STEROID RECEPTORS IN MENINGIOMAS

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Epidemiological and clinical data suggest that sex-steroid hormones may be involved in the biology of meningiomas. Two-thirds of all meningiomas are reported in women [14]. They are diagnosed most frequently between 35 and 55 years [27] and are known to progress during pregnancy or at the end of the menstrual cycle [1]. Furthermore, there is a significant association of meningiomas with breast carcinoma [16]. On addition, the efficacy of glucocorticoid treatment on clinical signs associated with intracranial tumors is well known, although this effect seems to be related to brain edema and not to tumor cell proliferation [6,23].

Studies on the mechanism of action of steroids have shown that the presence of cytoplasmic receptors is essential for initiation of target tissue response. As an initial step in evaluating the effect of steroids we submitted ten meningiomas to an estrogen (ER), progesterone (PR), androgen (AR) and glucocorticoid (GR) receptor binding analysis.

MATERIALS AND METHODS

Tissue samples were obtained from 10 patients: (6 female, 4 male). All patients were being treated with dexamethasone (16 mg/day) and phenytoin (300 mg/day) before and during surgery. After excision of the tumor from the patients, a representative sample was submitted for microscopic examination and classification. The remainder of the tissue was immediately frozen in liquid nitrogen and transported to the laboratory. Tissues were stored at -80°C until processing. The procedure used for cytosol preparation was a slight modification (addition of 20nMol/l Na_2MoO_4 to the extraction buffer) of the standard dextran-charcoal technique which is in use in our laboratory for the assessment of steroid hormone receptor activity in breast cancer (3). Estrogen (ER), progesterone (PR) and glucocorticoid receptors (GR) were assessed as previously described (3). The androgen receptor (AR) was determined according to Zava et al. (26). Radioactive ligands were: R5020 (Promegestone), 87.0

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Ci/mmol; dexamethasone 39 Ci/mmol; R1881 (Methyltrienolone), 87.0 Ci/mmol (New England Nuclear, Boston, MA) and Estradiol, 100 Ci/mmol (Amersham Corp. Arlington Heights, IL). Unlabeled 19-nor steroid were obtained from New England Nuclear; other steroids were from Sigma Chemical Co. (St. Louis, MO). We considered a positive assay for ER, PR, GR and AR to be $\geqslant$ 10 fMol/mg cytoplasmic protein and if a proper Scatchard plot could be constructed. In order to determine steroid specificity we compared the ability of various ligands to compete with radiolabeled ligand for binding to the progesterone, androgen or glucocorticoid receptor measured. Cytosol was incubated with 10nM tritiated ligand either alone or with 100 fold excess of the unlabeled competitors to be tested. Results were expressed as a percentage of the competition demonstrated by the appropriate unlabeled steroid (R5020 for PR, R1881 for AR and dexamethasone for GR).

RESULTS

Table 1 shows age and sex of each individual patients and hormonal receptor levels, expressed in fMol/mg protein. Mean age of the female group was 54,7±7,6 ys. All women were postmenopausal. Mean age of the male group was 57±8,9 ys. Female patients have a higher incidence and titer of ER, PR, AR and GR. Table 2 summarizes incidence of positivity, mean specific receptor content for each class of steroids and

Patient	Age	Sex	ER*	PR*	GR*	AR*
1	48	F	5,0	25,0	16,0	30,0
2	58	F	nd	103,0	50,0	23,0
3	51	F	6,0	70,0	77,0	15,0
4	46	F	14,0	98,0	65,0	53,0
5	59	F	10,0	53,0	60,0	36,0
6	54	F	nd	111,0	nd	53,0
7	50	M	8,0	32,0	10,5	13,0
8	60	M	nd	14,0	7,0	nd
9	66	M	nd	23,0	18,0	nd
10	67	M	nd	nd	nd	nd

*Table 1 — Patient's age, sex and steroid receptor in meningiomas: * expressed in femtomoles per milligran of protein; nd, not detectable.*

Receptor	% Positivity	Mean level *	Mean Kd **
ER	20 (2/10)	12,0	0,6
PR	90 (9/10)	60±38	1,06±0,67
GR	70 (7/10)	44±28,0	4,2 ±1,7
AR	70 (7/10)	32±14	1,57±0,48

*Table 2 — Incidence, mean receptor content and affinity (Kd) of steroid receptors in meningiomas: * expressed in femtomoles per milligran of protein; ** dissociation constant, expressed in nM.*

mean dissociation constant (Kd) for the respective class calculated by Scatchard analysis on the binding data.

