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RESEARCH ARTICLE

Design and Testing of a Device for Drug Infusion and Collection of Continuous or Batch Cerebrospinal Fluid (CSF) in Rats: Pharmacokinetic Study of CSF Fluorescein Injected Intravenously and Infused Minocycline

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ABSTRACT

Many devices for obtaining cerebrospinal fluid (CSF) have been published. However, none of them allows the infusion and collection of CSF in a continuous or discontinuous base. The purpose for the design of this system is based on the possibility of injecting drugs of rapid or slow absorption (encapsulated in nanoparticles). Also useful, for studying the pharmacokinetics of drugs injected outside the cerebrospinal compartment (i.e. intraperitoneal), which can be followed over time by collecting small samples of CSF. Among the advantages of this system are the high yield of the CSF withdrawal (102.5±17 µL), without the presence of blood (around 99% of the rats tested (n=20)). Also, the survival of rats approaches around 75 % after a weekly CSF withdrawal repeated for one month (n=6). Finally, there was no need of a surgical procedure to expose the dura in the cisterna magna to withdraw CSF. The use of a stereotaxic device for securing the rat head allowed us to insert the collecting needle in a fixed place, without the guessing of other methods. Even though the use of this system appears to be expensive, it is worthwhile using it in pro of obtaining blood free CSF samples. In order to test this device, we assayed CSF for blood contamination, CSF protein determination and the pharmacokinetics of fluorescein in the CSF after the intravenous injection of the dye. Also, we studied the kinetic of infused minocycline into the CSF compartment. We can conclude that this device might become an indispensable tool in studies that involve manipulating the cerebrospinal compartment composition.

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INTRODUCTION

The CSF bathes the brain and the spinal cord (Nau *et al*., 2010). This compartment is advantageous for the application of drugs acting on those structures, and not only the infusion, but by extraction of liquid from this compartment. With the advent among others, of the nanoparticles technology, the application of drugs directly into this compartment becomes a reality. The possibility of incorporating nanoparticles of slow release (Barthel *et al*., 2014) into the LCR is generating a high impact in the preclinical development of drugs. These kinds of molecules offer a vast therapeutic platform allowing the reduction of the number of doses, decreasing the toxicity, protecting the drug form inactivation. Among other advantages, this device allows both the infusion and withdrawal of LCR samples. Many other devices have been published, but all of them present some drawbacks. For example, Ceaglio *et al*. (2013) describes a device for withdrawing LCR samples that are invasive because they perform a midline incision of the neck skin in the cephalo-caudal direction until the prevertebral aponeurotic plane is visualized before performing the LCR withdrawal. No infusion was considered. Nirogi *et al*. (2009) describes a method for collecting rat CSF by using a drawing system consisting of a 23G needle that is covered with PE-50 in all the extension but 5 mm from the tip. This system does not need any incision in the rat neck skin, but, unfortunately relays in the experimenter skill to insert the needle without damaging the brain below the skin. Again, no infusion was contemplated. Pegg *et al*. (2010) describes a procedure for extracting CSF samples by

using a stereotaxic device, but again, the exposure of the atlanto-occipital membrane is made by an incision in the skin and no drug infusion is contemplated. Sharma *et al*. (2006) developed a percutaneous CSF collection technique, but, unfortunately, the rats are asphyxiated with CO_2 before CSF extraction, making them useless for long term experiments. Also, Shapiro *et al.* (2012) performed a cisterna magna cannulation, making the rats useful for periods no longer than 2 weeks. There are many other devices published (Consiglio and Lucion, 2000; Cassar *et al*., 2010; Mahat *et al*., 2012), but as can be seen from above, all of them present some problems, successfully solved by the device presented in this study.

MATERIALS AND METHODS

Animals: For the experiments, we used 22 normal adult rats Sprague-Dawley weighing 250-300 g. The experiments were done in accordance with the Ethics Committee of the University of Santiago of Chile and the Ethical Guidelines for Research on Experimental Pain in Conscious Animals (Zimmermann, 1983). The rats were maintained in a closed chamber with light/dark cycles of 12/12 hours, starting at 8:00 AM., and a temperature of 25±1 ºC. Also, they were fed with food and water *ad libitum.* For CSF withdrawal, the rats were anesthetized with a brief isofluorane application (1.8% in 100 % oxygen).

CSF cell counting and protein measurement: CSF fluid was placed in a Mossbauer chamber and the red blood cells were counted under a microscope. Results were expressed as number of cells/µL. The CSF protein concentration was determined in accordance with the method described by Smith *et al*. (1985), and performed using the Pierce™ BCA Protein Assay Kit from Thermo Scientific.

Experimental groups: Two experimental groups were prepared. Rats for CSF withdrawal, consisting of a) rats for measurement of CSF volume withdrawal (n=4), b) rats for CSF blood cells counting (n=4) and c) rats for batch CSF collection over time (n=6). Rats in pharmacokinetic studies consisting of a) rats for fluorescein pharmacokinetics (n=4) and b) rats for minocycline pharmacokinetics (n=4).

