Journal of Equine Veterinary Science 45 (2016) 8–16

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

Histological Evaluation of Equine Cadaver Skin Cores Dependent on Needle Type and Skin Preparation Method to Investigate Intramuscular Injection Complications in Horses

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ARTICLE INFO

Article history:
Received 15 June 2015
Received in revised form 25 January 2016
Accepted 4 February 2016
Available online 26 February 2016

Keywords:
Intramuscular injection
Complication
Skin core
Needle
Infection

ABSTRACT

To histologically determine number and size of skin cores and hair fragments resulting from intramuscular injection techniques using equine cadaver skin explants. Clipped (clip) and nonclipped (no.clip) skin specimens, of the hamstring region (n = 10 horses), were obtained from freshly euthanized horses and punctured with 18G needles (18G), 18G needles with stylet (18GM), and 22G needles (22G). Needles were flushed with 0.2 mL of sterile saline solution. The saline was collected on slides and stained (Pappenheim). Skin cores, produced with 18G, were fixed with formalin solution (4%) and embedded in paraffin. Sections were stained with Masson's trichrome. Light microscopy was used for evaluation. Each different injection technique resulted in the identification of skin cores within a subset of slides obtained from the different 10 skin samples. Displaced skin cores were identified more frequently in clip samples than no.clip samples; 18G needle punctures resulted in the highest number and largest surface area of skin cores in both no.clip and clip samples as compared to 22G and 18GM punctures ($P < .05$); 18G needles with stylet punctures in no.clip samples resulted in the lowest number of skin cores. Histology of paraffin sections identified connective tissue (14/16 samples), stratum corneum (13/16), epidermis (12/16), isolated hair shafts and sweat glands (11/16), hair shafts integrated with epidermis and sebaceous glands (6/16), and hair bulbs (5/16). Skin cores can contain bacteria which may initiate infective injection complications. The coring incidence and hair fragmentation correlate with needle size; therefore, smaller needle sizes should be used.

1. Introduction

Infective complications after injections include malignant edema and other inflammatory processes, which cumulate in cellulitis and abscess formation [1]. Cellulitis and abscesses both occur in 0.016% of annual intramuscular (IM) injections in horses [2]. There are two etiologies discussed in literature for injection site infections. Although it is suggested that bacteria gain access to injection sites via the blood stream, it is also suggested that with most injections, bacteria are directly advanced into deeper tissue layers by the needle either by displacement of skin cores or by translocation of microbes carried along the needle's surface [3–5]. The hypothesis of bacterial translocation was confirmed by isolating several bacteria species from the cultured needles after puncturing equine skin [6].

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http://dx.doi.org/10.1016/j.jevs.2016.02.231

Coring is a process also described by the pharmaceutical industry, defining it as particles cut out of the closure of drug vials, when puncturing it with needles. The skin itself is considered a semirigid material in which coring occurs [7]. Skin coring is described in numerous experimental studies in human medicine, comparing the coring potential of different needles with various types of skin specimens [8–18]. In these studies, the incidence of skin coring varies from 1.2% [13] to 97.1% [15], depending on the type of specimen, live or cadaver tissue from different body locations, and needles used. The types of cells and tissues identified by microscopic evaluation included epithelial cells [16,19], orthokeratotic keratin [17], adipose tissue [12], nucleated cells without mitotic activity [15], epithelial cell structures, and muscle fibers [14].

Skin cores occurring in lumbar punctures are discussed to be causative for the development of intraspinal epidermoid tumors in men [15–17]. Furthermore, skin cores occurring with intravenous punctures can be carried along the bloodstream and eventually reside in the lungs, in small arterioles or capillaries. Although these epithelial pulmonary emboli are likely removed after thrombus formation, it is suggested they introduce infective agents into the lung tissue [8]. The occurrence of hair and epidermal pulmonary emboli after intravenous treatments can be reported in mice [20], rats [21,22], rats and rabbits [23], dogs [24], and in one case report in men [25].

