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BRIEF COMMUNICATION

Chronic Intravenous Dosing and Blood Collection in the Unanesthetized Monkey[1]

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BEDFORD, J. A., A. H. KIBBE AND M. C. WILSON. *Chronic intravenous dosing and blood collection in the unanesthetized monkey.* PHARMAC. BIOCHEM. BEHAV. 6(5) 601–602, 1977. – A procedure is described which allows chronic dosing and blood collection from awake monkeys. The procedure involves only minor surgical anesthesia and allows the same vein of the animal to be used several different times with home-cage recuperation periods intervening between sequential chronic catheterizations.

Chronic intravenous dosing Blood collection

FOR the past two years, this laboratory has been engaged in a program of research which has often involved the necessity of chronically administering drugs intravenously to monkeys. In addition, our designs have often called for serial blood sampling at specific intervals postinfusion. Chronic administration of drugs by the intravenous route has been observed to produce numerous problems. These are: (1) swelling around the vein making subsequent injection difficult, (2) difficulty in accurately hitting the vein, (3) venous collapse, and (4) experimental contamination by stress factors as a result of physically restraining the animal.

Chronic blood sampling also presents several problems. Many analytical procedures require at least one ml sample volumes and most analytical chemists would rather have 2–3 ml samples so that they can obtain more than one analytical determination per sample. Attempts to obtain samples of this size utilizing standard butterfly infusion sets were found to be completely unsatisfactory. It has been our experience that one is rarely able to obtain more than two samples on the order of 2–3 ml before the vein collapses. In addition, the stress factor is also involved with this technique, in that the animal must be restrained while the butterfly is inserted. Femoral puncture procedures will allow the collection of samples of this size; however, because of the risk of unseen hemorrhage, this technique was felt undesirable. The aforementioned problems led us inevitably to the use of an indwelling catheter preparation which circumvented all or the worst of these problems.

Reports in the literature concerned with this problem have involved the catheterization of deep vessels, e.g., external iliac artery [1]; common iliac artery, [4]; iliac vein, [2,3]. These procedures necessitated the use of deep anesthesia (i.e., sodium pentobarbital) and a prolonged recovery time. The catheterization procedure which has proven most reliable and advantageous in this laboratory involved only minor tranquilization of the animals (Ketamine, HCl, 14 mg/kg, IM) and the use of a very superficial vein (Saphena parva). If the animals have been adapted to the restraining chair prior to surgery, in most cases the dosing and blood collection can be started the day following surgery. In addition, if the procedure discussed below is utilized, the same vein can be used several times.

PROCEDURE

The animals are given 15 mg/kg Ketamine, HCl intramuscularly as an anesthesia following which the calves of

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the legs are shaved, scrubbed with an antibacterial soap and painted with a commercial prepping solution (Acu-Dyne, Acme United Corporation). Next, using sterile technique, a one-inch incision is made dorsally, directly over the sapheneous vein at the level of the gastrocnemius muscle, being careful not to cut the vein as it lies very close to the surface. The skin is then separated from the muscle and fascia via blunt dissection, pulled out of the way and left in place with intestinal forceps. The vein is then carefully separated from surrounding tissue. In order to present venous occlusion by the fascia it is better to cut the fascia away from the vein rather than using blunt dissection. Once the vein is completely exposed, it is tied off at the most distal point along the incision with 00 silk. Following this a piece of sterile polyethylene tubing (PE50, Clay-Adams) approximately 1.5 m long, that has been connected to a syringe filled with sterile saline, is inserted into a hole which has been cut in the vein with iris scissors. The catheter is then pushed into the vein until the tip rests an inch or two below the level of the heart. The wound is then infiltrated with nitrofurazone powder (Furacin Soluble Powder-Eaton Laboratories) and sutured around the catheter with 00 silk. Thimerosal (Merthiolate, Lilly) is sprayed on the exterior surface to inhibit local infection. Immediately after surgery, the animal is given 600,000 units of penicillin (Bicillin, Wyeth) intramuscularly. The animal is then restrained in a primate restraining chair (Plas Labs), placed in a sound attenuating cubicle, and connected to an IV drip of sterile normal saline. For best results, a Veno-Set Micro Drip (Abbot) should be used to administer the saline, as other brands of intravenous sets do not allow small amounts to be administered accurately. Drip rate should be set at approximately 3–5 drips per min (dpm). Bottle height can be adjusted in order to obtain sufficient pressure for intraanimal variability with respect to venous pressure. A drip rate any higher than 3–5 dpm can result in swelling and edema in the lower extremities of some animals which necessitates removing them from the experiment. Occasionally after the animal has been chronically catheterized for several days the back pressure in the cannula increases to a point where the IV drip will not flow. When this occurs, we switch from the IV drip to a syringe pump set to deliver 1.5–2.0 ml of solution per hr. In addition, when back pressure increases, it can generally be alleviated or eliminated by putting 0.25–0.5 ml of a standard solution of Elase^{R} (Fibrinolysin and Desoxyribonuclease, Parke-Davis) in the catheter and allowing the pump to inject it slowly.

RESULTS

The aforementioned procedure has been utilized in approximately twenty rhesus and cynomolgus monkeys, maintaining catheter patency for both infusion and blood withdrawal for as long as 6 weeks with no apparent ill effects to the animal during or after treatment. Two to three ml blood samples may be drawn as frequently as one per minute. Using the recommended polyethylene tubing (PE50, Clay-Adams) very little edema has been seen. However, increasing the size of the tubing (PE60–90) did result in the production of edema which forced premature termination of the experiment.

Another problem occasionally incurred with this technique was the inability to get blood return from the catheter, while infusion was still possible. In all cases, this problem was solved by simply pulling the catheter out an inch or so or pushing it in a short distance. Apparently, the wall of the vein covers the tip of the catheter, precluding blood withdrawal. The operation of sliding the catheter in or out places the catheter tip in a position such that blood withdrawal is again possible.

When a particular experiment was completed, the animals were usually returned to the home cage to allow them to exercise, as the chairs do not permit extensive movement of the lower extremities. Standard procedure was to remove the catheter completely, and since the vein had been tied off, this would normally mean that the vein could not be used again. However, a technique has been developed which involves leaving the majority of the catheter in the vein which can, at a later date, be removed and a new catheter inserted into the same vein.

Briefly, following completion of a particular experiment the animal is anesthetized with 10 mg/kg Ketamine HCl; the area around the old incision is prepared in the usual manner. Following this, under sterile technique the incision is reopened and the point where the catheter enters the vein is exposed. Next, the catheter is pulled out about two inches, cut off, and heat sealed making sure there are no rough edges on the catheter tip. Next the entire catheter is inserted into the vein with the heat-sealed tip completely inside the vein. The vein is then tied behind the catheter with 00 silk such that the catheter cannot slide out. The wound is then closed in the usual manner and the monkey returned to the home cage. Following a sufficient recovery time (e.g., 2–3 weeks or when the animal is again needed in an experiment) the animal is again anesthetized and the vein exposed. The vein tie is then removed and the old catheter pulled out. After placing a new catheter in the vein the procedure, which was described earlier, is undertaken. With proper care the catheterization–recatheterization procedure can be accomplished several times with no apparent ill effects to the animal. With the cost of nonhuman primates continuing to rise and availability continuing to decline, any procedure which greatly enhances the experimental life of a monkey should be welcomed.

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