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Chronic cannulation in the small intestine of feral pigeons (*Columba livia*) to assess bioavailability

J.G. Chediack[1,2,3], F.D. Cid[1,2,3], S.V. Fasulo[1,2], E. Caviedes-Vidal[1,2,3]

[1]Laboratory of Biology "Prof. E. Caviedes Codelia", Facultad de Ciencias Humanas, Universidad Nacional de San Luis, San Luis, Argentina
[2]Laboratory of Integrative Biology, Institute for Multidisciplinary Research in Biology (IMIBIO-SL), Consejo Nacional de Investigaciones Científicas y Técnicas, San Luis, Argentina
[3]Dept. Biochemistry and Biological Sciences, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, San Luis, Argentina

ABSTRACT: We improved a method of chronic duodenal cannulation to study intestinal transport of solutes in an *in vivo* model (pigeon, *Columba livia*). A hypoallergenic cannula was inserted into the proximal part of the small intestine of pigeons and used for solution administration. Recovery from surgery was extremely rapid and animals started eating and drinking within a day. After surgery, the body mass of cannulated pigeons was stable, and no adverse effects in the weight could be detected. The method is simple, economical and useful to determine intestinal bioavailability of solutes, for nutritional and ecological studies, in intact animals without influence of anesthesia.

Keywords: chronic cannulation; small intestine; pigeons; bioavailability

The study of intestinal absorption is important for research in areas as diverse as clinical medicine, nutrition and ecotoxicology. In the first case, several studies have evaluated the passive permeability characteristics of the small intestine using a series of nonelectrolyte hydrosoluble probes, and they are routinely used in oral absorption studies to test for intestinal pathologies (Elia et al., 1987; Bjarnason et al., 1995). In the second and third cases, intestinal absorption is relevant for the different pathways of nutrient absorption and their regulation by different factors, because intestinal permeability is a major determinant of the oral bioavailability of hydrosoluble nutrients and toxins (Caviedes-Vidal, 2003; Chediack et al., 2003; Cid et al., 2005).

Many techniques have been developed for *in vitro*, *in situ* and *in vivo* studies of intestinal absorption processes. To understand the absorption process of a compound, a proper method has to be chosen. The *in vitro* methods to study intestinal absorption are: cell lines (e.g. Caco-2, HT-29 and MDKC), everted sleeves, brush border membrane vesicles and Ussing chambers. The *in situ* method involves perfusion of an intestinal segment, followed by recirculation of the substrate through the intestinal segment and the determination of the amount of substrate remaining in the solution (Roig and Vinardell, 1990; Perez et al., 1993). There are two main advantages to measuring absorption in the intact animal (*in vivo*) over in anesthetized animals (*in situ*) or by tissue preparations. First, while the latter methods can demonstrate conditions and mechanisms by which a process can occur, they cannot by themselves demonstrate the mechanisms by which it normally happens. Second, there is evidence that approaches not relying on intact animals may introduce artifacts in intestinal absorption (Uhing and Kimura, 1995).

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In this study we propose the use, for the first time, of another technique to measure *in vivo* intestinal bioavailability, the needle catheter duodenostomy. The duodenostomy is a surgical procedure where a feeding tube is inserted into the duodenum. In birds, it is a viable method of alimentation (Goring et al., 1986). We suggest the exploitation of this technique, developed in the beginning for duodenal alimentation, to assess bioavailability by pharmacokinetic techniques. The classical pharmacokinetic technique involves feeding (by gavage) and injecting probes and then measuring those probes in the blood at various times post-gavage or post-injection (Chediack et al., 2001). Studies of intestinal transport have historically been based on a variety of *in vitro* and *in vivo* methods, which in turn allows the assembling of a picture of how the intestine absorbs solutes (Karasov and Hume, 1997).

Pigeons are a good experimental model for studies of avian physiology *in vivo*. Some reasons for this are, (i) its cosmopolitan distribution, (ii) ease of capture and (iii) laboratory maintenance. Methodologically they are also a good subject because they allow intestinal cannulation for dosage administration, as we describe here, as well as easy serial blood sampling for pharmacokinetics studies (Chediack et al., 2001).

