Corticosteroid Therapy of Experimental Tumour Oedema

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I. Introduction

It is a well–established clinical fact that corticosteroids have a beneficial effect on peritumoural brain oedema (2, 6, 8, 10, 15, 23, 25, 31, 37). Although this observation has never been disputed, its mode of action is still poorly understood. One of the reasons is the fact that few experimental models are appropriate for the study of this question. Such a model should fulfil two requirements: 1. Oedema should develop around standard brain tumours resembling primary cerebral neoplasms and 2. the brain of the animal species in which such a tumour is produced must contain sufficient amounts of white matter to allow the accumulation and spread of peritumoural oedema in a similar way as in patients. In practical terms, this means that the animal species must be cat, dog or monkey, and that the tumour must be produced by implantation of neoplastic glial cells. Chemically induced brain tumours are of lesser interest because they are not standardised and they develop only in lower animal species such as rats in which the proportion of the white matter is very low. On the other hand, the hitherto available transplantation tumours in higher mammals were choriocarcinomas and melanomas (20, 40) but with the exception of ependymoblastomas (31), gliomas have not been used.

Only recently, a glial cell line has become available which can be transplanted into the brain of a cat (18). This cell line is a clone which has been derived from a glioma which was previously induced chemically in rats by the application of ethyl–nitrosourea. The cells of this clone resemble neoplastic astrocytes, and they have been kept in tissue culture for several years. By stereotactic implantation of a constant number of cells of this clone into the cat brain, a tumour can be produced which grows in a relatively sterotyped way in the same place, and which is accompanied by a reproducible type of peritumoural oedema. The pathophysiology of this type of oedema has been studied before, and information is available about the composition and the degree of oedema in untreated animals (17, 18, 38). In the present series of experiments this tumour was used for the investigation of corticosteroid therapy on peritumoural oedema.

II. Material and methods

Brain tumours were produced in nine adult cats by stereotactic implantation of 4 million cells of the rat glioma clone RG2 into the internal capsule of the left hemisphere, as previously described in detail (18). After two to three weeks, a crystalline suspension of 10 mg dexamethasone was injected as a single dose intramuscularly. One week later the animals were anaesthetized with 30 mg/kg pentobarbital (Nembutal^{R}), immobilized and mechanically ventilated with room air. Blood pressure was monitored in the femoral artery and intracranial pressure in the cisterna magna. EEG was recorded from the exposed calvarium using silverball electrodes. EEG frequency analysis was performed by fast Fourier transform, using a laboratory computer (PDP 12, Digital Equipment, Maynard, Md). A frequency index was calculated by dividing the intensity of the fast frequency bands by that of the lower ones. Cerebral blood flow was measured using the intracardiac microsphere injection technique (33). A catheter for injection of microspheres was inserted into the left ventricle of the heart via a femoral artery, and a reference blood sample was withdrawn at a calibrated speed from the other femoral artery. Permeability of the blood–brain barrier was tested by intravenous injection of 20 mg/kg Evans blue or 100 mg/kg horseradish peroxidase. 15 min later, the animals were killed by air embolism. The brain was quickly removed and dissected in a moist chamber. Brain samples were taken from the tumour, peritumoural grey and white matter and homotopic

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regions of the opposite hemisphere. Water content was determined by drying the samples to constant weight, and electrolyte content by flame photometry following tissue extraction in 0.75 mol nitric acid.

Control values and measurements obtained in ten untreated animals were taken from two previous series of experiments in which the same methods as in the present investigation were used for the production and evaluation of peritumoural oedema (17, 18).

III. Results

After tumour cell implantation and after recovery from anaesthesia, none of the animals exhibited neurological deficits until the end of the experiment. Two to three weeks after implantation the animals received a single dose of crystalline suspension of dexamethasone (10 mg intramuscularly). When the brains were dissected one week later (i.e. three to four weeks after implantation), tumours of varying size were found: three tumours were large (diameter more than 7 mm), two were medium–sized (3–7 mm) and four were small (less than 3 mm). In the previous group of untreated animals seven tumours were large, two were medium–sized and only one was small.

In both groups tumours were stained with peroxidase or Evans blue, indicating a breakdown of the blood–tissue barrier to circulating macromolecules (Fig. 1). However, only in the previous untreated group peritumoural brain oedema was present as evidenced by swelling of the ipsilateral hemisphere, a mass shift of the midline to the opposite side, and a distinct increase of water content in the peritumoural white matter. In the present series, peritumoural oedema was distinctly reduced, even in those animals in which tumours had a diameter of more than 7 mm. As summarized in Table I, peritumoural white matter water content increased from 69.1 to 73% as compared to 80% in the untreated group. Calculation of peritumoural tissue swelling according to the equation described by Elliott and Jasper (7) revealed an increase of 56.7% in the untreated animals but only 14.2% in the treated group. The associated electrolyte shifts were also significantly less pronounced after dexamethasone treatment, indicating that inhibition of oedema development was not solely the effect of tissue dehydration.