The presence of specific ER was demonstrated in 2/10 (20%) meningiomas. The receptor content was low, but the binding data of the two positive cases showed a good fit to Scatchard analysis. Ninety per center of all meningioma biopsies analyzed displayed significant quantities of specific progesterone binding activity, ranging from 23 to 103 fMol/mg prot. GR and AR were detected in 70% of meningiomas. GR levels ranged from 10 to 77 fMol/mg prot and AR levels from 10 to 53 fMol/mg prot. The results of progesterone specificity testing are shown in table 3, where the amount of binding with a 100-fold excess of cold R5020 was designated as 100% competition. It can be seen that only progestins effectively compete for the binding of (3H) R5020. Testosterone, estradiol and cortisol are poor competitors, thus establishing that the binding components is higher specific for progestins. Table 4 and 5 shows the results of specificity testing for androgen and glucocorticoid binding respectively. From table 4, we can see that unlabeled testosterone and dihydrotestosterone competed with labeled R1881. Dexamethasone, R5020 and DES where weak competitors, indicating that the androgen receptor can be differentiated from other receptors. Table 5 shows that all glucocorticoid compounds compete efficiently with (3H) dexamethasone for specific binding in meningioma cytosol. DES and testosterone competed to a lesser degree. Progesterone compete almost as well as dexamethasone.

Competitor	Relative percentage of binding
R5020 (promegestone)	100% *
Progesterone	86%
ORG2058	96%
Testosterone	35%
Estradiol	10%
Cortisol	10%

*Table 3 — Ligand specificity of (^{3}H) promegestone binding to meningioma cytosol: * as percentage of competition to R5020.*

Competitor	Relative percentage of binding *
R1881 (methyltrienolone)	100%
R5020 (promegestone)	10%
Testosterone	94%
Dihydrotestosterone	84%
DES (diethylstilbestrol)	30%
Dexamethasone	30%

*Table 4 — Ligand specificity of (^{3}H) R1881 binding to meningioma cytosol: * as percentage of competition to R1881.*

Competitor	Relative percentage of binding *
Dexamethasone	100%
Cortisol	100%
Triamcinolone acetonide	100%
Prednisolone	100%
Prednisone	70%
Testosterone	51%
DES (diethylstilbestrol)	4%
Progesterone	90%

*Table 5 — Ligand specificity of (^{3}H) dexamethasone binding to meningioma cytosol: * as percentage of competition by dexamethasone.*

COMMENTS

In this study, only a small percentage of meningiomas contained ER. The positive binding levels observed are very low and approach the sensitivity limits of the assay. This low incidence is in agreement with reports on ER by some authors [2,4,15,18,20,22], but in contrast to those of other investigators [5,10,25]. Differences between studies may be due to the extremely low levels of ER found in most cases and different criteria of positivity. Presence of high levels of progesterone repector in meningioma tissue have been reported previously [2,4,18,22]. It is confirmed in our studies. Besides low capacity and high affinity binding sites, specific steroid receptores have to display specificity for a given class of ligands. The binding observed is highly specific (table 3) and cannot be due to the presence of glucocorticoid or androgen receptor. Tumors arising from hormone responsive tissues can retain and at times magnify some of the characteristics of normal tissue receptor status. It was suggested that progesterone receptor is a common feature of normal leptomeninges [12].

The presence of ER-PR+ tumors is inconsistent with the general hypothesis that PR levels are regulated by estrogens, presumably through the estrogen receptor. Markwalder et al.[11] suggested that PR synthesis is not estrogen — regulated in meningiomas. However, as in our series and those of Poisson et al.[13] and Cahill et al.[4], PR levels have been quantitatively higher in women, it is possible that low contents of ER are present and that present biochemical techniques are not sensitive enough to detect them. It is also possible that the cellular expression of PR is controled by other steroid hormones (progestins, androgens, glucocorticoids) through their respective receptors. In fact, besides PR we have detected the presence of AR and GR in meningiomas. GR levels were lower than those reported [24] for intracranial tumors, but our patients were on glucocorticoid therapy. It has been suggested that down-regulation of glucocorticoid receptors in brain [21] follows glucocorticoid administration. GR was specific, except for progesterone but it is known that progesterone binds to glucocorticoid receptors [7]. Results of AR incidence are similar to those published

recently [13]. It is tempting to speculate that the higher percentage of females with AR-positive tumors may reflect lower levels of circulating androgens and a concomitantly higher percentage of unbound receptors available for assay.