Our main objective was to adjust and improve a needle catheter duodenostomy technique, to study intestinal bioavailability of compounds by pharmacokinetic techniques.

MATERIAL AND METHODS

Animal care and housing

Adult Feral Pigeons (*Columba livia*; Linneo, 1758) were captured with a live trap near the Universidad Nacional de San Luis Campus (San Luis, Argentina). The birds were housed individually in cages indoors under relatively constant environmental conditions (25.2 ± 0.3°C, relative humidity of 50 ± 9%) on a photoperiod of 12:12 hours (Light: Dark) with water and food *ad libitum* (normal food for breeding broilers, Cargill). We used a mix of a broad spectrum anthelminitc medication to treat internal parasites (Nevugon-Bayer 0.03% and Piperazine 1.5 g/l of water) and externally with Nevugon-Bayer 0.3%. Animals were acclimated to laboratory conditions prior to use in experiments. Animal care and trial protocols followed the guidelines of the Universidad Nacional de San Luis.

Preparation of the cannulas

Cannulas were made with a nasogastric catheter (PVC/silicone composition), a common hypoallergenic material used in surgery and suitable for chronic use. The nasogastric catheter was cut at a length of 45 mm. The barrel of the cannula was 25 mm long, the outer and inner diameters were 2 and 1.4 mm, the extremity was rounded and a little support consisting of the same material was added. We added a support made of PVC/silicone to fix in the abdominal muscle, and we adapted an *i.v.* catheter (FEP polymer catheter 20G) inside the cannula to avoid obstruction by intestinal fluid (Figure 1).

Surgical procedures

Before cannulation surgery, the pigeons were acclimated over 12–13 days in an animal room. The animals fasted 18 h before the cannulation surgery (enough time to empty the crop). Anesthesia was induced with ketamine (40 mg/kg) and xylacine (10 mg/kg) diluted in physiological saline solution and administered through an injection in the pectoral muscle (Paul-Murphy and Fialkowski, 2001). The bird was placed in an operating table and the body fixed with thread. The operation zone was dis-

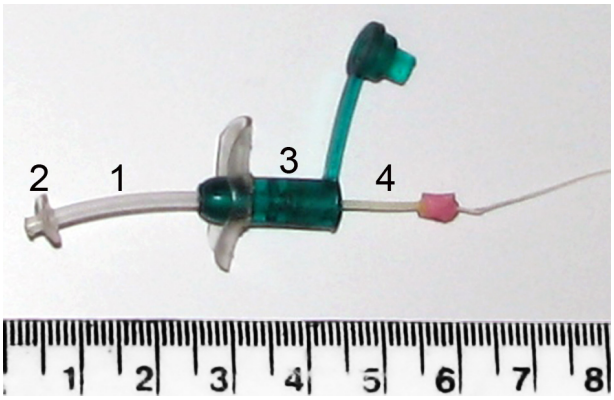

Figure 1. Cannula used for intraduodenal cannulation. The nasogastric catheter was cut at a length of 45 mm. The barrel of the cannula was 25 mm long (1), the extremity was rounded and a little support consisting of the same material was added (2). We add an additional support (3) to fix in the abdominal muscle. Finally, we adapted an *i.v.* catheter inside the cannula (4)

infected with chloroxylenol and the feathers were removed with vaseline. The abdomen was open longitudinally with a surgical knife, between the xiphisternum and the pubic bones. To access the intestinal cavity, a longitudinal incision was done (~ 3 cm), and the duodenum was exteriorized. The cannula (PVC/silicone catheter) was inserted in a small incision and fixed with thread to the intestine for one of the supports, and afterwards fixed to the abdominal muscle with another support (Figure 2). Finally, the skin was sewed.

Birds were moved to a recovery room outside the surgery room, and observed for three to four hours after the surgery. The birds were fully awake six hours after surgery. Drinking water was offered immediately and food a day after surgery. The bioavailability experiments were performed one week after surgery.

Material and reagents

Catheter K33 (Koler S-20. Argentina), FEP polymer catheter 20G (Becton, Dickinson. Brazil). L-rhamnose ($C_6H_{12}O_5$, MW = 164.2), $ZnSO_4$, acetonitrile and derivatization reagents were purchased from Anedra S.A. (Buenos Aires, Argentina) and Sigma Chemical Co. (St. Louis, MO, USA). Ketamine (Holliday-scott S.A.) and xylacine (Köning S.A.).

