**Experimental Research**

**A model for intratumoural chemotherapy in the rat brain**

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**Summary**

To achieve the best reproducibility in rat brain tumour models several injection techniques have been used. Although stereotactic cell injections have proved to be effective and reliable, they are expensive and time consuming. A new permanently implanted device is presented here. It allows precise cell delivery for best tumour reproducibility, and it can be left in place for future injections at the exact same location, such as intratumoural chemotherapy. A Teflon tube was mounted on a disc, inserted into the rat brain and sealed to the skull. The device was tested in two rat strains (Wistar and New Zealand Nude rats) with two different glioma cell lines (9L and C6). Rats were treated with placebo to determine if repeated treatments had an effect on the device placement, or if device-related morbidity was induced. Analysis of brain sections showed that the device path was always within the tumour. The device never moved or came off the scalp. Both Wistar rats and NZ nude rats tolerated the device well. No morbidity or mortality was observed, regardless of the presence of the device; no infections were seen. Biocompatible, non-irritating and well tolerated, such a device can be used for reproducible tumour cell injections and repeated intralesional delivery of drugs.

**Keywords:** Stereotaxy; rat brain; tumour model.

**Introduction**

Brain tumour models in large animals being rare and expensive [1, 2], the best solution is offered by brain tumour models in smaller animals, i.e. mostly rats [3–6]. Such a model allows the testing of chemotherapeutics in vivo, whether systemically (e.g. intraperitoneally) or intratumourally. The latter option has been shown to be particularly effective as it allows the delivery of a high drug concentration in the tumour with low systemic toxicity. In order to test any chemotherapeutic agent in a rodent brain tumour model intralesionally (i.e. intratumourally), the following points should be considered: 1) A rat brain has a volume of a few cc. 2) A rat brain tumour can lead to severe deficits or death after it reaches a volume corresponding to the lowest resolution limit of a conventional MRI-based stereotactic method. 3) Such a method is expensive and time consuming, thus not suitable for repetitive treatments. 4) It requires an additional surgical procedure with related morbidity and mortality. These arguments underline the difficulties that are encountered when a drug has to be tested intratumourally in an experimental rat brain tumour.

In order to circumvent these problems a new biocompatible device was developed. This can be used to inject the cells, and later to precisely deliver the treatment intratumourally without the need for a stereotactic frame. Such a device is sealed to the skull and does not move, thus allowing high precision and reproducibility.

**Materials and methods**

**Device**

The device was made of a silastic tube (e.d. 0.7 mm) mounted in an EVA hemisphere (e.d. 3 mm). The outer portion of the device was 15 mm long, and the intracranial part was 4 mm long (Fig. 1a and b). The device is easy to assemble and it is not commercially available or intended for commercial use. The application of the device to the skull was done with a metal stilet, that was removed after the fixation to prevent the device from bending during insertion.

**Surgical technique**

After reflection of the periosteum, a 1.5 mm burr hole was drilled in the calvaria 3 mm anterior to the coronal suture on the left side, 2 mm lateral from the midline. This location gave access to the left frontal
pole. The device was then cemented to the skull with cyano-acrylate (Histoacryl). Water-tight adhesion was tested with the injection of 10 μl of PBS. The cell suspension was injected through the device by means of a Hamilton syringe. In the control groups (A, C) the device was subsequently removed. In the B and D groups the device opening was sealed with a droplet of cyano-acrylate to prevent reflux, and the skin was sutured over the device, leaving the upper portion of the tube outside. For all later injections (20 μl PBS) the device was reopened by cutting off the closed end. No antibiotics were given prior, during or after the procedure. No occlusive dressings were used.

Cells

C6 and 9L cells [7] in exponential growth were harvested by trypsinization for 5 min. at 37°C (EDTA/Trypsin, GIBCO), resuspended in RMPI-1640-Medium and centrifuged at 800 × g. The pellets were then resuspended in medium without any supplement, at a final concentration of 5 × 10⁵/ml. Every animal received 1 × 10⁵ cells in a volume of 20 μl.

Animals

Thirty two adult Wistar rats and 8 New Zealand nude rats were used for all experiments. The mean age was 10 weeks and the mean weight was 200 ± 20 gr. The animals were handled in accordance with the Rules and Regulations of the Central Animal Laboratory of the Hannover School of Medicine. For all procedures requiring anesthesia, rats were anesthetized with 80 mg/kg body weight of Ketamin HCl 10% and 2 mg/kg body weight of Xylazin HCl 2%, i.m.

All animals were handled according to the Principles and Regulation of the Hannover School of Medicine. All studies were approved by the local Animal Ethics Board which regulates animal studies. The approved study bears the number: 604i-42502-96/918.

Groups

The rats were divided in the following treatment groups (each group, n = 8):

- a. Wistar rats implanted with 9L tumours, by means of the device.
- b. Wistar rats implanted with 9L tumours by means of a stereotactic frame.
- c. Wistar rats implanted with 9L tumours, free-hand.
- d. Wistar rats injected with Evan’s blue by means of the device.
- e. NZ nude rats implanted with 9L tumours, by means of the device.

Sacrifice and histological samples preparation

All rats of the groups a. to c. and e. were sacrificed on the 15th day post-implant, and fixed by in situ endovascular perfusion. The brains were subsequently dissected under a microscope, post-fixed in 10% buffered formalin for 24 hours, embedded in paraffin, cut and stained with Haematoxylin-Eosin. Consecutive slices were evaluated microscopically, in order to detect the tumours and the path left by the device. Rats from group d. were sacrificed immediately after injection (n = 4) and 6 hours later (n = 4) to establish the distribution of the injected drug.

Results

During all experimental procedures no morbidity or mortality induced by the device or by the treatment was observed. No rats experienced irritation at the wound site, no rats tried to remove their device or to scratch it. No infections were seen. The device never moved or came off the scalp. Both Wistar rats and NZ nude rats...