This was confirmed by plotting the increase of water against the increase of sodium. The calculated sodium content of oederna fluid of the treated and untreated animals was 132 meq/l, and correlation between the two findings was highly significant in both groups (Fig. 2). In the grey matter changes of water and electrolytes were absent in both treated

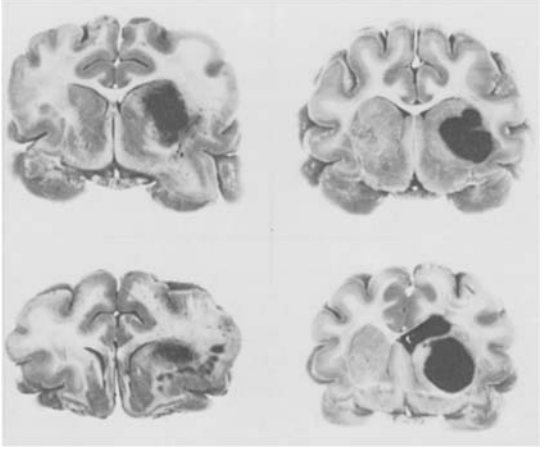

Fig. 1. Effect of corticosteroid treatment on peritumoural oedema following experimental tumour implantation into the cat brain. Left: untreated animal. Right: animal treated for one week with 10 mg/kg dexamethasone. Barrier permeability was tested with peroxidase. Note intense staining of the tumour in both the treated and untreated animals (peroxidase staining by courtesy of Dr. Hürter).

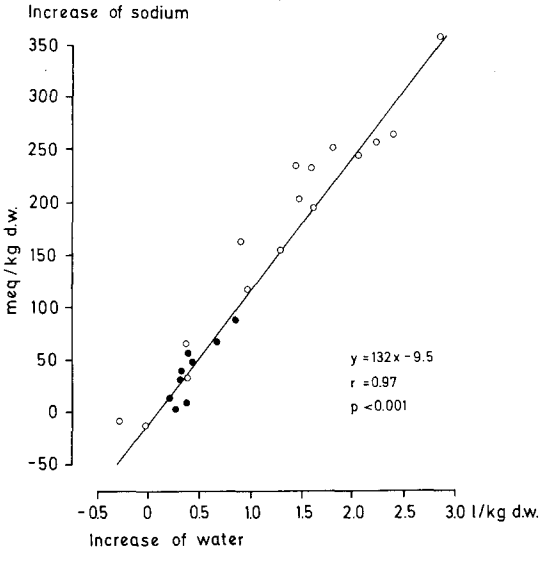

Fig. 2. Relationship between increase of sodium and increase of water content in peritumoural white matter of untreated animals (open circles) and animals treated with 10 mg/kg dexamethasone (filled circles). Common regression coefficients of both groups are indicated.

and untreated animals, confirming the previous notion that oedema was confined to peritumoural white matter alone (17, 18).

A well–known side-effect of oedema formation is the decrease of blood flow in the oedematous tissue (4, 14, 28, 29, 31). In the untreated group a decrease to about 60% from 32.2 to 18.9 ml/100 g/min was observed. This decrease was of the same order of magnitude as tissue swelling which suggests that the flow reduction was due to volume expansion of the oedematous tissue and not the consequence of increased vascular resistance (17). In the present treated group, blood flow was normal or even slightly increased although some tissue swelling was present (Table I). Under dexamethasone treatment, in contrast, vascular resistance was lower than in the control group. This difference apparently depended on local and not on global factors because intracranial pressure was identical in both groups: 5.8 ± 0.8 mm Hg in the treated and 6.1 ± 0.7 mm Hg in untreated animals.

Despite the pronounced differences in oedema formation, the incidence of EEG abnormalities was similar in both groups. The EEG frequency index which is a quantitative denominator of EEG background activity, varied over a wide range but did not correlate with the degree of oedema in either of the two groups (Fig. 3). Oedema, in consequence, does not seem to have a significant effect on EEG activity.