Pharmacokinetic method to assay bioavailability

To measure absorption we used a pharmacokinetic technique which involves feeding (by intraduodenal administration) and injecting probes and

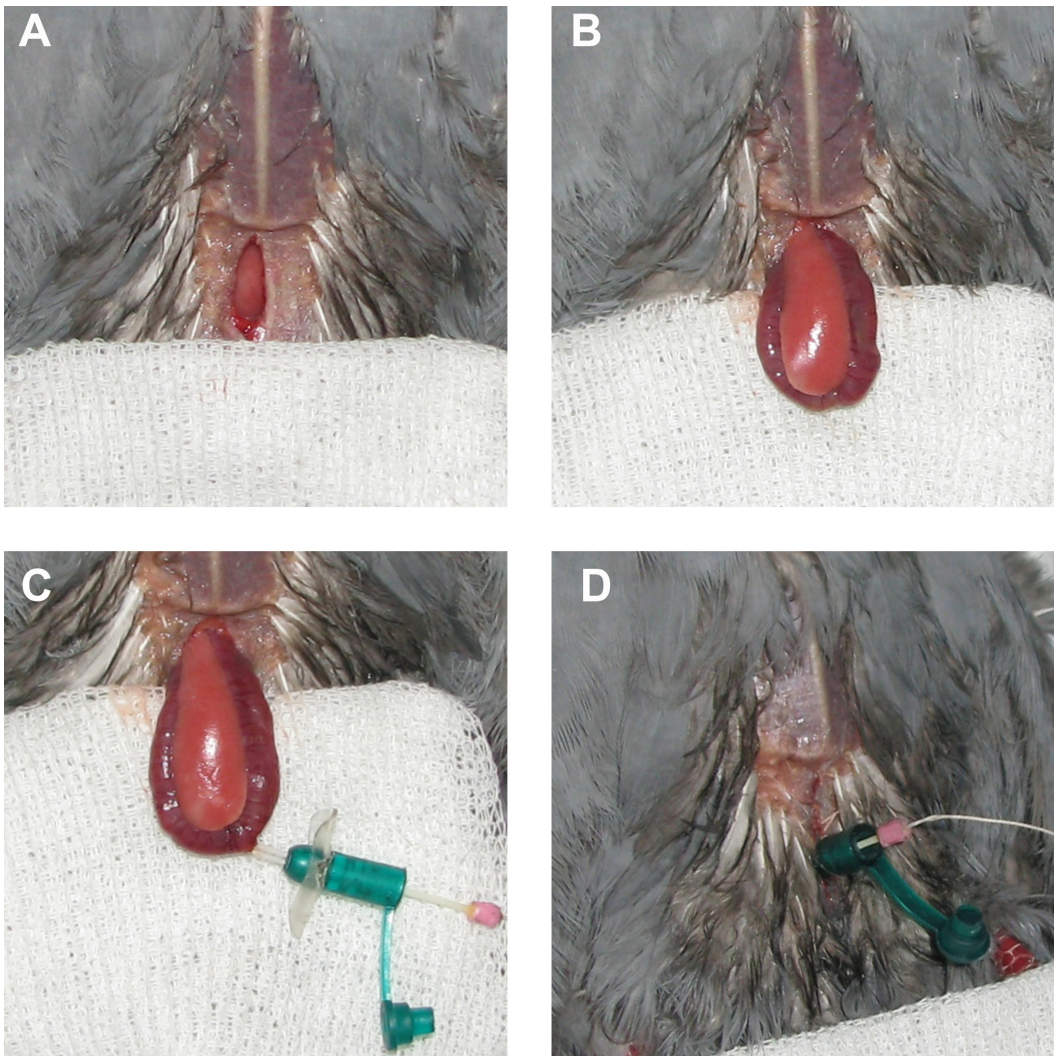

Figure 2. Steps of the surgical procedure. Abdominal incision between the xiphisternum and the pubic bones (A), the small intestine is exteriorized (B), the cannula is inserted and fixed to the intestine (C), and the duodenal cannula is in position to perform experiments (D)

then measuring those probes in the blood at various times post-gavage or post-injection (Chediack et al., 2001). Birds were placed in observation cages and iso-osmotic solutions of L-rhamnose were administered intraduodenally after 18 h of fasting. At this time the animal doesn't have any nutrients in the crop and/or stomach. The volume administrated was 3 ml and the administration was completed within one minute.

