The potential use of N-octanoyl-dopamine (NOD) in organ transplantation
Wedel, Johannes

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Simultaneous subcutaneous implantation of two osmotic minipumps connected to a jugular vein catheter in the rat

Johannes Wedel
Michel Weij
Annemieke Smit-van Oosten
Jan-Luuk Hillebrands

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Abstract

Subcutaneous osmotic pump implantation connected to a venous catheter is a well-established method to deliver compounds intravenously for intermediate duration (~2 weeks). When prolonged release is desired (~4 weeks) reduced flow rate is needed with similar pump volume. With a fixed intra-pump compound-concentration, reduced flow rate results in unwanted reduced bioavailability of the compound. Prolonged intravenous delivery would therefore need pump replacement resulting in increased discomfort and confounding effects on experimental outcome.

To overcome this, we here describe a method to double compound infusion rate for 4 weeks by implanting two low-flow rate osmotic pumps (2.5 µl/h for 28 days) connected to a jugular vein catheter in a single rat. Rats implanted with a single high-flow rate pump (5 µl/h for 14 days) served as control.

Double-pump implanted rats displayed similar post-operative weight gain and physical activity indicating similar levels of discomfort when compared with single-pump implanted rats. Double-pump implanted rats had an increased risk for pump-related complications (4 delivery failures [double pump] vs. 1 delivery failure [single pump]).

Our data show that double-pump implantation is a feasible alternative to changing pumps or extracorporeal pump systems connected via a long wire to partly restrained animal.
Introduction
In experimental animal research intravenous administration of therapeutic agents for prolonged duration, especially fast-degradable compounds with a low bioavailability and unknown pharmacokinetics or –dynamics, is often desired. To this end, intravenous catheter systems connected to an external pump have been developed. This method requires restraining the animal. Tight restrain is however unwanted as it causes stress and harms the animal, making it unethical [1]. The use of long tubes partly overcomes these disadvantages and allows relative freedom of movement to the rats [2,3]. Common complications are bitten through or twisted tubes causing blockade of continuous infusion or fatal bleeding. Another technique is the use of a subcutaneously implanted osmotic minipump connected to a venous catheter [4]. Osmotic minipumps with a fixed reservoir volume can have different duration rates of infusion implying that increasing delivery duration can only be established by lowering the flow-rate. In principle this could be overcome by increasing the concentration of compound, however some compounds do not dissolve at higher concentrations. Prolonged infusion can be achieved by replacement of fast-flow rate pumps during the experiment. Although implantation of osmotic minipumps is generally considered a mild surgical procedure, it definitely impacts on animal welfare, especially when combined with another major surgical intervention. Therefore, pump exchange will result in additional discomfort and certainly compromise proper comparison with rats that did not undergo replacement. To circumvent this, we developed a method to simultaneously implant two long-lasting, low-flow rate pumps combined with another major surgical intervention (i.e. aortic transplantation). This method allows comparative kinetic studies (2 vs. 4 weeks infusion) by introducing only one variable with fixed pump reservoir compound-concentration and no need for pump exchange.
Materials and methods

Animals
Male Brown Norway rats (BN, n=32) weighing 230-270 gram were obtained from Janvier (St. Berthevin Cedex, France), and female Wistar rats (n=2 used for pilot implantations) weighing 280-310 gram were obtained from our own breeding colony. Rats were kept under clean conventional conditions and fed standard rat chow and water ad libitum. All experimental procedures were performed according to European Commission guidelines and Dutch laws and were approved by the animal ethics committee of the University of Groningen (DEC 6624B).

Catheter and osmotic minipumps
To achieve a pump delivery rate of 5 µl/h for 4 weeks, two 2ML4 pumps (2.5 µl/h for 28 days) from Alzet (DURECT Corporation, Cupertino, USA) were used. One day prior to pump implantation, pumps were filled with 5% Tween80 in saline with or without additional N-octanoyl-dopamine (NOD, 11.16 mg/ml), attached to a saline-filled jugular vein catheter (DURECT Corporation, Cupertino, USA), cut after 1.5 cm and connected to an in-house made Y-tube (Figure 1). Filled pumps were incubated in saline overnight at 37°C according to supplier’s instruction. NOD is our compound of interest but will not be further discussed as this is beyond the scope of this article. For comparison, conventional single pump implantation was performed using an Alzet 2ML2 pump (5 µl/h for 14 days) without using the Y-tube.

Figure 1: Osmotic minipumps, catheters and Y-tube ex vivo.
Two osmotic minipumps are connected to a Y-tube that is attached to a jugular vein catheter. For implantation, the jugular vein catheter is replaced, separately implanted and connected to the Y-tube in situ again.
Figure 2: Procedure of double-pump implantation.
A: Submandibular skin incision for jugular vein catheterization.
B: Jugular vein is exposed by the ligation of the cranial end of the dissected vein.
C: Vein is punctured by a bended G20 needle and jugular catheter is introduced into the vein.
D: Catheter is fixed by the caudal suture (*). Cranial suture is knotted around the catheter (#) and loose ends of the cranial and caudal sutures are fixed together crosswise.
E: The jugular catheter is tunneled to the neck incision and subcutaneous pockets for pump implantation are made.

