

Positron Emission Tomography: An Animal Model of Spinal Distribution of Drugs After Intrathecal Administration

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ABSTRACT

An animal model has been developed in the Rhesus monkey for noninvasive monitoring of CSF transport of drugs by external detectors i.e. positron emission tomography. The model comprises the cannulation of the subarachnoid space (with a spinal needle), and has been used without any damage to the monkey. With the method it was shown that injection rate had a major influence on the transport rate of ⁶⁸GaCl₃ in the CSF. Injection of 0.5 ml over 60 sec gave the highest radioactivity near the injection site, whereas an injection rate of this volume over 10 sec resulted in high radioactivity more rostrally shortly after injection. This method have been of value for the determination of drug kinetics after spinal administration.

INTRODUCTION

Positron emission tomography (PET) is a noninvasive technique which measures the kinetics of biochemical processes and transport of radiolabelled compounds in vivo (5,6). The technique furnishes cross-sectional images of the distribution of radioactivity in the body and can be regarded as an in vivo autoradiographic method. The technique may be potentially useful in measuring cerebrospinal (CSF) bulk flow or the distribution of low molecular radiotracers after spinal (intrathecal or epidural) administration, since kinetics are monitored noninvasively by external detectors.

The rostral bulk flow of CSF has been studied by gamma scintigraphy using radiotracers such as iodinated human serum albumin (4). It is doubtful, however, if albumin will mirror the distribution of small drug molecules owing to differences in molecular weight and physio-chemical properties.

An experimental model using Rhesus monkeys was developed to study the distribution of intrathecally administered ^{68}Ga by PET. The objectives were to:

- * evaluate a model using PET for the measurement of the transport of small radiolabelled molecules in CSF after spinal administration
- * study the influence of injection rate on the distribution of the radiotracer in the CSF.

MATERIALS AND METHODS

Animals

Rhesus monkeys (*Macaca Mulatta*) weighing 6.3-10.5 kg from the Primate Laboratory of Reproductive Research, Uppsala university were used after an overnight fast. The animals were anesthetized with repeated doses of 50-100 mg of ketamine intramuscularly (Ketalar^R, Parke-Davis NJ, USA) given every 30-60 minutes. Diazepam (Diazemuls^R, Kabi-Vitrum Stockholm, Sweden) was administered intravenously as a supplement in doses of 5-10 mg every 30-60 minutes. After induction of anaesthesia, the monkey was placed left side down in a specially constructed plastic cradle, which was kept horizontal during the experiment.

Intrathecal administration

The spinal canal was punctured between the spinal processes L3-L4 with a modified spinal needle 40 mm long and with a dead space of 50 μl (Everett spinal needle^R, Avons Medical Ltd Redditch, England, 0.7 mm ODx90 mm). To stabilize the needle during the experiment it was passed through a plastic stopper, that was fixed to the needle with a screw and to the skin with a tape. The escape of one drop of CSF indicated puncture of the subarachnoid space. The needle had a short bevel, which was positioned with its opening pointing cranially. Before the injection of radioactivity, the position of the needle was verified with fluoroscopy, after the injection of 0.1 ml metrizamide (Amipaque^R, 170 I/ml, Nyegaard, Oslo, Norway). The CSF-pressure was monitored by connecting the spinal needle to a Gould pressure transducer via a saline filled polyethylene catheter.

The size of the spinal canal was calculated by computed tomography (CT; Siemens Somatom DR2) in a monkey weighing 9.3 kg. The cross-sectional area of the spinal canal was estimated at different levels by measuring the two axes in the axial CT scan and using the ellipsis. The volume of the spinal canal was calculated by multiplying the cross-sectional area with the length measured on the longitudinal scan.

CSF and blood sampling

A second spinal needle (Everett spinal needle, 0.5 mm ODx50 mm) was introduced into the subarachnoid space through a lateral puncture at C1-C2 level for CSF

sampling (1,9). A PE 50 polyethylene catheter was connected to the end of this needle through an adapter. Blood from a peripheral vein and CSF samples were collected up to 40 and 60 minutes after injection, respectively.

Radiopharmaceuticals

⁶⁸Ga as the chloride salt (⁶⁸GaCl₃) was produced in a tin dioxide open bed ⁶⁸Ge/⁶⁸Ga column (New England Nuclear Medicine, Boston, USA) eluted with a few ml of 1 M hydrochloric acid. This solution was neutralized with 1 M sodium hydroxide with phenol red as indicator.

