tr>
<tr>
<td>tumor:edema ratio</td>
<td>1.00</td>
<td>0.62</td>
<td></td>
<td>1.04</td>
<td>0.92</td>
<td>0.97</td>
</tr>
<tr>
<td>glioma</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tumor</td>
<td>12</td>
<td>83.9 ± 4.7</td>
<td>19.8 ± 16.0</td>
<td>716 ± 549</td>
<td>393 ± 120</td>
<td>224.3 ± 117.7</td>
</tr>
<tr>
<td>edema</td>
<td>5</td>
<td>83.8 ± 3.7</td>
<td>25.2 ± 20.5</td>
<td>496 ± 233</td>
<td>373 ± 67</td>
<td>233.1 ± 112.8</td>
</tr>
<tr>
<td>tumor:edema ratio</td>
<td>1.00</td>
<td>0.79</td>
<td></td>
<td>1.44</td>
<td>1.05</td>
<td>0.96</td>
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<tr>
<td>meningioma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tumor</td>
<td>17</td>
<td>83.3 ± 3.6</td>
<td>11.5 ± 8.93</td>
<td>662 ± 349</td>
<td>390 ± 129</td>
<td>227.1 ± 112.6</td>
</tr>
<tr>
<td>edema</td>
<td>6</td>
<td>83.3 ± 3.6</td>
<td>39.9 ± 12.1</td>
<td>610 ± 220</td>
<td>355 ± 67</td>
<td>217.7 ± 115.8</td>
</tr>
<tr>
<td>tumor:edema ratio</td>
<td>1.00</td>
<td>0.29</td>
<td></td>
<td>1.09</td>
<td>1.10</td>
<td>1.05</td>
</tr>
<tr>
<td>metastasis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tumor</td>
<td>9</td>
<td>84.2 ± 1.6</td>
<td>16.1 ± 5.5</td>
<td>427 ± 110</td>
<td>383 ± 156</td>
<td>ND</td>
</tr>
<tr>
<td>edema</td>
<td>4</td>
<td>84.9 ± 2.4</td>
<td>25.3 ± 4.3</td>
<td>473 ± 193</td>
<td>363 ± 57</td>
<td>ND</td>
</tr>
<tr>
<td>tumor:edema ratio</td>
<td>0.99</td>
<td>0.64</td>
<td></td>
<td>0.90</td>
<td>1.06</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Values are means ± standard deviations. ND = not determined.

made when average group values were compared. In all groups the mean water content was almost identical in the tumor and the peritumoral tissue, but the content of serum proteins was lower in the tumor while the sodium level was higher in the tumor. Among the four groups studied, the amount of extravasated serum proteins was highest in regions of brain tissue close to glioblastomas, followed by meningiomas, metastases, and gliomas. The rank order of sodium concentrations in peritumoral tissues was: glioblastomas, meningiomas, gliomas, and metastases. In all tumors the mean CBV was 2.5 ml/100 gm wet weight and in peritumoral brain tissue it was 1.6 to 2 ml/100 gm wet weight. No major difference in potassium content was observed. This seems to be in line with the previous notion that peritumoral edema does not disturb extracellular to intracellular ion homeostasis, 13 although knowledge of total tissue potassium concentrations does not allow conclusions to be drawn about cell membrane ion fluxes. The relationship between the accumulation of water and the content of sodium and extravasated proteins was more obvious when individual measurements were correlated. With the exception of metastases, positive correlations were obtained between water content and both serum protein concentrations and sodium content in tumors and peritumoral edema. A determination of the slope of these correlations allowed the estimation of the average protein and sodium content of edema fluid. The serum protein content of edema fluid varied between 4 and 16 mg/ml, with higher values being observed in glioblastomas and lower values in peritumoral edema surrounding metastases. The sodium content ranged from 130 to 170 µEq/ml in tumors and 130 to 150 µEq/ml in peritumoral tissue. Interestingly, glioblastomas exhibited a maximum protein concentration of 60 to 70 mg/gm dry weight which did not increase further, while the water content continued to rise. Similar high levels were not observed in other tumors, indicating that the water content did not increase as high as in glioblastomas (see Figs. 1 and 2).

Corticosteroid Treatment

Most of the patients included in the present study had been treated with dexamethasone prior to surgery. In order to determine a possible relationship between corticosteroid concentration and edema formation, we...
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FIG. 1. Correlation of water content with the content of extravasated serum proteins (upper row) and with sodium levels (lower row) in tumor biopsy material from glioblastomas, gliomas, meningiomas, and metastases. There is a positive correlation in all tumors except metastases. d.w. = dry weight.