The majority of meningiomas are surgically resectable, but in some patients surgery is inadvisable because of old age or tumor location. In addition, meningiomas can reccur, with a high percentage of reccurence for incomplety removed tumors [19]. Finally, a small percentage of meningiomas are malignant and are rarely cured by surgical excision alone [9]. Since meningioma represents a quantitatively important brain tumor accounting for 13-18% of all intracranial neoplasms [17], there is a need for adjuvant therapy. Studies on breast cancer have demonstrated that the presence of ER and PR are useful in the prediction of response to endocrine therapy [8]. The finding of steroid receptors in meningiomas suggests that some type of steroid hormone therapy may be of clinical use in the treatment of this disease.

SUMMARY

Cytosolic estrogen (ER), progesterone (PR), androgen (AR) and glucocorticoid receptors (GR) were evaluated in 10 meningiomas using a dextran charcoal coated method. We consider as positive specific receptor values $\geqslant 10$ fMol/mg protein. In this study 20% of the meningiomas contained very low titers of specific ER. PR was detectable in 90% of the tumors, at high levels. The mean PR content of PR+ tumors was 60 ± 38 fMol/mg prot. GR and AR were present in moderate levels, in 70% of the tumors. Competition studies demonstrated steroid specificity for these hormone-binding proteins. Female patients have a higher receptor incidence and titer. In conclusion, it can be hypothesized that the meningioma are a target tissue for steroids and that endocrine therapy may be relevant to unoperable and/or reccurent tumors.

RESUMO

Determinação de repectores esteroídicos em meningiomas.

Dados epidemiológicos sugerem uma associação entre meningiomas e hormônios sexuais. Há preponderância da incidência destes tumores em mulheres, sendo diagnosticados mais frequentemente entre 35 e 55 anos. Progressão tumoral pode ocorrer durante a gravidez e a associação entre meningiomas e câncer de mama é estatisticamente significativa. O efeito benéfico positivo dos glicocorticóides no tratamento clínico de tumores intracranianos está bem documentado, embora este efeito seja relacionado com o edema cerebral e não com proliferação tumoral. A presença de receptores intracelulares específicos parece ser condição necessária embora não suficiente para a expressão dos efeitos celulares destes esteróides. O objetivo do nosso trabalho foi a determinação de receptores de estrógeno (ER), progesterona (PR), andrógeno (AR) e glicocorticóide (GR) em 10 meningiomas através da metodologia do carvão-dextrana. Neste estudo verificamos que somente 20% dos meningiomas contêm

receptores de estrógeno e as concentrações determinadas são baixas. Receptores de progesterona estão presentes em 90% dos tumores, em alta concentração (média±dp = 60±38 fMol/mg prot). Cerca de 70% dos meningiomas contêm níveis intermediários de AR e GR. Testes de competição demonstram que todos os receptores são específicos. Incidência e concentração de todos os receptores são maiores em pacientes do sexo feminino. A ocorrência de receptores de progesterona, andrógeno e glicocorticóide pode indicar a possível utilidade de manipulações endócrinas em meningiomas não operáveis.