Note that pictures were taken without sterile drapes for pedagogical purposes only; the use of sterile drapes is highly recommended for this kind of surgery.
Implantation procedure
Rats were anaesthetized with isoflurane and 0.01 mg/kg body weight buprenorphin and placed in a supine position. At first post-operative day, same dosage of buprenorphin was used as analgesia. Aortic transplantation (using Dark Agouti donors) was performed as described previously [5]. For jugular vein canulation, a 1 cm long incision at the right shoulder close to the base of the neck was made (Figure 2A). The jugular vein was bluntly dissected and cranial end was tightly ligated with a 5-0 non-absorbable suture, loose ends were fixed at the table to expose the vein (Figure 2B). A second suture was loosely placed at the caudal end. The vein was punctured with a bended G20 needle. Through the curved part of the needle the saline-filled catheter was introduced 3.8 cm into the vein (Figure 2C) that the catheter tip ends in the cranial cava vein just before the right atrium. The catheter was fixed by tightening the caudal suture. The cranial suture was unfixed and knotted around the catheter (Figure 2D). Intravascular access was checked by withdrawing blood and flushing with saline. The jugular vein catheter was tunneled subcutaneously to the dorsal side.

For implantation of the double-pump, the rat was placed in a prone position. A 2 cm long skin incision was made across the neck (Figure 2E). Subsequently, a subcutaneous pocket at each flank was made and pumps, connected to the Y-tube, were implanted. Finally the Y-tube was connected to the jugular vein catheter and skin incisions were closed. Figure 3 shows a schematic representation of the anatomical location of the implanted pumps connected to the jugular vein catheter.

Figure 3: Scheme of the implanted double-pump.
Results

2 Wistar rats (pilot study, without aorta transplantation) and 16 BN rats (with aorta transplantation) were implanted with a double-pump and sacrificed after 28 days. 16 single-pump implanted BN rats (with aorta transplantation) served as controls and were sacrificed after 14 days. In 16 out of 18 (89%) double-pump implanted rats (14 BN and 2 Wistar) and 15 out of 16 (94%) single-pump implanted rats (BN) the anticipated follow-up time of 28 days (double-pump) respectively 14 days (single-pump) was achieved. Two double-pump implanted rats had serous fluid accumulation around the pumps or in the pleura at day 13 post-implantation (Table 1). Fluid accumulation was not related to signs of infection, catheter leakage, disconnection or penetration of the vessel. However, infection cannot be excluded as no further analysis on effusion fluids was performed. One single pump implanted rat was found with an opened abdomen at day 1 post-implantation; a complication related to the transplantation procedure and not to pump implantation.

Of the remaining, pumps were removed at 14 days (15 rats; 15 catheter connections [single-pump]) or 28 days (16 rats, 80 catheter connections [double-pump]). Inspection at sacrifice revealed that in the single-pump group 6.7% (1 out of 15) of the connections were leaking, whereas in the double-pump group 2.5% (2 out of 80) were leaking and another 2.5% (2 out of 80) were disconnected from the pump (total failure rate: 5% [4 out of 80]). No significant differences in weight gain (Figure 4) or physical activity were detected between both groups within the first two weeks.

<table>
<thead>
<tr>
<th>Complications</th>
<th># of animals</th>
<th>Adapted risk°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leading to premature termination</td>
<td></td>
<td></td>
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<tr>
<td>Single-pump</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Open abdomen</td>
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<td></td>
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<tr>
<td>Double-pump</td>
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<td></td>
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<tr>
<td>Serothorax unknown genesis</td>
<td>n=1 (5.6%)</td>
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<tr>
<td>Serous fluid around pumps</td>
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<tr>
<td>Catheter-associated problems</td>
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<tr>
<td>Single-pump</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leakage at catheter</td>
<td>n=1 (6.7%)</td>
<td>1/15 (6.6%)</td>
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<tr>
<td>Double-pump</td>
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<td></td>
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<tr>
<td>Leakage at catheter</td>
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<td>4/80 (5%)</td>
</tr>
<tr>
<td>Catheter disconnected</td>
<td>n=2 (12.5%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: List of complications.°

°taking the increasing risk for disconnection and leakage at connection sites into account: double-pump: 5 connection sites (1 at each pump and 3 at the Y-tube); single-pump: one connection to the pump. Formula: (delivery failure) / ((connection sites) x (number of animals))
Discussion

Several methods have been described for continuous intravenous administration of compounds in unrestrained rats. To increase infusion rate over a longer period of time, we showed that simultaneous implantation of two slow-flow rate osmotic pumps connected to a jugular vein catheter is feasible and not linked to additional signs of discomfort compared to single-pump implanted rats.

Both lethal complications in the double-pump group manifested at day 13 post implantation. As all single-pump implanted animals were terminated at day 14 (except for one, pump unrelated), this indicates that premature loss was indeed associated with double- vs. single-pump implantation. Double-pump implantation appeared to be associated with a higher risk for pump-related complications leading to delivery failure or earlier termination of the animal. One should take into account that these rats have two catheters with 5 connection sites (1 at each pump and 3 at the Y-tube) instead of 1 catheter with 1 connection to the pump thereby increasing the risk that one of these connections fails. When calculated for the individual rat, adapted relative risk was comparable between both groups (6.6% [single-pump] vs. 5% [double-pump] [Table 1]). We believe delivery failure is related to the 5-fold higher number of connections in double-pump implanted rats, although effects of the longer follow-up time (28 vs. 14 days) cannot be excluded.

In conclusion, simultaneous double-pump implantation is a feasible method to achieve prolonged intravenous administration in unrestrained rats.

Figure 4: Weight curves of animals.
Acknowledgments
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References