IV. Discussion

The present series of experiments confirms earlier clinical observations that corticosteroids have a beneficial effect on peritumoural brain oedema. Reulen et al. (31) observed in dexamethasone–treated patients a reduction of peritumoural white matter water content from 79 to 75.6% which was accompanied by an increase of blood flow by 38% from 26.3 to 36.3 ml/100 g/min. Similar findings have been made by other authors, either by direct evaluation of tissue water content (15, 26) or indirectly using computerized tomography (26) or intracranial pressure recording (1, 5, 21, 27).

Experimental data, on the other hand, are rare. Yamada et al. (40) studied peritumoural oedema after transplantation of human choriocarcinoma into the brain of the monkey and observed a reduction of tissue swelling from 68% to about 30%. A reduction of peritumoural oedema was also described by Shapiro and Posner (35) in brain tumours induced by ependymoblastoma, by Kotsilimbas et al. (20) following intracerebral transplantation of melanoma,

Tab. 1. Effect of dexamethasone on peritumoural white matter oedema

	CBF ml/100 g/min	H_2O ml/100 g w. w.	Sodium meq/kg d. w.	Na/K ratio	Swelling %
control	32.2 ± 5.6	69.1 ± 0.9	147 ± 7	0.62 ± 0.04	–
untreated	18.9 ± 0.05*	80.0 ± 0.8*	399 ± 32*	1.29 ± 0.12*	56.7 ± 6.0*
treated	35.7 ± 2.8$^+$	73.0 ± 0.5$^+$	215 ± 15$^+$	0.68 ± 0.04$^+$	14.2 ± 2.1$^+$

Values are means ± SE
* significantly different from controls ($p < 0.001$)
$^+$ significantly different from untreated group ($p < 0.001$)

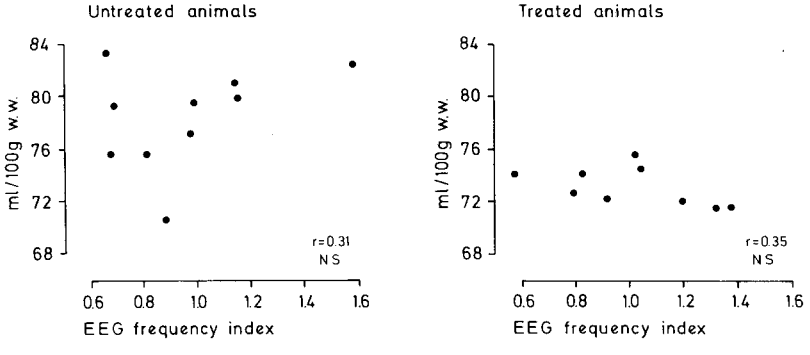

Fig. 3. Relationship between peritumoural oedema and EEG frequency index three weeks after experimental tumour implantation in untreated animals (left) and in animals treated with 10 mg/kg dexamethasone (right). Note the wide scatter of frequency index, indicating different degrees of EEG slowing, and the absence of a correlation with water content of peritumoural white matter in both treated and untreated animals.

and by Guracy et al. (13) in transplantable rat glioma.

A major difference between these and the present investigation is the fact that in our series for the first time a depot of dexamethasone was given as a crystalline suspension in a single dose. Such suspensions are used for local application, e.g. for the treatment of arthritis, but have never been applied for treating oedema. The present investigation demonstrates that this form of administration results in the same or an even better degree of oedema resolution than using the usual repetitive intravenous injections.

The mechanism of this effect is still unknown. Most authors suggest that dexamethasone interferes primarily with the formation of oedema (16, 22, 24, 32). Our findings suggest that this is not the main mechanism involved. In both treated and untreated animals, tumour vessels were permeable to circulating macromolecules as indicated by the intense staining with Evans blue or peroxidase. Dexamethasone, in consequence, does not prevent the leakage of serum proteins across the tumour vessels. A certain reduction of oedema formation, however, cannot be excluded, and experiments are under way to study by quantitative methods the exudation of oedema fluid.

A more likely mechanism for the action of corticosteroid is inhibition of spread, or improvement of resolution of oedema. Resolution of oedema has been attributed to three different mechanisms: Cellular uptake of serum proteins (19), resorption of oedema by transport from the extracellular space into the blood across the vascular wall (3), and bulk flow into the ventricles (30). An improvement of bulk flow under dexamethasone is not likely because intracranial pressure was identical in both groups, and there was no indication of a gradient of oedema density from the tumour toward the ventricles. Improved cellular uptake and vascular transport, on the other hand, are possible mechanisms of dexamethasone action which should be investigated in more detail. The distinct increase of blood flow in the peritumoural brain tissue may be associated with such a process because activation of metabolic activity which can be expected to occur in this condition, is generally coupled to a parallel improvement of blood circulation.

