NON- INVASIVE METHOD FOR THE STUDY OF LOCAL MUSCLE REACTIONS AFTER INTRAMUSCULAR (IM) INJECTION OF AN ANTIBIOTIC IN HORSES

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SUMMARY

This study describes muscle reactions to intramuscular injection of an antibiotic through ultrasonography and serum creatine kinase (CK) concentrations in horses. Three adult horses from the Equine Centre, Universiti Putra Malaysia were used. An advance real-time B-mode ultrasound machine connected to a linear array multifrequency (5-8 MHz) transducer was used throughout the study. Ultrasonographic examination was done before and after IM injections at approximately 6, 24, 48, and 72 hours and at 3-day intervals until the muscle structure returned to normal. Blood samples were obtained before IM injection and at 6, 24, 48 and 72 hours post-injection, and serum CK concentrations were measured. There were localised increased echogenicity or hyperechoic structures at site of drug deposition. The muscle returned to normal appearance after approximately 2 weeks of post-injection. In one horse, the drug was detected to be deposited intermuscularly. Serum CK analysis seems to increase after 24 hours of injection. Muscle reaction to IM injection of antibiotics can be defined ultrasonographically and the progress of muscle reactions until recovery to the normal muscle structures can be monitored with ultrasound. A slight increase in serum CK concentrations after antibiotic injection indicated that the drug used in this study did not cause significant damage to the muscle of these horses. Ultrasonography provides a clear definition of the location of drug deposition within the muscle and could be used as a meaningful diagnostic tool for monitoring of muscle reactions to IM injections of drugs.

Keywords : ultrasonography, creatine kinase, intramuscular injection, horse

INTRODUCTION

Intramuscular injection is a routine route for drug administration both in human and veterinary medicine. Intramuscular injection is generally a safe method that seldom cause complications. Besides , clinical observation (pain, induration, abcess and necrosis), post mortem findings (especially at slaughterhouse for domestic species), and experimental studies in animals have shown that some therapeutic agents can cause local tissue damage at injection sites (Svendson *et. al.*, 1979). Adverse reactions sometimes occur and may include sudden death (presumably when medication is inadvertently given intravascularly) and local, non-septic swelling or infection at the injection site. The infection may remain localised and form an abcess or may fulminate, often rapidly developing into fatal cellulitis or myositis or both.

Several methods have been used to measure tissue reactions to drug injections. The most common is the histopathological method, where the size and type of necrosis are investigated (Rasmussen and Svendsen, 1976). However, such an approach requires that the animals sacrificed and thus it is unacceptable both from an economical and ethical point of view.

Muscle damage may be monitored by measuring the activity of muscle specific enzyme including serum creatine phosphokinase (CK) (Diness, 1985; Braun *et. al.*, 1992). Intramuscular injections increases serum CK activity owing to local area of muscle necrosis (Steiness *et al.*, 1978). Recently, ultrasonographic evaluation of the injection site has been suggested as another method to define local tissue damage (Banting and Tranquart, 1991). The tremendous application of ultrasonography in recent years has largely resulted from the fact that this diagnostic modality is noninvasive and, as far as currently known does not cause any biological change in the imaged tissue. No biological hazard has been known to occur from the use of diagnostic ultrasonography either in domestic animals or to the operator of ultrasonographic machine using the proper technique. This method allows the size, shape, location and consistency of soft tissues to be evaluated and monitored (David, 1991).

The present study was undertaken to define ultrasonographically local muscle damage or reactions at the site of intramuscular injection of a commonly used antibiotic in horses. The serum creatine phosphokinase activity is also determined as evidence of local muscle damage or reactions.

MATERIALS AND METHODS

Experimental animals and drug

Three adult, clinically healthy thoroughbred horses of ages 5-16 years old were used in this study. The horses were kept in individual stables at Equine Centre, Universiti Putra Malaysia and fed on commercial diet. A commercial solution of antibiotic, (Pen-Hista -Strep®, V´etaquinol, Lure, France) with the main ingredients of Penicillin G (Procaine), dehydrostreptomycin base (as sulphate), dexamethasone acetate and chlorpheniramine

maleate was used for the intramuscular injection. The horses were restrained manually using a head collar. Area for the IM injection at the neck region was determined approximately at the level of fifth cervical vertebra, ventrally to the funicular part of the nucheal ligament and dorsally to the brachiocephalic muscle. The muscles involved at the injection site were *Rhomboideus cervicis*, *splenius*, *semispinalis capitis*, *trapezius* and *serratus ventralis cervicis*. The injection area was prepared on the left side of the neck of each horse by shaving the hair on a skin approximately 5 cm x 3 cm. An alcohol swab was applied at the shaved skin before injection. Ten (10) millilitre of antibiotic was administered intramuscularly to each horse at the shaved area using 18 G needle.

