

Technical Report: Technique of Bladder Catheterization in Female Mice and Rats for Intravesical Instillation in Models of Bladder Cancer

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Summary

Animal models offer a system that enables a better understanding of basic biological questions. Urinary bladder catheterization is a common procedure in models of female urothelial tumours and yet the technique does still need to be described further. The methods described in the existing literature do not outline how the procedure should be adapted for different research goals. In this report, we describe systematically catheterization of female mice and rats as well as analysing several anaesthetic protocols, which can be used to carry out this technical procedure.

Animal models offer a system that enables a better understanding of basic biological questions. With animal models, an adequate control of experimental design is possible and accurate experiments can be performed in order to test various hypotheses. Bladder cancer is one of the most prevalent forms of cancers in the western world (Franeikova *et al.*, 2007). In Europe, urothelial tumours are the fourth most frequently occurring cancer among men and the eighth among women (Stamouli *et al.*, 2004). Moreover, bladder cancer is likely to become more prevalent as the average age of the population rises (Pashos *et al.*, 2002). To induce the development of urothelial tumours or to test therapeutic drugs,

it is important to correctly select the model that is most analogous to the clinical setting. In this way research observations can be readily transferred to clinical studies for validation. There are many published papers describing several protocols that can be used to induce urothelial tumours in laboratory animals (Raghavan *et al.*, 1986; Crallan *et al.*, 2006; Oliveira *et al.*, 2006; Cohen *et al.*, 2007). Depending on the research aims, bladder tumours can either be chemically induced or transplanted. Tumours can be induced by oral administration (i.e. N-butyl-N-(4-hydroxybutyl) nitrosamine or N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide) or by the intravesical instillation (i.e. N-Methyl-N-nitrosourea) of chemical carcinogens (Cohen *et al.*, 1976; Fukushima *et al.*, 1981; Kunze & Gassner, 1996). Urothelial tumour cells, obtained using the *in vitro* exposure of animal cells to chemical carcinogens, can be administered in syngeneic models by intravesical instillation. However, in this case it is necessary to denude urothelial cells by the sequential applica-

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tion of hydrochloric acid and potassium hydroxide, using urinary bladder catheterization (*Ibrahiem et al.*, 1983.). If the experimental aim is to evaluate a drug's efficacy against human urothelial tumour cells, immunodeficient mice are frequently used in xenograft models. In these circumstances, human urothelial tumour cells can be instilled into the urinary bladder or administered via a subcutaneous route. In all these models, the therapeutic evaluation of anticancer drugs is commonplace. These drugs can be administered by intravesical instillation or by another route. When the tumour is chemically induced, it develops in the urinary bladder. In this particular case, intravesical drug instillation is necessary to evaluate a drug's efficacy in the tumour's natural environment. Intravesical instillations are only possible in females: anatomical differences render this technique impossible in males. Other alternative routes like intraperitoneal, subcutaneous, or gavage can be used to evaluate drug efficacy. Intravesical exposure to chemotherapy agents is easier to tolerate than systemic exposure, as the drug is only contained within the environment of the urothelium, therefore protecting the animals from systemic exposure. Systemic chemotherapy is also considered less effective than intravesical chemotherapy for the treatment of locally confined superficial bladder tumours. Urinary bladder catheterization is a common procedure in models of urothelial tumours and yet there is still a need to better describe the technique and to learn more about the mechanisms of action of the different anaesthetics available. Both these factors may profoundly affect experimental results. St. Clair & colleagues (1999) described the urinary bladder catheterization of female mice and rats and referred to an anaesthetic protocol using ketamine and xylazine. In this report, we systematically describe female mice and rat catheterization applied to rodent models of bladder cancer, and furthermore we also consider the different anaesthesia that can be used to perform the technical procedure. To perform bladder catheterization in rodents it is necessary to maintain females under anaesthesia to prevent distress, to carry out the technique safely

