

CSF – injected contrast medium enhances post-traumatic spinal cord cysts. An experimental study in rats

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Abstract. – OBJECTIVE: Spinal cord injury (SCI) is still one of the most challenging problems in neurosurgical practice. One of the major obstacles to neural regeneration following trauma is the formation of glial scarring and post-traumatic cysts which acts against proper growth of axons through the site of injury. Cerebrospinal fluid (CSF) delivery of bioactive agents into cystic cavities could represent a promising therapeutic strategy. In the present study, we investigated specifically the dynamics of intradural delivery of contrast medium and its relocation into post-traumatic cysts in an experimental model of spinal cord cryoinjury in rats.

MATERIALS AND METHODS: 32 male Sprague Dawley SPF rats were submitted to injury as previously described. Omnipaque-240 was injected either into the cisterna magna or at the level of the cauda equina. Subsequently, cerebral CT scan examinations were performed in order to check the CSF dynamics of the contrast medium.

RESULTS: There was a steady accumulation of contrast medium into post-traumatic cysts as early as five minutes after injection. A dosage of 65 mg of iodine per kilogram ensured an adequate filling of the cysts at an average of 30 minutes.

CONCLUSIONS: Our data indicate that intraspinal injection of bioactive agents can easily reach the site of injury and fill post-traumatic cysts. This could represent an interesting potential therapeutic protocol for SCI.

Key Words:

Spinal cord injury, Syringomyelia cysts, Central canal, Contrasting, Computer tomography (CT).

Introduction

Spinal cord injury (SCI) represents a social emergency mostly in developed countries^{1,2}. The

development of post-traumatic so-called “syringomyelia” cysts is a frequent evenience following SCI and represents a formidable barrier against potential axonal regeneration across the site of the traumatic damage, which in turn, explain why functional recovery is a quite unsatisfactory rule³⁻⁵. Intracystic manipulation with curative purposes has been recently suggested^{6,7}. However, the technique for intracystic delivery is particularly sophisticated in those small animal experimental models used^{8,9}. We studied the dynamics of cerebrospinal fluid (CSF) delivery of contrast medium in a recently published experimental model of SCI¹⁰. Our data seem to indicate that other ways of bioactive material delivery into post-traumatic cysts can be used. We present here the results of this study.

Materials and Methods

Animals and Model of Glial Scar Simulation in the Rat Spinal Cord

32 male rats SD with an average weight (\pm SEM) of 367.6 g (\pm 24.6) were used for the present experiment. All animals were housed under standard conditions in the Animal Breeding Facility of BIBCh, RAS (Unique Research Unit Bio-Model of the IBCh, RAS; the Bioresource Collection – Collection of SPF-Laboratory Rodents for Fundamental, Biomedical and Pharmacological Studies), which has international accreditation by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALACi). All experiments and procedures were approved by the institutional animal care and use committee (IACUC No. 831/21, dated 20/04/21).

A glial scar rat SCI model by cryoapplication early described^{10,11} was used. Briefly, following microsurgical T₁₃ laminectomy, a controlled cryoinjury, approximately 1 mm wide, was created on the right side of the cord. Animals were allowed to recover from isoflurane anesthesia and subsequently used according to the study protocol.

Contrast Injection into Subarachnoid Space

Omnipaque-240 (Iohexol, 240 mg of iodine/ml, GE Healthcare AS, Oslo, Norway), diluted with saline or CSF collected from other rats 1:1 by final volume 200 µl, was used for the present study. A mixture of contrast was warmed to body temperature and injected using a 29G needle in the subarachnoid space either *via* the cisterna magna or the caudal lumbar region (L1-L2 level). Subsequently, rats' spine CT-imaging (45 days after cryoinjury) was performed 5 min after contrast injection.

Experimental Groups

The animals were randomly assigned to four study groups:

Group 1: injection of Iohexol with saline into cisterna magna by atlanto-occipital puncture (n = 8).

Group 2: injection of Iohexol with CSF into cisterna magna by atlanto-occipital puncture (n = 8).

Group 3: injection of Iohexol with saline into the caudal lumbar region (n = 8).

Group 4: injection of Iohexol with CSF into the caudal lumbar region (n = 8).

Collection of CSF from Cisterna Magna

CSF was collected under close sterile conditions from 20 other rats, and the volume of CSF ranged between 50 and 150 µl. A needle (29G) connected to a 1 ml syringe was used for CSF collection, under visual control using an operating microscope (OptikaMicroscopes, Pontenica, Italy). Propofol (Hana Pharmaceutical Co. Ltd., Seoul, South Korea) was used as an anesthetic agent for this procedure. The posterior side of the neck was first shaved and then disinfected with skin antiseptic (Sterisol, Vadstena, Sweden). A midline incision was made, beginning at the level of the ears and ending approximately 2 cm caudally. The aspiration of CSF was achieved by pulling back the syringe plunger. The collected CSF was ejected into a 1.5 ml Eppendorf tube and frozen at -196°C until the next use.