Blood samples (120 µl) were collected from the wing veins with a heparinized capillary tube. Ten to twelve blood samples (1.2–1.5 ml total, which accounts for < 10% of total blood volume were collected (Stangel, 1986). The samples were centrifuged for 3 min at 10 000 rpm in a hematocrit centrifuge and plasma was separated and stored at 0°C for analysis. Plasma proteins were precipitated using 10% $ZnSO_4$ and acetonitrile (Lam and Malikin, 1989). The rhamnose was derivatized for high performance liquid chromatography (HPLC) fluorescence detection by reductive amination with anthranilic acid (Du and Anumula, 1998). The HPLC system consisted of a Beckman automated binary system with a pump (model 126), an autosampler (model 507) and an interface (Model 406). Derivatives of carbohydrate probes in samples and standard solutions were detected with the Gilson 121 fluorescence Detector.

Experiments

Experiment 1. To test the efficiency of the cannulation surgery procedure, we used ten pigeons weighing from 260g to 320g, five were cannulated and the other five were treated as controls (without cannulation). Each pigeon was housed in an individual cage following a random procedure. The body mass of each pigeon was registered in the morning (08:00–09:00 h) at different intervals of time (see Figure 3). Additionally, the surgery site of each pigeons was carefully examined and the correct operation of each cannula was tested.

Experiment 2. To test the applicability of this model in the intestinal absorption research, four pigeons were cannulated and rhamnose (a passive absorption probe) bioavailability was measured following the pharmacokinetic method described above.

Statistical analysis

The Shapiro-Wilk W-test was used to test for normality and the Levene Test was used to test for homogeneity of variances. Analysis of variance (ANOVA) was used to examine the effect on pigeons with and without surgery. The significance level was set at $P < 0.05$.

RESULTS AND DISCUSSION

The body mass of pigeons was stable after two weeks of acclimation in the animal room (Figure 3). The cannulation surgery was performed at day 12–13 after arrival of the pigeons at the animal

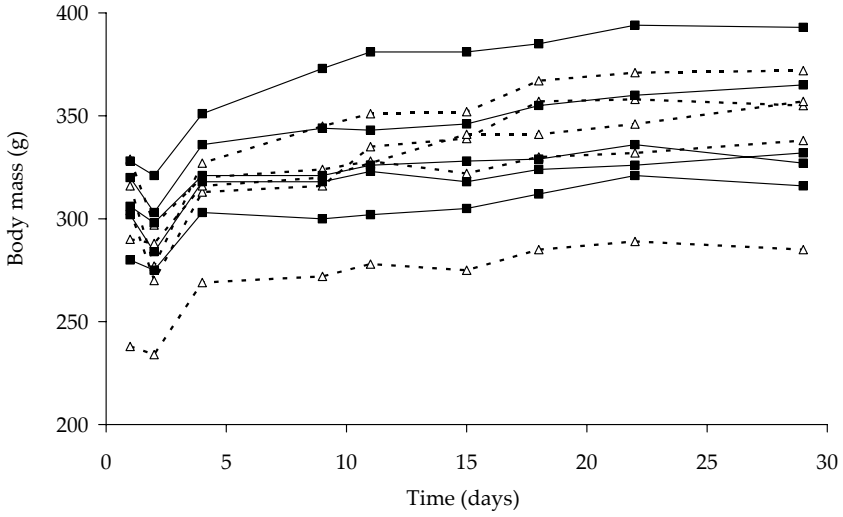

Figure 3. Stability of body mass in pigeons in the animal room. Filled square and solid line: cannulated pigeons. Empty-triangle and dashed line: control, non-cannulated pigeons

room, and was successful in 100% of cases. Recovery from surgery was rapid and animals consumed water within a day, and food the next day. After the surgery, the body mass of cannulated pigeons continued to be stable until the end of experiments (Figure 3), and no significant differences could be detected between cannulated and non-cannulated animals (ANOVA $P > 0.25$). In the same way, Goring et al. (1986), found no adverse effects in the weight of pigeons fed by needle catheter duodenostomy versus control animals.