Drug infusion and collection device: A device was tested for the infusion and collection of continuous or batches of CSF for which the details are given in Fig. 1. Fig.1A shows a scheme of the device. From left to right: the 100 µL Hamilton syringe whose needle is inserted into the T-shaped structure by means of a Luer adapter. This T-shaped structure comprises two interconnected needles. The second needle (25Gx1") is the one inserted into the rat cisterna magna. In the middle, a sample collection system formed by a glass vial with two polyethylene tubes of 0.58 mm internal diameter. One carries the CSF to an Eppendorf tube of 200 µL located inside the vial. The other goes into the vacuum system (right side) consisting of a 60 ml syringe positioned vertically, which hangs in its plunger a weight of 400 g to form a vacuum into the vial, allowing the CSF flow in a slow, smooth manner. In the case of performing drug infusion, the Hamilton syringe is used. Fig.1B shows the complete system. This consists of a stereotaxic system in which is positioned the head of the anesthetized rat ears secured by rods. Then the head is positioned downwards at an angle of 45° from horizontal, then inserting a needle into the cisterna magna using a descender device. The needle is positioned in the stereotaxic device exactly at the center of the rods. Fig.1C shows the Hamilton syringe connected to the T-shaped structure and also to the glass vial. Fig.1D shows the glass vial with two polyethylene tubes and inside, the Eppendorf tube. Fig.1E The vacuum system consisting of the 60 ml syringe, the 400 g weight and a three way valve that allows to select between vacuum, closed or to return the syringe to 0 ml.

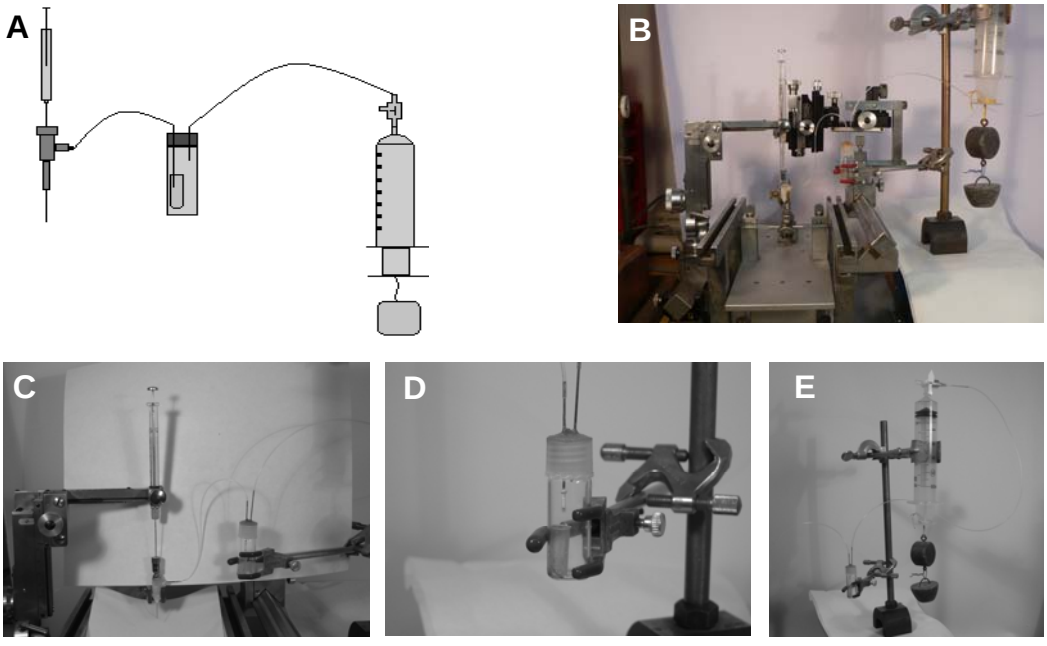

Fig 1: Drug infusion and collection device prepared.

RESULTS AND DISCUSSION

By the drug infusion and collection device, CSF withdrawal was 102.5±17 µL in tested rats (Table 1). Average RBC count and protein concentration in CSF withdrawal fluid were 179±9.4 cells/µL and 100±1.8 mg/dl, respectively (Table 2). Table 3 shows the withdrawal of CSF of 6 rats on a weekly basis. It can be seen that only one rat did not survive the second week CSF withdrawal (marked with an X), constituting a 75% of survival. With the purpose of testing this device, normal rats were injected with 500 µL i.t. fluorescein 2% w/v in serum. Then, samples of 20 µL CSF were withdrawn from the rats (n= 4) at 15 minutes intervals and measured with a fluorimeter (Biotek, Synergy HT). Values (mean±SD) were then converted to ng/ml by means of a standard curve whose r^2 was equal to 0.998 (Fig. 2). Minocycline (1 mg/ml) was infused into the CSF by means of the Hamilton syringe. Then, samples of CSF (20 µL) were withdrawn from the rats (n= 4) at time zero, one, two, four and 18 hours after the minocycline infusion (mean±SD) (Fig. 3).