Computer Tomography of the Spinal Cord

CT was carried out using a CT scanner MRS* PET/CT (MR Solution, Guildford, Surrey, UK).

Scan parameters

Energy where as follows: 40 kVp, exposure 100 ms, current 1 mA, stepping angle 1°. For the purpose of the diagnostic procedure, the animals were anesthetized with a 3% mixture of isoflurane and air and maintained in a specialized bed at a temperature of +37 °C. The CT images obtained were processed using the VivoQuant (Invicro, Needham, MA, USA) and Inobitec (Inobitec llc, Voronez, Russia) software. Axial scans were reconstructed in 3 standard planes, 3D-rendered, and a curvilinear reconstruction using manually placed fiducials along the central spinal canal was carried out. Regions of interest were determined as contrast-enhanced post-traumatic syringomyelic cysts. Segmentation of these was performed using a semi-automatic threshold mode to determine their mean density in standard Hounsfield units (HU) and their volume in mm³.

Determination of the Density of Contrast Agent in Vitro and in Vivo

First, for the quantification of the data, the density of reference contrast agent samples was measured. The contrast agent was diluted in different saline volumes (see Table I) into a 1.5 ml Eppendorph's tube, then sample density was measured on CT. The obtained reference points were subjected to regression analysis using a simple linear regression graph. This was used to calculate the predicted contrast agent concentration in the region of interest (ROI), and its dilution rate was related to the initial concentration. The concentration of the contrast agent in the cysts was determined in a similar way. The density of contrast agents in CT-imaging studies was measured using the VivoQuant (Invicro, Needham, MA, USA). Microsoft Excel 2019 software was used for statistical calculations.

Histological Studies

Animals were sacrificed, and histological studies were performed on day 45, as previously described^{10,11}. Briefly, samples of the rat spinal cord encased in the bone of three vertebrae were fixed in 10% neutral buffered formalin, rinsed in tap water, and processed for decalcification in Trilon B at room temperature for 12-16 days. The biomaterial was oriented for further microtomy in the sagittal planes. Hematoxylin and eosin staining was used for serial 4-5 µm thick paraffin-embedded sections. The sections were examined by standard light microscopy with an Axio Scope, A1 microscope (Carl Zeiss, Jena, Germany).

Photomicrographs of the histological sections were made with an Axiocam 305 color high-speed camera (Carl Zeiss, Munchen, Germany).

Statistical Analysis

Data by tissue density and contrast agent concentration were analyzed using Statistica software (StatSoft®, v.12.6, Tulsa, OK, USA). The results are presented as mean \pm SD.

Results

Histological analysis of the rat spinal cord at day 45 after cryoinjury demonstrated that all animals had large thin-walled cystic cavities with a size of up to 1.0 mm² in the projection of the gray matter and dorsal funiculi of the white matter. Most of the cavities contained an amorphous component (Figure 1). In some animals, cystic cavities were located cranially from the site of the injury, along the central canal of the spinal cord, a fact which would likely indicate impairment of the cerebrospinal fluid circulation.

Since the basal density of CSF is very close to the density of saline (about 0-5 HU), the main contribution to density level after contrast enhancement of ROI is made by contrast agent, so the

density increase should be directly proportional to the iodine concentration. For quantification of the data, the density of reference contrast agent samples (Iohexol) in different saline dilutions *in vitro* was measured by CT (Table I).

Iohexol concentration within post-traumatic cysts calculation is presented in Table II. So, the distribution of contrast in the cysts after 120 mg/ml initial Iohexol solution was approximately the same in all groups (Table II). Injected iodine concentration ends in the cyst, a fact that may be of some interest for later pharmacokinetics studies.

Immediately after Iohexol-saline injection into the cisterna magna 6 out of the 8 rats (75%) died in 1 to 10 minutes from respiratory and cardiac arrest. 2 rats survived up to 3-12 hours, and died with signs of progressive focal (ataxia, paraparesis) and general (seizures) neurological symptoms. A similar situation was observed in Group 2, after Iohexol-CSF injection into the cisterna magna. 5 out of the 8 rats (62.5 %) died in 1 to 10 minutes, and 3 rats survived up to 24 hours but were euthanized with signs of progressive heavy neurological disorders.