Before administration, the solution was filtered through a 0.22 μm membrane filter for sterilization. The radioactive dose varied between 10 and 60 MBq in a 0.5 ml sample. The injections were given in the same animal following each other (with an 2-hour interval) and given at a constant rate by a syringe pump (Model 220 Sage Instruments) over 10 or 60 sec, respectively (Table 1).

Table 1. Maximal radioactive uptake in the spinal canal after intrathecal administration at L3-L4. Registration with positron emission tomography.

| Exp | Radiotracer | Duration of injection (sec) | Spinal level | Maximal uptake * | Time after injection min |
|-----|---------------------------------|-----------------------------|--------------|------------------|--------------------------|
| 1 | ⁶⁸ GaCl ₃ | 60 | T8 | 11 | 15 |
| | | | L1 | 300 | 8 |
| | | | L6 | 580 | 27 |
| 2 | ⁶⁸ GaCl ₃ | 10 | T8 | 80 | 20 |
| | | | L1 | 350 | 6 |
| | | | L6 | 166 | 26 |

$$\hat{\text{Uptake}} = \frac{\text{nCi/cm}^3}{\text{Dose} \times \text{bodyweight}^{-1}} \quad (1)$$

Instrumental

For the imaging of different spinal levels, the cradle was moved to radio-logically predetermined positions. Imaging of the monkey was performed at the spinal levels L6, L1 and T8 in a PC 384-3B positron tomograph (Scanditronic Instrument AB, Uppsala, Sweden) equipped with two rings of detectors that gave three simultaneous slices of 13 mm thickness each (5). Images were recorded for 60 sec throughout the study with the initial kinetics of radioactivity monitored at the L1 level.

The influence of the injection rate was studied by using two collimated beta-scintillation detectors (BGO) positioned at L6 and L1 and with the positron camera at the T8 level. The distribution of radioactivity could thus be monitored at three different levels simultaneously.

Calculations

The radioactivity per cm^3 was calculated as a function of time at each spinal level corresponding to L6, L1 and T8 and was corrected for physical decay of the radionuclide from the time of administration. The values were divided by the amount of radioactivity given per gram body weight. A normalized uptake of 1.0 corresponded to the uptake that would have been achieved if the administered radioactivity was equally distributed in the whole body of the monkey assuming 1 cm^3 is equal to 1 g. The standard deviation of measured radioactivity was always below 15%. Radioactivity in blood and CSF were measured in a well-counter and expressed as uptake values as described above.

RESULTS

Animal model

Intrathecal drug administration, CSF-sampling, anesthesia and positioning of the cradle in the positron camera were tested in 4 animals before the experiments started. Satisfactory anesthesia of the monkeys was achieved with ketamine and diazepam with no signs of involuntary movements or hyperventilation. The animals could be kept in the same position for several hours and they did not show neurological sequela.

The intrathecal positioning of the needle was crucial, since a constant CSF-flow did not ensure a correctly placed needle. This may also occur when the needle is partly localized in the subdural space after disconnection of the arachnoidea (8). Puncture of the subarachnoidal space at C1-C2 could be carried out without problems and samples of 100 μmol of CSF were taken every 15 min.

The frontal and sagittal diameters at all levels of the spinal canal were less than 8 and 6 mm, respectively (Table 2). The estimated volume of the dural sac was 7-9 ml.

Table 2. Anatomic dimensions of the spinal canal calculated from CT scans in a Rhesus monkey (weight 9.3 kg). Length of spinal dural sac (foramen magnum - S2): 350 mm. Estimated volume of the spinal canal (foramen magnum - S2): 7-9 ml. Distance between the puncture site (L4-L5) and different vertebrae levels: L7: 50 mm; L1: 75 mm; T8: 170 mm; C7: 270 mm.

| Vertebral level | Frontal and sagittal diameters (mm) | Crosssectional area (elliptic) (mm ²) |
|-----------------|-------------------------------------|---|
| C5 | 6.7 x 4.7 | 25 |
| T4 | 4.0 x 3.7 | 12 |
| T11 | 5,3 x 3.7 | 15 |
| L1 | 6.7 x 5.3 | 28 |
| L3 | 6.7 x 5.3 | 28 |
| L5 | 7.5 x 5.3 | 30 |

Positron emission tomography

Injection rate had a pronounced influence on the spread of gallium (Table 1) and the model was tested by using measurements of the CSF-pressure and external monitoring of radioactivity at several levels by collimated detectors. The distribution of radioactivity at different spinal levels after injection times of 60 and 10 sec is shown in Fig. 1 and 2, respectively. Injection over 60 sec gave a linear increase of activity at all recording sites starting immediately and continuing during the whole injection period (Fig. 1). The increase is steep at L6, i.e. 1 cm caudal to the injection site. At T8, 15 cm from the injection site, the increase is smaller but linear over the whole period. After end of the injection no further increase in the radioactivity was measured at L6. At L1 and T8 the increase is linear up to 8 min with an approximate doubling of the activity within 12 and 6 min, respectively. Before injection the recorded CSF pressure was 1 mmHg. It was 3 mmHg at 2 min after end of injection.