It is also suggested that subcutaneous or intramuscular skin cores in combination with a suitable microenvironment may result in superficial or deep postinjection infections such as abscesses or produce dermoid cysts [8,10] because bacteria may be advanced into deeper tissue layers adherent to skin cores [5,10]. Given the sterile production of pharmaceuticals, it is considered that skin cores be the primary carriers of pathogenic bacteria. Moreover, because injection sites are not sterile, the frequency of complications following IM injections may depend on the amount of translocated skin, hair fragments, and foreign debris [4]. However, given the relatively low frequency of reported postinjection infections [2], translocation of pathogenic bacteria adhered to skin cores may not necessarily produce infections in well perfused subcutis or muscle tissue. More likely, such pathogenic bacteria may initiate injection site infections in combination with administered pharmaceuticals, which cause tissue toxicity and thereby reduce local and systemic immune defense mechanisms [10]. Skin cores are suggested to carry a higher bacterial risk than hair fragments because they consist of sweat glands, sebaceous glands, and hair follicles [4]. Although few studies in humans including puncture of the thoracic cavity and disinfected knee joints isolated bacteria from skin cores [5,26,27], one study in horses did isolate bacteria from skin cores [28].

Few studies exist on skin coring in equids. An early study concerning intravenous punctures in horses indicated smaller sized needles had a lower coring potential and that coring occurred more frequently when injection sites were clipped, and most frequently when injection sites were shaved [29]. Another study identified hair fragments introduced into the injection canal via the needle insertion and that the highest incidence of hair fragment introduction was observed in the clipped injection sites [4].

Skin coring associated with equine arthrocentesis was studied by producing skin cores from skin sites including the fetlock joint capsule [28,30]. Tissue cores were observed with an incidence of 89% to 97% of punctures, whereas hair contamination was observed in 22.3% to 47.5%, depending on needle size, type, and preparation methods [30]. Wahl et al [28] published similar results, with a tissue coring incidence of 88.5%, hair contamination in 42%, and both hair and tissue contamination occurring in 39.2%. Hair fragments and hair follicles were embedded in tissue cores.

Considering the potential impact of skin coring on potential infective complications after needle punctures, and despite the existing human and equine research, the impact of cell and tissue transfer needs further investigation [12,19].

Therefore, one aim of this study was to compare the incidence, number, and size of the skin cores resulting from injections performed with different sizes and types of needles and different skin preparation protocols. Another aim was to determine the exact histologic components of skin cores so as to further investigate the impact of skin coring on IM complications in equids.

2. Materials and Methods

The skin specimens for this study were obtained from horses euthanized at the Clinic for Horses and the Department of Physiology of the University of Veterinary Medicine Hannover. All skin specimens were obtained within 2 hours of euthanasia. All horses were euthanized for reasons independent of this study ($n = 10$). Criteria for skin specimen donors included warmblood horses aged 1 year and older presenting with good general condition. Horses were not suffering from skin diseases or conditions such as systemic disease, endotoxemia, vasculitis/vasculopathy, or hemodynamic shock, and their hair coat was not secondarily contaminated or sweated. Sampling was performed with the euthanized horse in lateral recumbency. Square skin specimens, measuring 5×5 cm, were taken from the hamstring area, approximately 15 cm distal to the ischial tuberosity in the central region of the semimembranosis muscle. Peripherally, the sampling areas were clipped and cleaned with a 70% ethanol solution (Softasept N, B. Braun Melsungen AG, Germany), for minimizing contamination during sample acquisition. The skin was incised laterally and ventrally of the squares with both ventral corners fixed with surgical forceps. With the forceps kept under traction, the skin was separated from the underlying muscle tissue, allowing a clean sampling procedure. Eventually, the remaining dorsal tissue bridge was incised, providing the complete skin specimen. Each horse had skin samples obtained from two different skin preparations. Skin specimens type 1 (no.clip) were not clipped, whereas skin specimens type 2 (clip) were clipped. The skin specimens were placed on a sterilized test tube rack (Heinz Herenz Medizinalbedarf, Germany), between two platforms lying on top of each other and tightly fixed with