Laboratory Investigation

The RG2 rat glioma model

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Summary

The ethylnitrosourea-induced cell line RG2 grows very well in infinite cell culture in vitro, and provides a simple, reproducible glioma model when inoculated into the brains of syngeneic Fischer 344 rats. We have used this tumor model in a series of therapy studies. We here report our experiences of the untreated (= tumor bearing control) animals, e.g. in terms of the techniques employed and also the growth, histology and effects upon the blood-brain barrier of the tumors. Weight loss as a measure of systemic effects during tumor development is also described. The RG2 model has considerable potential as a suitable tool for experimental neuro-oncology.

Introduction

An abundance of different animal models for experimental neuro-oncology have been developed [1]. One of the oldest in use employs a transplantable cell-line, RG2, induced in rats more than 20 years ago [2]. The model has been extensively used during the following years [3–10]. We have used it in our laboratory in a series of experiments [11–22] and have found it pertinent to characterize the model as used by us after the hundreds of passages the cell line has gone through since it was originated. As we use one control animal for every treated animal in most of our various therapy studies, a large control material comprising several hundred rats now exists and makes the basis for this publication. During all surgery, all animals are under general anesthesia with chloral hydrate given i.p. The experiments were examined and accepted by the local animal ethics committee. The aim of the study was to find and define a reproducible glioma model for basic research and therapy design studies.

Materials and methods

Animal model

The Fischer 344 rat strain used in the Division of Experimental Neurooncology (DENO) was provided in 1985 by professor H. Wigzell, Department of Immunology, Karolinska Institutet, Stockholm. The rats have since been continuously bred at DENO. Animals of both sexes, weighing 120–344 g were included in the study. The animals were kept in plastic cages with free access to water and pellets (SAN-bolagen, Malmö, Sweden). It should be noted that the Fischer 344 rats are sensitive to draught and are prone to develop respiratory tract infections. They also need a higher cage temperature than most of our other rat strains. Thus, a room temperature of 19 to 20°C gives a cage temperature of about 23 to 24°C which is preferable for these rats. Fischer 344 rats are more difficult to breed than e.g. Wistar and Sprague-Dawley rats, at least in our hands. We have found Fischer 344 rats to be more sensitive to noise and irregularities in
their environment than our other strains. The majority of the animals reported here have been used as individual controls for treated tumor-bearing animals in a series of studies concerning the effect upon brain tumor growth, e.g. whole body hyperthermia, calmodulin inhibitors and non-steroid anti-inflammatory drugs (NSAID's). None of the reported tumor-bearing control animals were sham operated or treated in any other way after inoculation with RG2 cells.

**Tumor cell line**

The rat glioma cell line RG2, utilized in our laboratory was established by Wolfgang Wechsler in Cologne from an ethylnitrosourea-induced glioma in Fischer-344 rats more than 20 years ago [2]. The cell-line which grows very well in infinite cell culture was given to us by professor D. Bigner, Duke University Medical Center, Durham, USA. The mean doubling time of the cells is 20 hours with a minimum of 18.5 hours and a maximum of 22 hours (own unpublished results). Cells are either stored in liquid nitrogen or kept in continuous serial passages in nutrient medium (RPMI 1640, Labdesign, Stockholm, Sweden) with addition of 5.5% fetal calf serum. Gentamycin (50 μg/ml) is added to the nutrient medium to avoid infection. Continuing controls are performed to check for mucoplasma.

Immediately before inoculation into rat brains the RG2 cells were collected by washing twice in a trypsin buffer, trypsination for 1 min, followed by 5 minutes incubation at 38°C, dilution in appropriate amount of nutrient solution, counting in Bürkér chamber and transport to the animal laboratory in a small sterile plastic vial. This was repeatedly shaken in order to avoid accumulation of the cells on the bottom. The whole procedure takes less than half an hour.

**Tumor cell inoculation**

All animals were under general anaesthesia during all surgical procedures. Chloral hydrate, 5%, 6 ml/kg body weight was given i.p. initially. One femoral vein was cannulated and during the treatment, additional chloral hydrate could be given i.v. to control and maintain anesthesia. A sagittal skin incision was made in the midline over the anterior part of the brain. The coronal suture was identified and a burrhole was made 2 mm to the right of the midline, 1 mm anterior to the suture. By stereotactic technique (Kopf small animal stereotaxic frame, Model 900 with the Kopf microinjector Model 5000), 500 to 100000 RG2 cells in 2–10 μl nutrient solution (5 or 10% fetal calf serum) were injected with a Hamilton 10 μl syringe into the head of the right caudate nucleus in the rats. After slow insertion of the needle to 5 mm depth, the content of (in most cases) 5 μl nutrient solution containing the RG2 cells was injected during 2 minutes. Thereafter the syringe was left in place for another 2 minutes before slow withdrawal during 30 seconds, in order to minimize spread of the tumor cells to the injection canal or the surface of the brain. By injecting the cells in the head of the caudate nucleus, we avoided contamination of the ventricles and CSF with cells and thereby early metastases through this route to other parts of the brain.

Groups of 6 to 16 animals were inoculated at each instance with cells harvested immediately prior to the procedure. The vial with nutrient solution and cells was shaken between each inoculation in order to maintain an even distribution of cells, and for each animal the appropriate amount of solution was aspirated in the Hamilton syringe before the inoculation. In order to avoid tumor growth extracranially, the injection site was cleaned with 70% ethanol after injection and the burr hole was sealed with wax (Professor Wechsler, Cologne, Germany is acknowledged for personal advice). Finally the wound was closed with single nylon sutures and the animals were allowed to wake up under observation. Animals with obvious and early neurological signs of hemorrhage were not included in the studies. As every treated animal at inoculation was matched to a control animal which had been inoculated with the same number of identical tumor cells immediately before the treated animal, the number of simultaneously injected control animals amounts 3 to 8. In all studies we have sacrificed the whole group of simultaneously inoculated animals at the point in