Simple Cannulation Procedure for Serial Blood Sampling Through Cutaneous Ulnar Vein in Chickens

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Simple Cannulation Procedure for Serial Blood Sampling Through Cutaneous Ulnar Vein in Chickens

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The objective of the study was to collect repeated, low-stress blood samples from the ulnar vein of chickens required for pharmacokinetic studies or hormonal assays. The study used 5 apparently healthy, unsexed, commercial broiler chickens about 6 weeks old and weighing 1.7–1.9 kg for serial sampling of blood. The study prepared the birds prior to cannulation and penetrated the catheter through the skin and into the lumen of the ulnar vein. The study successfully carried out serial blood samplings in 4 of 5 cannulated birds. Heparin (10%) solution maintained patency and prevented blood clot formation inside the cannula. However, the study found repeated clotting occurring in 1 bird. Cannula failed to maintain patency; the study could not carry out blood sampling properly, which was attributed to air embolism that might have occurred during catheter manipulation or repeated filling of cannula with heparin solution. The study observed no hematoma or inflammation at the site of cannulation. Owing to the advantages and to facilitate compliance with nonhuman animal welfare, this technique seems simple and efficient, allowing adoption for serial blood collection in chickens.

Chickens are presently being used in the areas of biomedical research, namely, fundamental biological and medical research, development of antibodies for...
diseases, pharmacokinetic and safety evaluation of chemicals, and drugs. Pharmacokinetic studies require serial blood sampling to understand the kinetic profile of a drug and to establish dosage regimens in any species. However, collection of serial blood samples from poultry is a tedious task.

Hematoma formation at the site of blood collection is a common phenomenon (Campbell, 1994) observed following blood collection in chickens that forces researchers to look for alternate avenues for serial blood sampling. One of the alternatives that can be followed for blood sampling is the serial sacrificing of birds at various intervals. However, issues pertaining to the welfare of nonhuman animals disallow such inhumane practice to be put into use. The technique to obtain recurrent, low-stress blood samples from birds for pharmacokinetic studies and hormonal assays is very much in need. Further, employing such a technique in animals not only reduces the number necessary to acquire such information but also facilitates compliance with the welfare of animals “4Rs” (replacement, reduction, refinement, and rehabilitation; Shetty & Sureshchandra, 2007). To this end, a simple surgical technique has been described to collect serial blood samples through the cutaneous ulnar vein in chickens.

MATERIALS AND METHODS

Birds

Five apparently healthy, unsexed, commercial broiler chickens (Cobb strain) about 6 weeks old with body weight in the range of 1.7 to 1.9 kg were procured from a commercial poultry farm. The birds were caged individually in experimental houses for nonhuman animals and maintained under standard laboratory housing conditions. All birds were provided with ad libitum commercial poultry feed and water throughout the experimental period. They were acclimatized to the laboratory housing condition for a period of 5 days. The experimental trials were approved by the Institutional Animal Ethics Committee. The experimental procedure met the National Guidelines as per recommendation of the Committee for Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India.

Catheter

Introcan®-W, Germany—with dimensions of 24G, $\frac{3}{4}$" (0.7 × 19 mm), flow rate of 22 ml/min, and flaplike extensions on either side of the cannula with a pore at periphery on each flap—was used in this study. To avoid occurrence of blood clots in the lumen of the cannula, it was flushed with heparin solution (10%) and air dried prior to inserting in to the vein (Figure 1).
Heparin Injection

A 10% heparin solution (heparin sodium 1,000 IU/ml [HEP 5®️, Gland Pharma Ltd, Hyderabad, India]) was prepared using double-distilled water.

Preparation of the Area

Using clipping scissors (Figure 2), feathers on the ventral aspect of each bird’s right wing were clipped. The cutaneous ulnar vein was clearly made visible by wiping the area of the vein with the cotton swab soaked in 70% ethyl alcohol (Figure 3). Lignocaine gel (lidocaine HCL) was applied topically at the site of cannulation and immediate adjacent area to mitigate pain in birds during the cannulation process. The vein was dilated and further stabilized by applying digital pressure for insertion of the catheter.

Cannulation Procedure

The catheter (stylet with the cannula) was penetrated through the skin and farther into the lumen of the vein (Figure 4). Successful insertion into the lumen of the vein was indicated by oozing of blood in the distal part of the stylet (Figure 5).
FIGURE 2  Preparation of area for cannulation (color figure available online).

FIGURE 3  Clearing and exposing the cutaneous ulnar vein (color figure available online).
FIGURE 4  Insertion of catheter into cutaneous ulnar vein (color figure available online).