and to avoid animal pain. In intravesical treatment, micturition should be induced before catheterization, allowing a longer and better contact between the chemical compound (carcinogenic compound or anticancer drug) and urothelial cells. Consequently, it is necessary for the anaesthesia to inhibit micturition. Some anaesthetics such as pentobarbital sodium, propofol and isoflurane are more suitable because they have a substantial depressant effect on micturition (*Matsuura & Downie*, 2000). Sodium pentobarbital is an anaesthetic with low pain control, however it inhibits diuresis via the release of antidiuretic hormone from the pituitary gland (*Hall*, 2001). Therefore, pentobarbital sodium is a frequently used anaesthetic in this type of procedure. It has also been described that this compound has a narrow safety margin in small rodents and induces severe visceral pain after intraperitoneal administration. For this reason, some countries have forbidden the use of pentobarbital sodium as an anaesthetic drug (*Hellebrecken et al.*, 2001; *Richardson & Flecknell*, 2005). Propofol can be used to induce sedation and anaesthesia in rats and mice when administered intravenously. The duration of anaesthesia is short and recovery is rapid and smooth with minimal cardiac and respiratory depression (*Glowaski & Wetmore*, 1999). Furthermore, it can suppress micturition at sedative or light anaesthetic levels (*Matsuura & Downie*, 2000). Volatile anaesthesia with isoflurane is an alternative anaesthetic protocol that can be applied if specific equipment (anaesthetic machine, breathing systems and vaporizer) is available to perform this procedure. Isoflurane is a potent anaesthetic that produces rapid induction and recovery from anaesthesia and an easy control of the depth of anaesthesia; it can therefore provide safe and effective anaesthesia in all laboratory animal species (*Hellebreckers et al.*, 2001; *Matthews*, 2007). Isoflurane has also been shown to abolish the micturition reflex (*Matsuura & Downie*, 2000). Anaesthetic combinations using alpha-2-adrenergic agonist agents, such ketamine/medetomidine or ketamine/xylazine, are often used in rodents studies because they provide good sedation and analge-

sia, and can be reversed with a specific antagonist (atipamezole), thereby considerably reducing the duration of the recovery (Hellebrekers *et al.*, 2001; Murrel, 2007). However, this combination is contraindicated for carrying out intravesical instillation, as alpha-2 agonist increases urine production due to a reduction in vasopressin and renin secretion (Cabral *et al.*, 1997; Sinclair, 2003; Murrel, 2007). This diuretic effect consequently decreases contact between the instilled compound and urothelial cells. In our experiments with rodent models of bladder cancer we have used sodium pentobarbital, because in preliminary studies we observed that the combination of ketamine and medetomidine induces intense diuresis and epiphora.

To execute bladder catheterization we use standard sterilized endovenous catheters and surgical gloves. The catheter diameter is selected according to animal size; in female mice we use 24 Gauge catheters while in female rats 18 Gauge catheters are used. The diameter of the urinary catheter should be the maximum that can be inserted into the urethra. Procedures for urinary catheterization are similar for both female mice and rats. After beginning anaesthesia, the animal is positioned in dorsal recumbence, and before performing urethra catheterization micturition must be induced through mild caudal abdominal massage (Figure 1). When the bladder is empty, contact with urothelial cells is more intimate and the urine lubricates the urethra, helping catheterization. Then, animals are held by the base of the tail and hind limbs are clasped by index finger and thumb on both sides to better expose the urinary papillae (Figure 2). The endovenous catheter is held by the free hand as if it were a pen, while taking care to do not touch the tip of the catheter. The annular finger from the same hand is used to make a mild compression near the vulva to permit a better visualization of the external urinary meatus. The endovenous catheter is carefully introduced into the urethra, firstly perpendicular to the urethra (Figure 3) and latterly parallel to spinal cord (Figure 4). If correctly performed there should be no obstacles during catheter insertion, if some difficulties in insertion are felt then



Figure 1. Induced micturition by mild caudal abdominal massage in a female mouse.



Figure 2. Female rat contentment.



Figure 3. Endovenous catheter is introduced perpendicular to urinary meatus in a female rat.

the catheter must be removed to avoid injuring the urethra. Minimal resistance is usually noted during



Figure 4. Endovenous catheter is introduced parallel to spinal cord in a female rat.

catheterization of female mice. However, female rats may present moderate resistance, and in this situation we would apply, via the catheter, a few drops of lidocaine into the urethra. The chemical compound should now be administered into the urinary bladder and then generally the catheter is removed. Depending on the aims of the experiment, the substance may be retained in the urinary bladder lumen for a greater or lesser period of time. During this period animals must be frequently rotated in order to facilitate whole bladder exposure to the substance instilled. At the end, micturition should be again induced by soft abdominal compression to remove bladder content and to promote a constant time of contact. During anaesthesia the body temperature must be controlled. For this reason we wrap animals in a homeothermic bandage to prevent hypothermia. Some side effects may appear after urinary bladder catheterization such as irritation, haematuria and bladder inflammation. As in all invasive techniques, these unpleasant side effects may vary widely.