REFERENCES

1. BICKERSTAFF, E.R.; SMALL, J.M. & GUEST, I.A. — The relationship course of certain meningiomas in relation to pregnancy and menstruation. J. Neurol. Neurosurg. Psychiat. 21:89, 1958.
2. BLANKENSTEIN, M.A.; BLAAUW, G.; LAMBERTS, S.W.J. & MULDER, E. — Presence of progesterone receptors and absence of oestrogen receptors in human intracranial meningioma cytosols. Eur. J. Cancer clin. Oncol. 19:365, 1983.
3. BRENTANI, M.M.; NAGAI, M.A.; FUJIYAMA, C.T. & GOES, J.C.S. — Steroid receptors in a group of Brazilian breast cancer patients. J. surg. Oncol. 18:431, 1981.
4. CAHILL, D.W.; DASHIRELAHI, N.; SOLOMAN, L.; DALTON, T.; SOLOMAN, M. & DUCKER, T.B. — Estrogen and progesterone receptors in meningiomas. J. Neurosurg. 60:985, 1984.
5. DONNEL, M.S.; MEYER, G.A. & DONEGAN, W.L. — Estrogen receptor protein in intracranial meningiomas. J. Neurosurg. 50:499, 1979.
6. GALICICH, J.M. & FRENCH, L.A. — Use of dexamethasone in the treatment of cerebral edema resulting from brain tumors and brain surgery. Amer. practit. Diag. Treat. 12:169, 1961.
7. HASLAM, S.Z.; McBLAIN, W.A. & SHYAMALA, G. — An empirical basis for the competition by dexamethasone to progesterone receptors as estimated with the synthetic progestin R5020. J. Receptor Res. 2:435, 1982.
8. HORWITZ, K.B. & McGUIRE, W.L. — Estrogen and progesterone: their relationship in hormone-dependent breast cancer. In W.L. McGuire, J.P. Raynaud & E.E. Baulieu (eds.): Progesterone Receptors in Normal and Neoplastic Tissues. Raven Press, New York, 1977, pg. 103.
9. JELLINGER, K. & SLOWWIK, F. — Histological subtypes and prognostic problems in meningiomas. J. Neurol. 208:279, 1975.
10. MAGDELENAT, H.; PERTUISET, B.F.; POISSON, M.; MARTIN, P.M.; PHILIPPAN, J.; PERTUISET, B. & BUGE, A. — Progestin and oestrogen receptors in meningiomas. Biochemical characterization, clinical and pathological correlations in 42 cases. Acta neurochir. 64:199, 1982.
11. MARKWALDER, T.M.; ZAVA, D.T.; GOLDHIRISCH, A. & MARKWALDER, R.V. — Estrogen and progesterone receptors in meningiomas in relation to clinical and pathologic features. Surg. Neurol. 20:42, 1983.
12. POISSON, M.; MAGDELENAT, H.; MARTIN, P.M.; PERTUISET, B.F.; HAUW, J.J.; FOHANNO, D.; SICHEZ, J.P.; VIGOURAUX, R.P. & BUGE, A. — Recépteurs de progestérone de la leptoméninge humaine normale chez l'adulte. Rev. neurol. (Paris) 139:163, 1983.
13. POISSON, M.; PERTUISET, B.F.; MAQUILEWSKY, M.; MAGDELENAT, H. & MARTIN, P.M. — Les recépteurs de stéroides du système nerveux central: implications en neurologie. Rev. Neurol. (Paris) 140:233, 1984.
14. RAUSING, A.; YBO, W. & STENFLO, I. — Intracranial meningioma: a population study of ten years. Acta neurol. scand. 46:102, 1970.
15. SCHNEGG, J.P.; GOMEZ, F.; LE MARCHAND-BERAUD, J. & TRIBOLET, N. — Presence of sex steroid hormone receptors in meningioma tissue. Surg. Neurol. 15:415, 1981.

16. SCHOENBERG, B.S.; CHRISTINE, B.W. & WHISNASIT, J.P. — Nervous system neoplasms and primary malignancies of other sites: the unique association between meningiomas and breast cancer. Neurology 25:705 ,1975.
17. SCHOENBERG, B.S. — Nervous system. In Schollenfeld & Fraumeni (eds.): Cancer Epidemiology and Prevention. W.B. Saunders Co., Philadelphia, 1982, pg. 968.
18. SCHWARTZ, M.R.; RANDOLPH, R.L.; CECH, D.A.; ROSE, J.E. & PANKO, W.B. — Steroid hormone binding macromolecules in meningiomas. Cancer 53:922, 1984.
19. SIMPSON, D. — The recurrence of intracranial meningiomas after surgical treatment. J. Neurol. Neurosurg. Psychiat. 20:22, 1957.
20. TILZER, L.L.; PLOPP, F.V.; EVANS, J.P.; STONE, O. & ALWARD, K. — Steroid receptor protein in human meningiomas. Cancer 49:633, 1982.
21. TORNELLO, S.; ORTI, E.; DE NICOLA, F.A.; RAINBOW, T.C. & McEWEN, B.S. — Regulation of glucocorticoid receptors in brain by corticosterone treatment of adrenalectomized rats. Neuroendocrinol. 35:411, 1982.
22. VAQUERO, J.: MARCOS, M.L.; MARTINEZ, R. & BRAVO, G. — Estrogen and progesterone receptor proteins in intracranial tumors. Surg. Neurol. 19:11, 1983.
23. YAMADA, K.; BREMER, A.M. & WEST, C.R. — Effects of dexamethasone on tumor-induced brain edema and its distribution in the brain of monkeys. J. Neurosurg. 50:361, 1979.
24. YU, Z.; WRANGE, O.; BOETHIUS, J.; HATAM, A.; GRANHOLM, L. & GUSTAFSSON, J. — A study of glucocorticoid receptors in intracranial tumors. J. Neurosurg. 55:757, 1981.
25. YU, Z.; WRANGE, O.; HAGLUND, B.; GRANHOLM, L. & GUSTAFSSON, J.A. — Estrogen and progestin receptors in intracranial meningiomas. J. steroid. Biochem. 16:451, 1982.
26. ZAVA, D.T.; LANDRUM, B.; HORWITZ, K.B. & McGUIRE, W.L. — Androgen receptor assay with (^{3}H) methyltrienolone (R1881) in the presence of progesterone receptors. Endocrinology 104:1007, 1979.
27. ZÜLCH, K.J. — Brain Tumors: Their Biology and Pathology. Springer Publ. Co., New York, 1965, pg. 62.

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