A simple and reliable device has been developed for the safe CSF extraction that does not involve an invasive procedure (except, a needle insertion) and can be performed in around five to ten minutes after the anesthetized rat is positioned in the stereotaxic apparatus.

The purity (i.e. blood contamination) of the CSF collection is crucial to obtain (Witwer *et al*., 2013), since the amount of total CSF in the rat approaches around 400-550 µL (Frankmann, 1986), and the amount withdrawn was around 103 µL. A minimal amount of contamination with blood will seriously alter the CSF composition (Lardinois *et al*., 2014). For example, in one of our CSF samples, a barely visible reddish color increased the protein composition in around 25%. The knowledge of the CSF compartment composition is crucial for drug concentration determinations, mainly those injected intravenous or intra- peritoneally (Nau *et al*., 2010). In our case, the fluorescein infused intra-peritoneally was possible to be quantified in the CSF. Other ways of administration are also possible (Kawai *et al*., 2012). Also, substances infused into the CSF are possible to be traced with this system. We selected minocycline for our kinetic assay because of the importance of their properties, not only because their antibiotic effect but the presence of anti-inflammatory (Zychowska *et al*., 2013), anti-apoptotic (Levkovitch-Verbin *et al*., 2014), neuroprotective (Rojewska *et al*., 2014), anti-ischemic stroke (Fagan *et al*., 2011) effects. Minocycline concentration was detected even after 18 hours without problems. One of the main advantages of this system is precisely the possibility of injecting large amounts of substances into the CSF compartment (Misra *et al*., 2003). The reason why we developed this apparatus was the possibility of injecting nanoparticles loaded with DNA (Yurek *et al*., 2015) or antiarthritic drugs. The idea behind this was to withdraw a large volume of CSF and then infuse the same amount of nanoparticles. This procedure will not alter (except may be transient) the CSF volume, avoiding high pressure developments in the brain.

Table 1: Volume (mean±SD) of CSF withdrawal by the device tested

Rat number	CSF withdrawn (µL)
1	100
2	120
3	80
4	110
Mean CSF withdrawal	102.5±17

Table 2: CSF blood cells counting (mean±SD) and protein determination

Rat number	RBC count (cells/µL)	Protein Concentration (mg/dL)
1	192	101
2	170	98
3	180	102
4	175	99
Mean	179±9.4	100±1.8

Table 3: Batch CSF collection over time

Rat	Week 1	Week 2	Week 3	Week 4
1	√	X		
2	√	-	√	√
3	√	√	√	√
4	√	√	-	√
5	√	√	√	√
6	√	-	√	√

The "–" indicates that the CSF withdrawal was not performed in that particular week.

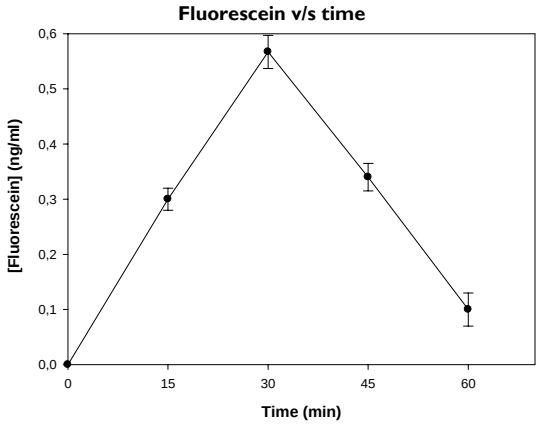

Fig. 2: Pharmacokinetics of intravenously injected fluorescein

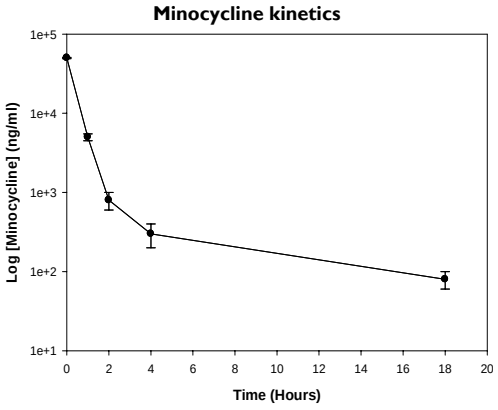

Fig. 3: Pharmacokinetics of infused intra CSF minocycline

Conclusion: We have developed a system for CSF withdrawal and infusion of drugs. The main advantage is to be almost completely noninvasive, with only one brief needle puncture. Also, this procedure permits the reutilization of the rats, since they recover completely at the following day.

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