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Invited review

Animal models of tumour-associated epilepsy

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ABSTRACT

Brain tumours cause a sizeable proportion of epilepsies in adulthood, and actually can be etiologically responsible also for childhood epilepsies. Conversely, seizures are often first clinical signs of a brain tumour. Nevertheless, several issues of brain-tumour associated seizures and epilepsies are far from understood, or clarified regarding clinical consensus. These include both the specific mechanisms of epileptogenesis related to different tumour types, the possible relationship between malignancy and seizure emergence, the interaction between tumour mass and surrounding neuronal networks, and – not least – the best treatment options depending on different tumour types. To investigate these issues, experimental models of tumour-induced epilepsies are necessary. This review concentrates on the description of currently used models, focusing on methodological aspects. It highlights advantages and shortcomings of these models, and identifies future experimental challenges.

Contents

1. Introduction

Epilepsies are estimated to be associated with brain tumours in 4–17% of cases (from newly diagnosed patients to those undergoing epilepsy surgery, respectively) (as reviewed in Lhatoo et al., 2013 and van Breemen et al., 2007). Conversely, brain tumours will present with epilepsy as one of their cardinal symptoms in approx. 30% of all cases, with the probability of developing epilepsy ranging from 10% in primary lymphomas to 100% in dysembryoblastic neuroepithelial tumours. Of the most common primary brain tumours, low-grade astrocytomas carry a 75% risk, and glioblastomas a 29–49% risk of being linked to epilepsies, sometimes with later onset (in 30% of the Moots et al., 1995). This indicates that in general, epilepsy will develop more likely with low-grade malignancies (reviewed in van Breemen et al., 2007), an observation which is also valid in pediatric patients (Prayson, 2010). Seizures hence complicate the clinical management of a large

Abbreviations: E_{GABA}, equilibrium potential of the GABA-receptor mediated current; GFP, green fluorescent protein; $[K^+]$, extracellular potassium concentration; KCC2, potassium chloride exchanger type 2 (neuronal); NKCC, sodium-potassium-2chloride co-transporter; SCID, severe combined immunodeficiency; TSC, tuberous sclerosis.

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proportion of patients with low- to high-grade gliomas (Kurzwelly et al., 2010), and often prove to be relatively therapy resistant (Moots et al., 1995). Because tumour-associated epilepsies do pose a clinical challenge still, but also because the mechanisms of epileptogenesis due to cerebral tumours are far from completely understood, we are still in need of viable experimental models. This review addresses this issue by giving a brief overview on available brain tumour models. Focusing on epileptological aspects, this overview condenses on two main topics, glioma and tuberous sclerosis (TSC) models, as these are the models practically exclusively used in the epileptology context. Since there are already a number of reviews on putative mechanisms of epileptogenesis in different models (Beaumont and Whittle, 2000; Kirschstein, 2012; Köhling, 2012; Shamji et al., 2009), we will only briefly touch this aspect, and will mainly address the methodological pros and cons of these models.

2. Animal models of glioma-associated epilepsy

As already outlined in the Introduction, most models of tumour-associated epilepsies actually focus only on glioma models, and even more precisely, on high-grade glioblastoma-like models. This is perhaps not surprising, since most work on glioma models actually originated in the oncology field, which naturally focuses mainly on invasive and malignant tumour types (cf. Barth and Kaur, 2009). It also imposes some methodological limitations, which will be discussed in the following section.

2.1. Methodological aspects

Brain tumour models usually are used in the context of oncological treatment research, and this aspect has been reviewed extensively (e.g. in Barth and Kaur, 2009; Chen et al., 2012, 2013; Daphu et al., 2013; Janbazian et al., 2014; Oh et al., 2014; Schmid et al., 2012; Simeonova and Huillard, 2014; Wu et al., 2011). By contrast, publications on the epileptological aspect of such models are indeed quite rare–an overview is presented in Table 1.