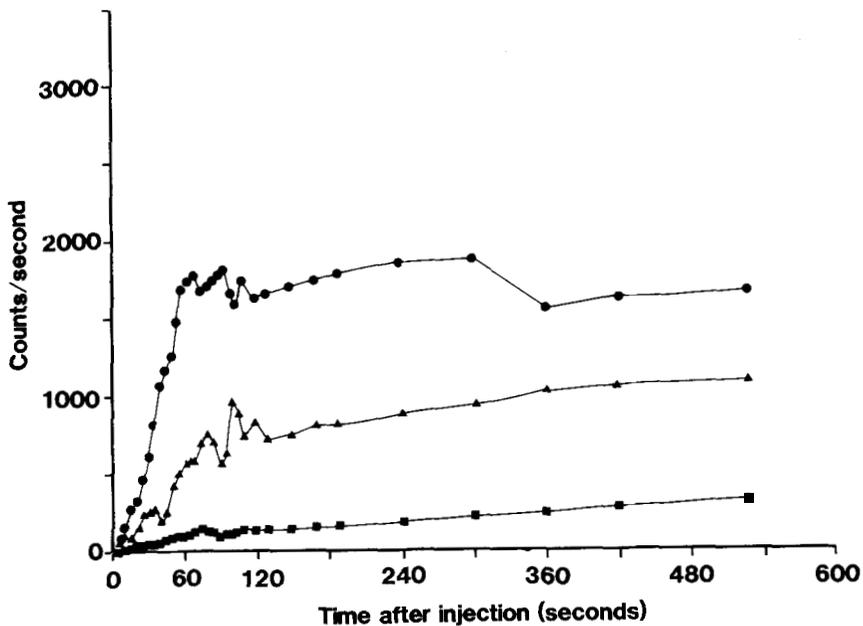


Fig. 1. Initial kinetics of radioactivity (counts per second) at vertebrae levels L6 (●) and L1 (▲) monitored by external detectors BGO and radioactivity at T8 (■) registered by positron emission tomography. The dose was given intrathecally in 0.5 ml of $^{68}\text{Ga-Cl}_3$ over 60 seconds at L3-L4.

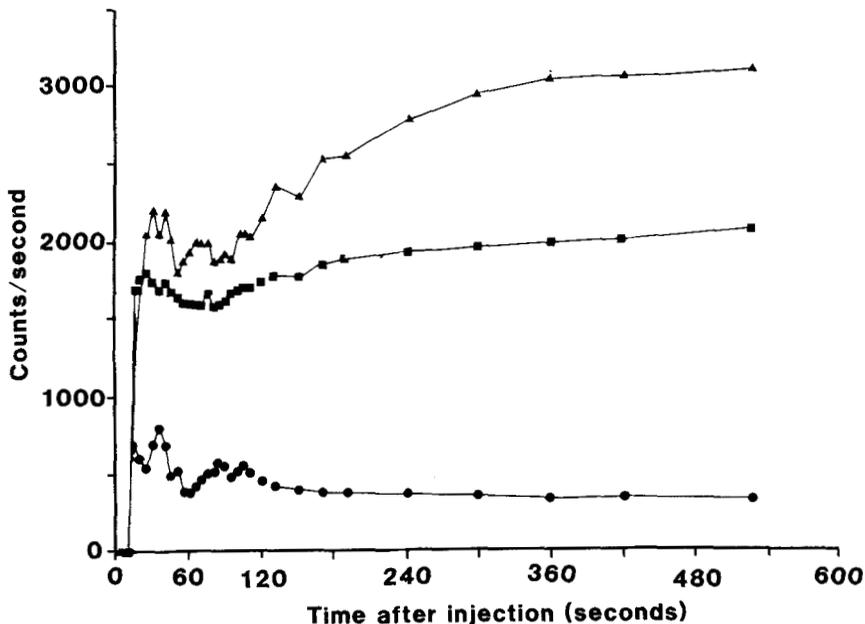


Fig. 2. Initial kinetics of radioactivity (counts per second) at vertebrae levels L6 (●) and L1 (▲) monitored by external detectors BGO and radioactivity at T8 (■) registered by positron emission tomography. The dose was given intrathecally in 0.5 ml of $^{68}\text{Ga-Cl}_3$ over 10 seconds at L3-L4.