Efficiency of cannulation

We tried several types of catheter with different flexibility: a 100% silicone catheter, FEP polymer catheter and a mix of silicone/PVC catheter. The most flexible cannula (silicone 100%) was better than rigid ones (FEP polymer and mix of PVC-silicone). A 100% silicone cannula stayed in the intestine for around two months, while the plastic catheter remained for about 3–4 weeks and a mix of silicone/PVC continued to work properly for at least one month. After this time the cannula was expelled by peristalsis without any harmful effect for the animal. Moreover, pigeons did not show any problem with regard to infection and cicatrisation. Three weeks after the expelling of the cannula, the condition of the intestinal and muscular tissue was normal, and we could make a new re-cannulation in the same site. Likewise, another study using a radiographic technique has reported that the duodenum of a pigeon appeared normal four weeks after catheter removal (Goring et al., 1986).

We decided to perform the two experiments using a PVC-silicone catheter, because both of its stability over time its economic convenience.

Application of the model in absorption experiments

The technique developed for surgery and chronic studies has permitted us to obtain consistent and reproducible results in the same animal for different absorption experiments. We used the cannula for infusion of 3 ml of an iso-osmotic solution with L-rhamnose as an intestinal permeability marker. The administration was performed in < 1 min without anesthesia. Then, we took several blood samples to analyze the absorption profile (Figure 4). The absorption profile of L-rhamnose determined in this study was in agreement with the results previously reported by other authors in pigeons (Lavin et al., 2007).

Implications

The method described herein is simple, economically attractive and useful for nutritional and ecological studies of the absorption of nutrients in the intestine *in vivo*. By using this cannulation method

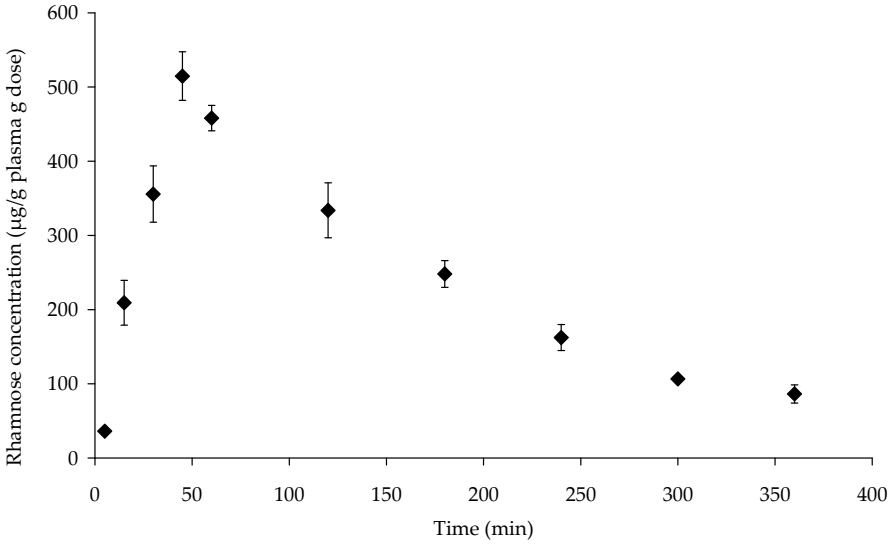

Figure 4. Absorption profile of L-rhamnose after intestinal administration of 3 ml of an iso-osmotic solution in cannulated pigeons. The data points are means + S.E.M. (n = 4 pigeons)

in combination with intraduodenal administration and intramuscular injection of compounds, it is possible to analyze the intestinal bioavailability in an *in vivo* model. In addition, it allows minimal use of anesthesia, which has previously been shown to be a problem in these kinds of studies (Uhing and Kimura, 1995).

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Corresponding Author:

Juan Gabriel Chediack, Dept. Biochemistry and Biological Sciences, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Chacabuco 917, 5700 San Luis, Argentina
Tel. +54 2652 423789 int.129, Fax +54 2652 430224, E-mail: jg.chediack@gmail.com