The influence of the technique used should never be underestimated, even when performing simple studies. During intravesical instillation, it is important to fully understand the anatomy and physiology of the animal model chosen as well as the limitations of animals and anaesthesia in this procedure. Females are commonly used due to the relative ease of performing bladder catheterization, as the urinary

orifice is external and slightly anterior to the vaginal opening.

References

- Cabral AM, KJ Varner & DR Kapusta:* Renal excretory responses produced by central administration of opioid agonists in ketamine and xylazine-anesthetized rats. *J. Pharmacol. Exp. Ther.*, 1997, 282, 609-616.
- Cohen SM, JB Jacobs, M Arai, S Johansson & GH Friedell:* Early lesions in experimental bladder cancer: experimental design and light microscopic findings. *Cancer Res.*, 1976, 36, 2508-2511.
- Cohen SM, T Ohnishi, NM Clarck, J He & LL Arnold:* Investigations of rodent urinary bladder carcinogenesis: collection processing, and evaluation of urine and bladders. *Toxicol Pathol.*, 2007, 35, 337-347.
- Crallan RA, NT Georgopoulos & J Southgate:* Experimental models of human bladder carcinogenesis. *Carcinogenesis.*, 2006, 27, 374-381.
- Franekova M, E Halasova, E Bukovska, J Luptak & D Dobrota:* Gene polymorphisms in bladder cancer. *Urol Oncol.*, 2007, 26, 1-8.
- Fukushima S, GH Friedell, JB Jacobs & SM Cohen:* Effect of L-tryptophan and sodium saccharin on urinary tract carcinogenesis initiated by N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide. *Cancer Res.*, 1981, 41, 3100-3103.
- Glowaski MM & LA Wetmore:* Propofol: Application in veterinary sedation and anesthesia. *Clin Tech Small Anim Pract.*, 1999, 14, 1-9.
- Hall LW, KW Clarke & CM Trim:* in *Veterinary anaesthesia*. WB Saunders, London, 2001, 10-11.
- Hellebrekers LJ, LH Booj & PA Flecknell:* Anaesthesia, analgesia and euthanasia. In: *Principles of laboratory animal science*. Elsevier, Amsterdam, 2001, 277-311.
- Ibrahiem EH, VN Nigam, CA Brailovsky, P Madarnas & M Elhilali:* Orthotopic implantation of primary N-[4-(5-Nitro-2-furyl)-2-thiazolyl]formamide-induced bladder cancer in bladder submucosa: an animal model for bladder cancer

- study. *Cancer Res.*, 1983, 43, 617-622.
- Kunze E & G Gassner*: Modification of N-methyl-N-nitrosourea-induced urinary bladder carcinogenesis in rats following stimulation of urothelial proliferation by a partial cystectomy. *J Cancer Res Clin Oncol.*, 1986, 112, 11-18.
- Matsuura S & JW Downie*: Effect of Anesthetics on Reflex Micturition in the Chronic Cannula-Implanted Rat. *Neurourol. Urodyn*, 2000, 19, 87-99.
- Matthews NS*: *Inhalant anaesthetics*. In: BSAVA manual of canine and feline anaesthesia and analgesia. BSAVA, London, 2007, 150-155.
- Murrel JC*: Premedication and sedation. In: BSAVA manual of canine and feline anaesthesia and analgesia. BSAVA, London, 2007, 120-132.
- Oliveira PA, A Colaco, LF De la Cruz P & C Lopes*: Experimental bladder carcinogenesis-rodent models. *Exp. Oncol.*, 2006, 28, 2-11.
- Raghavan D, F Debruyne, H Herr, D Jocham, T Kakizoe, E Okajima, A Sandberg. & I Tannock*: Experimental models of bladder cancer. A critical review. In: *Developments in Bladder cancer*: Alan R. New York: Liss, Inc., 1986, 171-208.
- Richardson, CA & PA Flecknell*: Anaesthesia and post-operative analgesia following experimental surgery in laboratory rodents: are we making progress? *ATLA.*, 2005, 33, 119-127.
- Sinclair MD*: A review of the physiological effects of α 2-agonists related to the clinical use of medetomidine in small animal practice. *Can Vet J.*, 2003, 44, 885-897.
- Stamouli MI, AD Panani, AD Ferti, C Petraki, RT Oliver, SA Raptis & BD Young*: Detection of genetic alterations in primary bladder carcinoma with dual-colour and multiplex fluorescence in situ hybridization. *Cancer Genet Cytogenet.*, 2004, 149, 107-13.
- St Clair MB, AL Sowers, JA Davis & LL Rhodes*: Urinary bladder catheterization of female mice and rats. *Contemp Top Lab Anim Sci.*, 1999, 38, 78-79.