LOMA LINDA UNIVERSITY
HEALTH

Louis R Rose
President and CEO
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12 Jun, 2014

Re: Use of the Clo-Sur P.A.D. in Fetal Vascular Surgery

Dear Mr. Rose:

Our experimental goal was to administer a custom-made adenovirus to a fetal sheep. We chose the fetal sheep because prior to delivery, many of the cells of the body are not fully differentiated, which renders them highly receptive to adenoviral transfection. In addition, levels of multiple growth factors circulating in the blood are very high in the fetus, and this further encourages successful transfection. One major problem with fetal transfection, however, is that intraperitoneal or even intravenous administrations of adenoviral particles typically fail. This is because most of the virus enters the venous system and is cleared by the lung with a secondary fraction cleared by the liver. This is a major challenge for my group, as our interest is in transfecting the brain and cerebral vasculature.

To selectively transfect the fetal brain, we chose a large species that would enable vascular access. For this, the fetal lamb was ideal, and we selected an age of 125 days gestation, which was about 90% of term. To preferentially deliver the adenovirus to the brain, we chose to administer the virus via intra-arterial injection into the internal carotid artery. The challenge here was that at 125 days, the fetal lamb internal carotid artery consists of only 3 or 4 layers of smooth muscle cells and does not have an extensive outer adventitial layer. This means that any needle puncture, even with a very small 27ga needle, will bleed after withdrawal of the needle. Such bleeding would of course cause loss of the adenovirus from the vascular compartment and compromise the efficiency of our transfection. To solve this problem, we cut a very small square of the Clo-Sur P.A.D., approximately 4 mm X 4 mm. Through the center of this pad we passed the tip of the needle, prior to injection. Upon injection of the adenovirus, we used a fine forcep to position the small square of the Clo-Sur P.A.D. to lie immediately on top of the artery surface. We then used the forcep to hold the Clo-Sur P.A.D. in place as we slowly removed the needle. As expected, the puncture wound in the internal carotid artery did bleed back, but this blood was immediately absorbed by the Clo-Sur P.A.D. Within approximately 60 seconds, the Clo-Sur P.A.D. was saturated with blood, but the bleeding stopped. At this point, the small piece of Clo-Sur P.A.D. was very sticky and swollen, but remained in place. As an extra precaution, we placed a second piece of Clo-Sur P.A.D. that measured approximately 1 cm X 1 cm, directly on top of the internal carotid artery, which put it in contact with the small piece of Clo-Sur P.A.D. used to stop the initial bleeding. We had also previously placed a 1 cm X 1 cm square of Clo-Sur P.A.D. underneath the internal carotid artery at the site of injection. This arrangement created a “sandwich” structure with the artery running in between two layers of Clo-Sur P.A.D. As soon as we had placed the upper pad, we closed the wound in the fetal neck, returned the fetus to the womb, then repaired the fetal membranes, the myometrium, and then closed the peritoneum and skin. We returned the ewe to her cage, where she recovered from anesthesia in less than an hour. Three days later, we harvested the fetal brain and vasculature.

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During dissection of the fetus 72 hours after initial surgery, there was no evidence of post-surgical bleeding from the injection site. The Clo-Sur P.A.D. had become quite enlarged and dark brown, but retained its shape. It was very sticky and adherent, and there was no evidence of vessel bleeding. Correspondingly, our adenoviral transfection was a success, and we observed evidence of the adenovirus throughout the fetal brain on the side of the injection. As an aside, you should know that I will be presenting these results at the meeting I'm organizing in Kuala Lumpur next August.

In the past I have used a product called OxyCell to promote local coagulation in tissues following surgery. This product was useful for low pressure (venous) bleeds, but was only marginally successful in stopping high pressure bleeds. In contrast, the Clo-Sur P.A.D. was very effective in stopping the high pressure bleed resulting from a needle puncture in the internal carotid artery of a 125 day fetal lamb. I am convinced that the Clo-Sur P.A.D. contributed greatly to the success of our in vivo transfection experiment, and for that I am grateful for the opportunity to use this product. I hope to use it again in similar future experiments.

If there is any other information I can provide about our procedure, please do not hesitate to contact me. I will be pleased to acknowledge your company when we publish our results from the fetal lamb adenoviral transfection studies.

Sincerely,

William Pearce, Ph.D.
Professor of Physiology
Associate Director, Center for Perinatal Biology