Another factor which may indirectly affect the degree of oedema, is inhibition of tumour growth. Our observation of a lower incidence of large tumours in the treated animals is in line with earlier clinical and experimental data which also demonstrate that steroids may slow down tumour development (8, 9, 13, 20, 35, 39). The mode of tumour inhibition is presumably the suppression of DNA synthesis because uptake of thymidine in tumour cells is distinctly reduced by steroids (9, 35). It is obvious that oedema formation may be reduced in small tumours; however, the fact that in treated animals with large tumours, oedema was also absent, is at variance with such an interpretation.

An interesting side observation is the relatively high incidence of EEG changes in the treated animals. It has been previously suggested that EEG slowing associated with tumour development is the consequence of oedema formation because tumours are electrically silent (36). This view has been challenged more recently by several authors who were not able to detect a consistent correlation between EEG changes and oedema under experimental or clinical conditions (11, 12, 34). The present finding of EEG changes in a number of tumour animals without oedema formation confirms this observation and corroborates the notion that EEG abnormalities result directly from the local lesion produced by the tumour and not indirectly by peritumoural oedema.

Summary

Experimental brain tumours were produced in cats by stereotactic implantation of 4 million suspended cells of a rat glioma clone into the internal capsule. Three weeks after implantation a spherical tumour developed with a diameter of up to 10 mm which was surrounded by vasogenic white matter oedema. In untreated animals water content in the peritumoural white matter increased from 69.1 ± 0.9 to 80.0 ± 0.8 ml/100 g w. w., and regional blood flow reciprocally decreased from 32.2. ± 5.6 to 18.9 ± 0.05 ml/100 g/min. A single injection of a crystalline suspension of 10 mg/kg dexamethasone given intramuscularly one week before the animals were killed, led to a significant amelioration of brain oedema. Peritumoural white matter water content decreased to 73.0 ± 0.5 ml/100 g w. w. and blood flow rose to 35.7 ± 2.8 ml/100 g/min. These changes were accompanied by parallel shifts of electrolyte content but they did not correlate with EEG activity, as assessed by Fourier frequency analysis. Corticosteroids did not prevent extravasation of peroxidase or Evans blue across the tumour vessels. The beneficial

effect, therefore, is attributed to either an acceleration of resorption or an inhibition of the spread of oedema from the tumour into the peritumoural brain tissue.

Keywords:

Blood flow – Brain oedema – Brain tumours – Cats – Corticosteroids – EEG

Zusammenfassung

Bei Katzen wurden experimentelle Hirntumoren durch stereotaktische Implantation von suspendierten Tumorzellen eines Rattengliomklon in die innere Kapsel hervorgerufen. Innerhalb von drei Wochen entwickelte sich am Implantationsort ein kugelförmiger Tumor mit einem Durchmesser von etwa 10 mm. Um den Tumor herum entstand ein ausgedehntes vasogenes Ödem, das sich in der weißen Substanz ausbreitete. Bei unbehandelten Tieren stieg der Wassergehalt im ödematösen Mark von 69.1 ± 0.9 auf 80.0 ± 0.8 ml/100 g Feuchtgewicht an, und die Durchblutung sank in diesem Gebiet von 32.2 ± 5.6 auf 18.9 ± 0.05 ml/100 g/min ab. Die einmalige intramuskuläre Injektion einer kristallinen Suspension von 10 mg/kg Dexamethason bewirkte innerhalb einer Woche eine signifikante Abnahme des Hirnödems. Der Wassergehalt sank auf 73.0 ± 0.5 ml/100 g Feuchtgewicht und die Durchblutung stieg über den Kontrollwert hinaus auf 35.7 ± 2.8 ml/100 g/min an. Die Veränderungen wurden von entsprechenden Elektrolytverschiebungen begleitet, korrelierten jedoch nicht mit EEG-Veränderungen, die mit der Fourier Frequenz-Analyse quantitativ ausgewertet wurden. Die Kortikosteroid-Behandlung verhinderte nicht die Extravasation von Peroxidase oder Evans Blau aus den Tumorgefäßen. Die therapeutische Wirkung wird deshalb auf eine Beschleunigung der Resorption oder eine Inhibition der Ausbreitung der Ödemflüssigkeit von dem Tumor in das peritumorale Hirngewebe zurückgeführt.

Schlüsselwörter:

Hirndurchblutung – Hirnödem – Hirntumoren – Katzen – Kortikosteroide – EEG

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