FIGURE 5  Oozing of blood from cannula (color figure available online).
The stylet was slowly withdrawn from the cannula, and the depth of the cannula was adjusted by carefully pushing farther into the lumen of the vein (Figure 6). The patency of the cannula was confirmed by drawing blood from the vein using a 2 ml Disopovan syringe (Figure 7). The cannula was closed with the stopper and was fixed to the skin in situ by suturing it on both the sides using Ethilon®, a nonabsorbable surgical suture material (Monofilament Polyamide Black, 3-0 [Ethicon, Jhonson & Jhonson Ltd, Himachal Pradesh, India]; Figure 8).

Each individual cage provided enough freedom for movement. All the birds were given ad libitum feed and water.

Serial Blood Sample Collections

Blood samples were drawn at an interval of 0, 10, 20, and 30 min and 2, 4, 6, 8, 10, 12, and 24 hr. At each time interval, approximately 1 ml of blood was collected. During serial blood sampling, immediately after blood collection, the lumen of the cannula was filled with 50 μl of 10% heparin solution. An insulin syringe was used; in addition, the stopper of the cannula was rinsed in heparin solution before it was plugged back. This was done to avoid formation of a
FIGURE 7  Ascertaining patency of the cannula by drawing blood (color figure available online).

FIGURE 8  Cannula fixed to the skin in situ by suturing it on both sides (color figure available online).
blood clot at the tip of the cannula, situated inside the lumen of the vein and at the distal part of the cannula, which is in proximity with the stopper.

RESULTS

Serial blood samplings were successfully carried out in 4 out of 5 cannulated birds. Because repeated clotting occurred in 1 bird, the cannula failed to maintain patency and blood sampling could not be done properly. The cannula fixed in the lumen of the cutaneous ulnar vein of all the birds remained in situ up to 72 hr. Even after repeated blood samplings, formation of hematoma, visible inflammation at the site of cannulation, and incidence of self-mutilation at the cannulation site were not observed.

DISCUSSION

Cannulation avoids repeated puncturing of veins and reduces stress to the birds induced while they are restrained for serial blood sampling. Insertion of the cannula into the vascular system for serial blood sampling has been widely used for physiological and pharmacological studies in rats (deJong & Raaij, 2001), turkeys (Liu & Bacon, 2002), and in broilers (Liu & Bacon, 2004). Cannulations via jugular vein were tried in laying turkeys and broiler birds and were successful in collecting serial blood samples to evaluate patterns of hormones associated with spontaneous ovulation and oviposition (Bacon & Kirby, 2002; Liu & Bacon, 2004).

To carry out various physiological and pharmacological studies, repeated puncturing of the ulnar vein in birds for serial blood sampling at different time intervals has been carried out by various researchers. However, this technique may lead to injury and hematoma formation at the site of blood collection (Campbell, 1994). An alternative to this technique is to use a large number of birds to establish a pharmacokinetic profile of a drug by serially sacrificing birds at various time points. In either case, birds are nonetheless subjected to an enormous amount of stress and increased chances of injury and inflammation. However, in the absence of an available refined technique, issues pertaining to ethics and animal welfare do not allow the researcher to use a large number of birds when minimizing the number of birds can establish a similar pharmacokinetic profile.

While fixing the cannula in situ, care should be taken during suturing to avoid the needle’s puncturing the artery; otherwise, hematoma formation may occur.

The primary cause of failure of serial blood sampling reported by earlier workers is due to blood clot formation at the tip of the cannula or bending
of the cannula and formation of a kink. Heparin is a glycosylated, single-chain polypeptide and functions as antithrombin (Majerus & Tollefsen, 2001). Coating or filling of the cannula with heparin improves patency of cannula for a longer duration and thereby prevents clot formation either at the tip or in the lumen of the cannula (Foley & Brausa, 2002). Hence, in the present study, filling of the cannula with 10% heparin solution after each point of blood collection prevented formation of blood clots and assisted in maintaining the patency of the cannula.

A secondary cause of failure of cannulation has been ascribed to self-mutilation by physically damaging cannula by pecking or scratching at the site of cannula insertion (Liu & Bacon, 2001). However, the incidence of self-mutilation was not observed in any of the birds cannulated in the present study.

CONCLUSION

In one of the birds, blood sampling could not be done successfully due to an air embolism, which might have occurred during catheter manipulation or repeated filling of cannula with heparin solution. Venous air embolism, the entry of gas into the peripheral or central vasculature, takes places secondary to iatrogenic complications; this may be due to peripheral intravenous or central venous catheters, pulmonary artery catheters, and hemodialysis catheters (Anonymous, 1993).

The concept of 3Rs (replacement, reduction, and refinement) was first mentioned by Russell and Burch (1959). The word refinement refers to techniques that reduce the pain and distress to an animal. Considering the aforementioned premise, in the present study, catheterization of the ulnar vein was deemed to be a refined technique that avoids repeated puncturing of the vein for blood sampling and thereby minimizes undue stress and pain to birds.

It was concluded that, owing to the advantages and to facilitate compliance with the welfare of animals, this technique was considered relatively simple and efficient; hence it can be pursued for serial blood collection in chickens.

REFERENCES