In all cases, when Iohexol in a concentration higher than 24 mg of iodine per injection (65 mg/kg) was used, a distinct contrast enhancement of subarachnoid cerebrospinal fluid spaces and central

Table I. Density of reference contrast agent samples (Iohexol) in different saline dilutions.

Starting contrast agent dilution, %	Starting contrast agent (iodine) concentration, mg/ml	Sample density, HU	Predicted contrast agent (iodine) concentration in sample, mg/ml
100	240	11,320	222
90	216	10,550	207
80	192	9,800	192
70	168	8,960	175
60	144	7,600	148
50	120	7,100	138
40	96	5,380	103
30	72	4,390	84
20	48	2,800	52
10	24	750	11
5	12	268	1

Table II. Density of contrast agent samples (Iohexol) in spinal cord tissues.

Number of groups	Starting contrast agent dilution, %	Starting contrast agent (iodine) concentration, mg/ml	Tissue density, HU \pm SD	Predicted contrast agent (iodine) concentration in tissue, mg/ml \pm SD
1	50	120	920.75 \pm 226.91	15.76 \pm 4.74
2	50	120	882.29 \pm 208.88	17.21 \pm 4.14
3	50	120	954.71 \pm 110.97	18.61 \pm 2.19
4	50	120	929.14 \pm 185.64	18.11 \pm 3.65

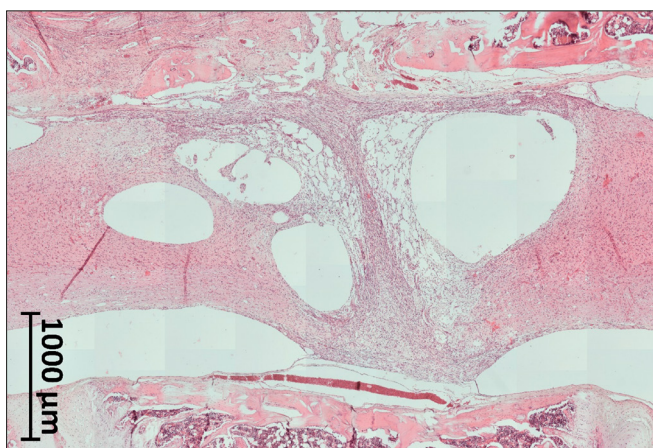


Figure 1. Panoramic view of the spinal cord lesion at day 45 after cryoinjury: large thin-walled cystic cavities (shown by the arrows) in the projection of the gray matter and dorsal funiculi of the white matter, containing an amorphous component. Hematoxylin and eosin staining.

canal in the cervical and thoracic regions was observed on CT scans. Distinct post-traumatic cyst enhancement was observed (Figure 2A-B). Unlike what happened with the central canal, no signs of connection of the cyst with subarachnoid spaces were observed. Subjectively, cyst enhancement intensity correlated with injected contrast agent concentration.

In all animals where injection into the cisterna magna of mixture Iohexol-saline had been performed, a fresh hemorrhage was observed at the cryoinjury (Figure 2C). Interestingly, such changes were not observed in the animals which were euthanized 24 hours after Iohexol injection using the same technique (Figure 2D).

After contrast agent injection (with either saline or CSF) in the lumbar region, all experimental animals (16 rats) survived. In these animals, enhancement of subarachnoid cerebrospinal fluid spaces and central spinal canal in all regions was observed. In all cases, they showed distinct staining of post-traumatic cysts (Figure 3A).

A panoramic view of the injury site in the spinal cord after injecting a contrast agent in the lumbar region showed cystic enlarged cavities, while their walls showed no signs of disintegration (Figure 3B).

Discussion

The present study gives evidence of the fact that Iohexol, if injected intracisternal, reliably and consistently shows the pathway of gradual contrast agent migration into a post-traumatic cyst *via* the central spinal cord canal. Moreover, it demonstrated that a contrast agent dosage of 65

mg of iodine per kg ensures adequate filling of the cyst within an average of thirty minutes.

This data, if translated into clinical practice, would indicate that potential neuroprotective/neuro restorative factors could be easily and effectively delivered into a post-traumatic cyst provided their density is equivalent – or even lesser – than the one of Iohexol. These facts have two important implications:

a) it would not be any more necessary to rely on very sophisticated technology in order to precisely – stereotactically – puncture the cyst(s), if the curative strategy would include local agents delivery;

b) the presence of multiple microcysts – which is the most frequently encountered actual situation following spinal trauma – would not be a problem anymore for effective local pharmacological agent delivery.