A different pattern is shown with a 10 sec injection (Fig. 2). A fast but non-linear increase in radioactivity is seen at all recording sites. The caudally located detector at L6 close to the injection site only recorded a small fraction of the total radioactive dose. The measured radioactivity at T8 is three times higher than at L6. After the injection fluctuating value over 200 sec indicate turbulence in the system. At 200 sec a slow increase in radioactivity at T8 and a slow decrease at L6 was measured. At L1 the radioactivity increased by 50 per cent to constant values after 6 min. Recorded CSF pressure prior to administration was 8 mmHg. It increased rapidly to a maximal value of 25 mmHg and decreased then slowly. The CSF pressure was stable at 4-6 mmHg at 6 min after injection.

The radioactivity in CSF samples taken from C1-C2 remained low throughout the experiment.

DISCUSSION

The present study demonstrates a new method to monitor drug distribution in the spinal canal and is also a new application of PET. The size of the Rhesus monkey allows the body to be freely moved in the PET but the animal is still large enough for injections of rather large volumes (0.5 ml) into the spinal canal. The model and the detection technique were, thus, adequate for studies of distribution after intrathecal administration of the radiotracer ^{68}Ga in the spinal canal.

The rapid initial distribution after an intrathecal dose was documented with the model. The kinetics of the radioactivity could be monitored at three levels simultaneously by using two external detectors and the PET-camera. Together with the sampling of CSF at C1-C2 a good measure was given of the distribution of radioactivity. It was documented that the injection rate highly determined the spread of radioactivity in the CSF.

A considerable change of volume may occur in the spinal dural sac, which acts as a pressure reducing chamber for the cranial CSF (8). Changes in the spinal CSF space are balanced by a decreased volume of the epidural veins of the spinal canal. The measured diameters of the spinal canal (Table 2) indicates that the maximal size of the spinal dural sac can be estimated to 7-9 ml. Assuming that the spinal cord diameter is about 2/3 to 3/4 of that of the spinal canal, a spinal CSF volume of 3-5 ml can be calculated which is in accordance to the data of Rieselbach(10). The injected sample (0.5 ml) thus represents 10-15 per cent of the spinal CSF space, which in humans would be a slightly larger volume than used to achieve spinal anaesthesia (2).

The injected volume will displace CSF and expand the dural sac. In the lumbar region this volume would correspond to a column of 3-4 cm and in the thoracic region to 7-9 cm (Table 2). Due to the direction of the bevel of the needle the displacement of CSF will mainly be cranially. With the rapid injection surprisingly small amounts of the tracer were found even just caudal to the injection site. It can be concluded that very little expansion apparently occurred in the caudal dural sac during the rapid injection with concomitant high CSF pressure. The cranial CSF space on the contrary may accommodate some 10 per cent of the spinal CSF volume by changing the blood pool by 0.5% of its intracranial volume.

The high radioactivity observed at T8 at the end of the fast injection cannot be explained simply by displacement of CSF. A considerable turbulence with mixing of the sample and the CSF has to be postulated. Turbulence may also explain the early increase in activity detected at T8 during the slow injection. The distribution rates of the radioactivity that are measured during the initial minutes after intrathecal injections of this volume (0.5 ml) are therefore not representative of CSF bulk flow. This affects the distribution of the injected tracer within the spinal dural sac during at least the first 5 minutes.

The anatomical dimensions of the spinal canal (cf Table 2) are smaller than can be resolved in the PET-system used (spatial resolution about 8x8x13 mm). The calculated uptake values (nCi/cm³/dose/bw) are relative and do not give exact quantitative measurements (5,6). Consequently the measurements from PET images represent concentrations in the spinal canal as a whole and cannot be strictly related to CSF or the spinal cord. However, in the case of ⁶⁸Ga almost all radioactivity would be within the CSF because of the hydrophilicity of the ion.

Another limiting factor of positron emission tomography is the observation time. The positron emitting tracers have a rapid physical decay with half-lives of ⁶⁸Ga and ¹¹C of 68.7 and 20.4 min, respectively. This makes prolonged studies impossible. More long-lived positron emitters like ⁷³Se (t_{1/2} = 7.1 h) can, when they are available, overcome such drawbacks.

Despite the limitations, PET gave an in vivo quantitation of variations in concentrations at different spinal levels and made it possible to study factors which influence rostral transport after intrathecal application. The animal PET model including technique for cannulation of the subarachnoid space may be applied to study the transport of several types of drugs such as e.g. opioid analgesics, in the CSF after intrathecal injection. Such studies using this

technique is presented elsewhere (7).

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