These models from a methodological point of view are quite similar, at least those focusing on glioma. Thus, they either rely on human cell lines (Campbell et al., 2012), human primary cell cultures maintained in nude, i.e. immunocompromized, mice (Buckingham et al., 2011; Campbell et al., 2015), or on a rat cell line (Köhling et al., 2006; Senner et al., 2004), with mixed cell lines being used in some cases (Beaumont et al., 1996). Cell lines have the advantage of a relatively easy cultivation under cell culture conditions in appropriate media such as DMEM/F12 (Invitrogen) supplemented with 7% bovine growth serum and glutamine (Campbell et al., 2012), or DMEM supplemented with 10% fetal calf serum and antibiotics (Köhling et al., 2006). They also have the advantage that they can be labelled stably with e.g. green fluorescent protein by transfection (Campbell et al., 2012; Köhling et al., 2006) and hence made visible for e.g. in vitro slice experiments, enabling the experimenter to delineate the tumour mass or even identify single glioma cells. Primary cell cultures, in turn, necessitate more effort in cultivation, as they need to be maintained in serial passage in immunodeficient (athymic nude) mice, and are then harvested for transplant, being intermittently kept in more expensive media such as Neurobasal A medium (Invitrogen) supplemented with epidermal and fibroblast growth factors, B-27 supplement without vitamin A (Invitrogen) as well as antibiotics (Campbell et al., 2015; Buckingham et al., 2011). Another disadvantage is that stable GFP-labelling is not possible, and that they apparently are working mainly in immunodeficient (scid) mice only. These disadvantages, however, are compensated by the fact that primary cell cultures are likely one of the best models, as they mimic the actual biology of the glioma, being derived from actual human gliomas.

In either case, using cell lines or primary cultures, tumour cells are usually stereotactically injected into the brain as cell suspension (in e.g. 5% methylcellulose, 10 μl), usually with a total cell number of 2.5×10^5 to 2×10^6. This results in tumour growth within 7–15 days to a size of 1–2 mm in diameter, and in the case of neocortically injected tumour cells, in seizures, or at least EEG abnormalities and in vitro hyperexcitability, including spontaneous discharges (Buckingham et al., 2011; Campbell et al., 2012, 2015; Köhling et al., 2006). As it can be inferred form Table 1, one of the main problems of such models appears to be that in most cases, seizures are not being reported, even cursorily under the heading of e.g. “clinical signs”. The reason for this may be that the authors (often concentrating on oncological aspects) did not look for such signs, and indeed overlooked them since in many cases, even if seizure-like activity is being observed in the EEG, behavioural signs are often quite subtle, comprising freezing, facial automatisms and head tremor, or massive startle response in reaction to audiogenic stimuli, and only very seldom generalised tonic-clonic convulsions (Buckingham et al., 2011; Campbell et al., 2015; Köhling et al., 2006). Another reason may be that tumour cell injection into areas other than the neocortex such as capsula interna, corpus striatum or even cerebellar subdural space, are probably not ideal for the purpose of an epilepsy model (Aas et al., 1995; Beaumont et al., 1996; Hossmann et al., 1989; Krajewski et al., 1986; Linn et al., 1989; Rewers et al., 1990; Wechsler et al., 1989). Another problem is also revealed in Table 1. In most cases, if not sacrificed before, or treated with cytostatic interventions, all animals die within approx. 30–35 days, with a 50% survival of 16–25 days (Aas et al., 1995; Beaumont et al., 1996; Desmarais et al., 2012; Krajewski et al., 1986; Rewers et al., 1990; Stafford et al., 2010; Scheck et al., 2012), and own unpublished observations in the course of pilot experiments to glioma-implantation studies (Köhling et al., 2006; Senner et al., 2004). This somehow matches the clinical course of high-grade gliomas e.g. glioblastoma in patients (at least relative to life-span; 20 days in rodents can be seen as corresponding to 1.5 years in humans), but not that of low-grade tumours, which clinically are expected to be more epileptogenic. And it is to be expected, as the models were designed to match glioblastoma. As a downside to the currently available models, there is an apparent lack of slowly growing and less invasive tumour models, at least from the epileptological standpoint.

Other malignant tumours besides gliomas have not been studied at all in in vivo tumour-grafting models, at least not in the context of epileptology. As Table 1 shows, there are some implantation models, mainly based on mammary adenocarcinoma, but also covering histiocytoma and melanoma (the latter actually resulting in real brain metastases after intracardial injection of cell suspension), but the authors, focusing on oncological aspects, did not explicitly look for seizures, EEG abnormalities, not to speak of signs of any hyperexcitability in in vitro preparations (Abramovitch et al., 2004; Colak et al., 1995; Tekle et al., 2012; Yuan et al., 1994).

2.2. Mechanisms of epileptogenesis in glioma models – a condensed overview

Even though the question of mechanistic insights into epileptogenesis is explicitly not at the central focus of this review, a brief summary on this issue seems justified. Several hypotheses have been put forward (cf. Beaumont & Whittle, 2000; Shamji et al., 2009; van Breemen et al., 2007), the earlier ones of which comprise the following: (a) an increased intracranial pressure as such, (b) a de-afferentation of cortical structures, (c) an increase in glial excitability, (d) a loss of gap junctions, and consecutively a loss of spatial buffering of extracellular potassium, and (e) glia mediated