FIG. 2. Correlation of water content with the content of extravasated serum proteins (upper row) and with sodium levels (lower row) in biopsy material from peritumoral edematous tissue of patients with glioblastomas, gliomas, meningiomas, and metastases. There is a positive correlation of water with both extravasated serum proteins and sodium content. d.w. = dry weight.
measured dexamethasone in the tumor and the peritumoral tissue of 56 patients with glioblastomas, gliomas, or meningiomas. Although a large variation of individual tissue samples was obtained, mean values of dexamethasone concentrations were almost identical in tumor and in peritumoral brain tissue, as well as in different tumor groups (Table 2). Here a suggested correlation of dexamethasone with water content and amount of extravasated proteins revealed an inhomogeneous pattern (Figs. 3 and 4). In glioblastomas, both water and serum protein levels decreased in a dose-dependent way, starting from a threshold of about 500 ng/gm wet weight. Steroid concentrations of meningiomas and gliomas did not show correlations with either water or serum protein content. Also, the peritumoral edema responded to dexamethasone only in the glioblastoma group but not in the glioma or meningioma groups. Interestingly, the therapeutic threshold of dexamethasone was reached in gliomas and glioblastomas only when the water content was below 6 ml/gm dry weight, whereas in meningiomas and peritumoral edema there was no restriction from tissue water. This suggests a passage of corticosteroids from the blood to the tissue that depends on both the tumor type and the degree of tissue hydration.

Discussion

In several previous studies, the water and electrolyte content of human or experimental brain tumors and peritumoral edema has been investigated with and without dexamethasone treatment. The present data, which depend on tissue measurements of the corticosteroid rather than on doses given to patients, are in general agreement with these studies, indicating that increases of water and sodium are concomitant and that both water and electrolyte content of edematous tissue is reduced under the influence of corticosteroids. However, to our knowledge no previous analysis has dealt with the relationship between serum protein extravasation and water retention in human peritumoral edema.

The present study is directly comparable with a previous investigation of vasogenic edema induced in cats by xenoinplantation of an anaplastic glioma, in that we observed close correlations in content between water and sodium as well as between extravasated serum protein and water. Calculations of serum protein concentrations in edema fluid revealed a protein value of about 12 mg/ml, whereas the average sodium content amounted to 132 μEq/ml. These values are within the range of the present measurements performed on human biopsy material, in which protein concentrations varied between 4 and 16 mg/ml and sodium content ranged between 130 and 150 μEq/ml, depending on the tumor type. The average protein and sodium concentrations in blood serum of these patients were 62 mg/ml and 140 μEq/ml, respectively. Edema fluid, therefore, contained about the same amount of sodium but only 6% to 25% of the protein content of blood serum. The low protein content in our study is in contrast to...
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FIG. 4. Effect of dexamethasone on the water (upper row) and serum content (lower row) in biopsy material from peritumoral brain tissue of patients with glioblastomas, gliomas, and meningiomas. There is a clear relationship between the tissue concentration of dexamethasone and the water and protein content in glioblastomas, but not in gliomas or meningiomas. d.w. = dry weight; w.w. = wet weight.

previous measurements of cold-injury edema carried out by Gazendam, et al.,8 and by Clasen, et al.,5 who obtained a protein concentration of edema fluid close to that of serum. However, these measurements are not directly comparable with the present investigation because edema fluid was mechanically separated by centrifugation5 or collected from the brain using implanted wick catheters.8 Edema samples, in consequence, were withdrawn mainly from the extracellular space, whereas in our study the total amount in edematous tissue was measured.

In several previous studies, extravasated proteins have been suggested as factors responsible for the retention of edema fluid in the tissue. One of the most forceful arguments in favor of this concept is the observation by Klatzo, et al.,15 that resolution of vasogenic edema is closely associated with the clearance of serum proteins from the extracellular compartment. Marmarou, et al.,19 came to the same conclusion by infusing serum and mock CSF directly into the white matter of cats. Bothe, et al.,4 noticed a more prolonged retention of water in the tissue after serum infusion, which would be in line with the assumption that edema formation is allied to the dynamics of protein extravasation. It should be stressed that, in these experiments also, protein-free mock CSF was quantitatively retained in the tissue for at least 24 hours, and that the protein content of serum is several times higher than the protein content of edema fluid, as determined in their and in our experimental studies. The presence of extravasated proteins, therefore, seems to modulate rather than to determine the amount of fluid retained in the tissue.