Intracisternal injection of contrast media has been a routine procedure in neuroradiological pre-MRI clinical activity, and potential toxicity of contrast agents has been extensively studied and shown mostly tolerable¹². Intracisternal delivery of potentially therapeutic agents is still routinely practiced in certain oncological protocols¹³, thus, CSF pharmacological agents' delivery toxicity evaluation is an acceptable practice and can be done with any other potential curative agent.

There are suggestions in the recent literature that surgical manipulation of the traumatized cord tissue, though with the aim of creating the best possible local environment for delivering therapeutic agents following spinal trauma, carries on a significant risk of increasing the level of functional damage by manipulating swollen, ede-

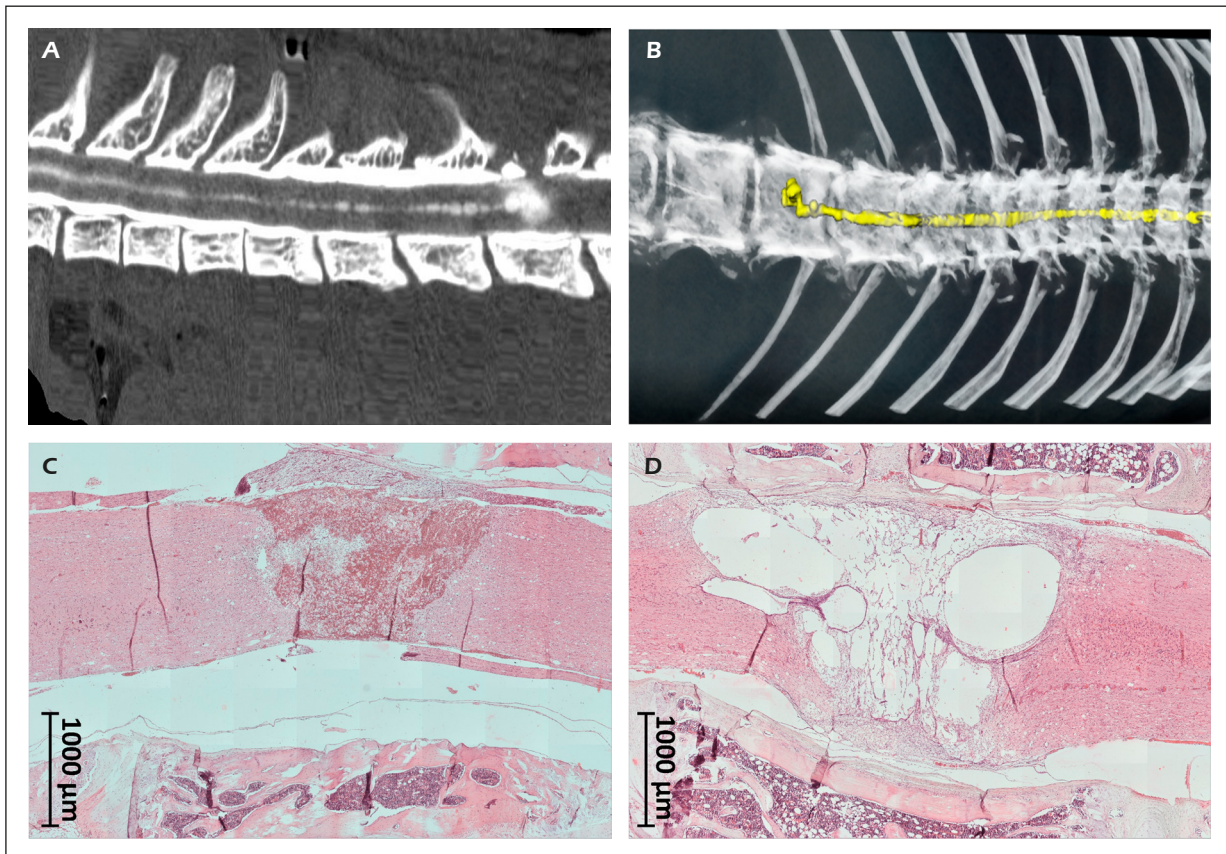


Figure 2. CT of the spinal cord after injection of contrast into cisterna magna. **A**, Curvilinear reconstruction using manually placed fiducials along the central spinal canal after contrast injection; **(B)**, 3D reconstruction of central spinal canal after contrast injection. **C**, Panoramic view of the injury site in the spinal cord after Iohexol injection into the cisterna magna: massive fresh hemorrhages fill the entire injury site of the spinal cord. **D**, Panoramic view of the injury site: cystic cavities are enlarged, while their walls show no signs of disintegration. Hematoxylin and eosin staining.

matous tissue where some living neurons could still be present^{14,15}. This is quite understandable, and if the goal is to deliver locally bioactive material – stem cells, scaffolds, and other – the present study shows that there is another effective, though definitely less invasive and risky, method that can be used in order to achieve such a goal.