This interpretation is substantiated by the present data. The slope of the protein/water relationship was distinctly more variable than that of the sodium/water relationship, indicating that the amount of water in the tissue is influenced initially by the electrolyte content of edema fluid. This conclusion becomes more evident when data from peritumoral edema of all patients are combined: the correlation coefficient of sodium versus water (slope 135 µEq/ml) was r = 0.928, whereas the correlation coefficient of protein versus water (slope 8 mg/ml) was only r = 0.528.

The concept of a constant electrolyte level and a more variable protein content of vasogenic edema is supported by theoretical considerations about the mechanism of edema formation.23,24 In intact brain vessels, the permeability of endothelial cells for both electrolytes and macromolecules is very low, resulting in an osmotic reflection coefficient close to 1. The hydrostatic pressure difference between plasma and tissue is, therefore, counteracted by the osmotic and oncotic pressure difference between the two compartments. If breakdown of the blood-brain barrier is consistent with a gradual opening of trans- or interendothelial channels, small electrolyte molecules will pass more easily through these openings than will proteins, resulting in an earlier and more pronounced decrease of the reflection coefficient for electrolytes. Since the net flux of solutes by hydraulic forces across the vas-
cicular wall is inversely related to the respective reflection coefficients, electrolyte concentrations of the edema fluid would approach those of serum more easily than serum proteins — a situation that was actually observed in this and in the experimental study of Bothe, et al. 4 The interpretation is also in line with the well-known observation that vasogenic brain edema contains more albumins than globulins, having a higher molecular weight and, therefore, a higher apparent reflection coefficient when the blood-brain barrier starts to break down (Unkelbach, et al., in preparation).

Provided this concept is correct, the presence of serum proteins in vasogenic edema may be a sensitive indicator of the severity of the barrier disturbance. Its contribution to the formation and retention of edema, however, is still not clear. As discussed previously, 2 increased protein concentrations in the extracellular space of edematous tissue completely shift the ion equilibrium across the cell membrane.

According to the Gibbs-Donnan law, the resting membrane potential and the ion equilibrium are maintained under physiological conditions only by the presence of polyanions (proteins) in glia as well as neurons. A sudden cellular uptake of serum-derived proteins by nerve cells, as observed by Klatzo, et al., 15 undoubtedly shifts potassium ions out of glial cells and also changes the sodium-potassium equilibrium of neurons. Resolution of edema would then depend initially on the reduction of tissue electrolytes associated with the extracellular to intracellular passage of serum proteins.

An interesting aspect of the present study is the influence of dexamethasone on edema resolution. Since the original communication by Galicich and French, 1 numerous authors have confirmed that corticosteroids reduce the extent of peritumoral edema. Possible mechanisms discussed include a reduction of the blood-tissue permeability, 22,27 increased transport of sodium, potassium, and water across the capillary tissue interface, 17 stabilization of lysosomal membranes, 1 reduced edema spread, 20 and an inhibition of tumor growth. 9 Yu, et al., 29 identified glucocorticoid receptors in human brain tumors and related the efficiency of corticosteroid therapy to the number of such receptors. However, in a later communication from the same laboratory, 11 edema resolution was found to be more pronounced in gliomas than in meningiomas although receptor densities were higher in meningiomas. A difference in sensitivity to dexamethasone among the various tumor types was also noted by Reulen, et al. 25 They observed greater clinical improvement in patients with metastases and glioblastomas than in those with meningiomas. Our study confirms that meningiomas respond poorly to dexamethasone, whereas a dose-dependent relationship between the drug and the reduction of edema does exist in glioblastomas and may (to a lesser degree) also occur in gliomas. The parallel reduction of water, sodium, and proteins points to a reduction of barrier permeability as the most likely mechanism. However, it should be noted that, in a previous experimental study of implanted gliomas, dexamethasone alleviated edema without significant reduction of blood-tumor permeability. 20 Dexamethasone may therefore improve resolution of edema in addition to reducing edema formation.

The reason for the failure of dexamethasone to improve edema around meningiomas remains unclear. Hatam, et al., 14 suggested that meningiomas may produce edema by mechanical pressure on the brain (that is, an ischemic type of edema without damage to the vessels). This interpretation is not supported by the present study because the content of extravasated proteins in meningiomas was almost as high as in glioblastomas. A more likely explanation is that the vessels of meningiomas are fenestrated, allowing free passage of plasma constituents into the tumor parenchyma. 15 It is conceivable that corticosteroids are unable to reduce extravasation across these relatively large openings, whereas they may be effective in acting on glial tumor vessels which exhibit a closed endothelial lining. Treatment of edema induced by brain tumors requires a specific approach that should be adjusted to the particular pathophysiology of edema formation.

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References

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