The main problem in finding an effective methodology for post-traumatic spinal cord recovery remains how to overcome the negative effect against the potential intrinsic restorative capacity of the damaged spinal cord exercised both by glial scar and post-traumatic cysts formation¹⁶. This latter represents a formidable barrier against axon regrowth, in particular if they produce a gap of at least 0.8 mm⁶.

The influence of the size of post-traumatic spinal cord cysts on possible neural regeneration – and consequently functional recovery – following trauma is not being extensively investigated in the literature. However, recently, this possibility

has been strongly suggested^{9,17}. Using an experimental model of controlled SCI in rats followed up with serial MRIs, it was established that a gap of 0.8 mm represented the critical value after which no axonal regeneration across the injury site would be possible. The same group of investigators in a more recent study⁶ has described a very sophisticated stereotactic technique for injecting precisely bioactive nanoparticles into the syringomyelic cysts developed after experimental SCI using the same rat model.

This technique requires a particularly sophisticated technical armamentarium as well as specific competence with micro stereotactic small animals surgery.

It is an extremely interesting hypothesis that local delivery of bioactive agents known to be pro-neuroregeneration, stem cells, scaffolds, neurotrophic factor, etc., could help in overcoming the obviously negative effect of the loss of anatomically already and ultimately could



Figure 3. A, CT of the spinal cord after contrast injection into caudal lumbar region (cyst enhanced with contrast is indicated by red arrows); (B) panoramic view of the injury site of the spinal cord after injection of the Iohexol-CSF into the lumbar region (cauda equina). Hematoxylin and eosin staining.

promote functional neuroregeneration following spinal cord trauma¹⁸⁻²⁰.

The present study shows that caudal-lumbar medium delivery leads to intracystic migration of the injected material in our experimental SCI model in rats. This would indicate that other bioactive agents can follow the same dynamics if injected into the CSF spaces and ultimately could migrate into the post-traumatic cysts. If so, technically demanding strategies for intracystic injection of bioactive material

would not necessarily represent the only way for optimal local delivery of potentially therapeutic agents following SCI.

The high mortality observed in the group of animals submitted to intracisternal delivery of contrast medium was not surprising. We attempted to decrease it by performing the procedure with extreme caution (experience acquired with time might have been of help) however, it appeared to be still a relevant issue. The use of the alternative technique of cauda equina injection

allowed us to collect sufficient data in order to terminate the experimental study and give strong support to the aforementioned statements.

This would also be an important indication in the perspective of future clinical studies since it would not be unexpected that CSF bioactive agents delivery close to vital areas could carry on a significantly higher risk of dangerous reactions than CSF delivery by lumbar puncture

The possibility of using CSF delivery for therapeutic purposes following SCI is a very attractive possibility, which deserves future consideration and future animal studies. Our model, which creates relatively controlled, consistent unilateral spinal cord traumatic injury, appears to be an ideal substrate for testing this interesting hypothesis.

Conclusions

CSF delivery of bioactive agents appears to reach cysts cavities in experimental SCI in rats, a fact which would suggest several potentially interesting therapeutic implications in SCI.

Moreover, present observation would also suggest that intracisternal delivery of contrast does not enhance the central canal of the spinal cord in rats in physiologic conditions, a fact which is considered the rule by most scientists up-to date²¹ since foramina of Magendie and Luschka are as rule thought to act as one-way valve^{21,22}. Our data, however, seem to give strength to Thouvenin's hypothesis of a bidirectional CSF of the spinal cord canal²³.

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Ethics Approval

All experiments and procedures with animals were approved by the Institutional Animal Care and Use Committee IACUC No. 831/21 dated 20/04/21.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Authors' Contributions

Conceptualization, Georgii Telegin, Aldo Spallone, Alexey Belogurov Jr.; methodology, Aleksandr Chernov, Alexey Minakov, Elena Malyavina, Vitaly Kazakov, Maksim Rodionov; formal analysis, Georgii Telegin, Aldo Spallone; investigation, Aleksandr Chernov, Alexey Minakov, Elena

Malyavina, Alexey Belogurov Jr.; data curation, Aleksandr Chernov, Vitaly Kazakov, Maksim Rodionov; writing-original draft preparation, Aldo Spallone, Aleksandr Chernov; writing-review and editing, Georgii Telegin, Alexey Belogurov Jr.; supervision, Georgii Telegin. All authors have read and agreed to the published version of the manuscript.

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