Ultrasonographic examination

Ultrasonographic examination was done prior to and immediately after the IM injection of the drug at 3, 6, 24, 48, 72 hours and at 3 days intervals until the muscle becomes normal again.

For ultrasonographic imaging, the ultrasound coupling gel was first applied to the shaved skin. Both the transverse and longitudinal scans were obtained by positioning the transducer at the injection site transversely and longitudinally across and along the muscle fibres, respectively. An Advance real-time B- mode ultrasound machine (TOSHIBA Just Vision 200) connected to a high resolution linear array multifrequency (5-8 MHz) transducer was used. The images were recorded digitally using a still image capture adaptor (SONY Mavicap).

Serum collection and analysis

Blood samples were collected from the jugular vein before and after IM injections of antibiotic at 6, 24, 48 and 72 hours using an 18G venoject needle and dispensed into plain tubes. The samples were then centrifuged at 10,000 rpm. for 5 minutes and the serum stored at 20°C until analysed. The serum creatine kinase (CK) was analysed using a standard diagnostic kit (Roche) in an automated Clinical Chemistry Analyzer (Cobas Mira Plus Hoffman-la Roche Ltd., Switz.).

RESULTS

Serum Creatine Kinase (CK) level

The CK concentration in each horse was slight increased after IM injection of antibiotic and decreased after approximately 24 hours post-injection.

Ultrasonographic examination

The ultrasonograph of normal muscle demonstrated fine, linear echogenic striae of muscle connective tissue representing the endomysium scattered throughout the parenchyma (Fig. 1).

Immediately after IM injection the affected site revealed an area of highly hyperechoic structure with acoustic shadowing artefacts indicating an accumulation of the antibiotic within the muscle (Fig. 2). The accumulated antibiotic was quickly resorbed and within 6 hours the ultrasonograph of the injection site shows an area of increased echogenicity with mild acoustic shadowing artefacts. Between 24 to 48 hours of IM injection the antibiotic deposited within the muscle can still be detected as hyperechoic foci with acoustic shadowing artifacts. The area of muscle reaction surrounding the hyperechoic foci appeared as slightly increased echogenicity with mild disorganised muscle structure. The hyperechoic foci became smaller with time. Between 72 and 96 hours of IM injection, the antibiotic was completely resorbed and the ultrasonograph revealed an area of increased echogenicity with homogenously disorganized muscle structure (Fig. 3). In one of the horses, the injection went intermuscularly. In this case, the ultrasonograph revealed the drug to be deposited between *splenius* and *semispinalis capitis* muscle as a linear hyperechoic stripe with acoustic shadowing artifacts. The muscle structures reverted to normal appearance approximately twoweeks after the injection.

DISCUSSION

An advance real-time B-mode ultrasound with a multifrequency linear array transducer allows good visualisation of the neck muscles on horses. Normal muscle ultrasonograph appears as fine linear echoic striations caused by muscle fasciculi and the muscle perimysium while the muscle tissue itself is hypoechoic (Smith et al., 1996). A similar muscle ultrasound characteristic was demonstrated in this study. The ultrasonographic appearance of the muscles at the antibiotic injection site in the first few days was increased echogenicity and the loss of fine linear echoic striations indicating muscle reactions to the injected drugs. The loss of normal muscle characteristic was due to accumulation of the drug within the muscle. The area of increased echogenicity or hyperechogenicity was found to be localised. However, the intensity of hyperechogenicity abated with time. It is believed that the localised hyperechoic area was caused by muscle reactions to the antibiotic deposited within the muscle.

The injected drug, which was a foreign material within the muscle appeared as hyperechoic structures with acoustic shadowing artefact within the first 24 hours post-IM injection. A similar finding was reported by Kramer *et al.* (1997). Two out of three IM injections went intramuscularly (within the splenius muscle) while one injection was injected intermuscular accidentally. This could have occurred because a number of muscles in the neck are small (Boyd, 1987), increasing the possibility of the antibiotic injected going intermuscularly. Thus, this study has shown that the ultrasound could precisely determine the area of drug deposition within the muscle from intramuscular injections.

The muscle structure at the antibiotic injection site returned to normal appearance approximately two weeks post-IM injection. In the case of intermuscular injection, the reaction was mild and the muscle returned to normal three days after drug injection. The antibiotic deposited within

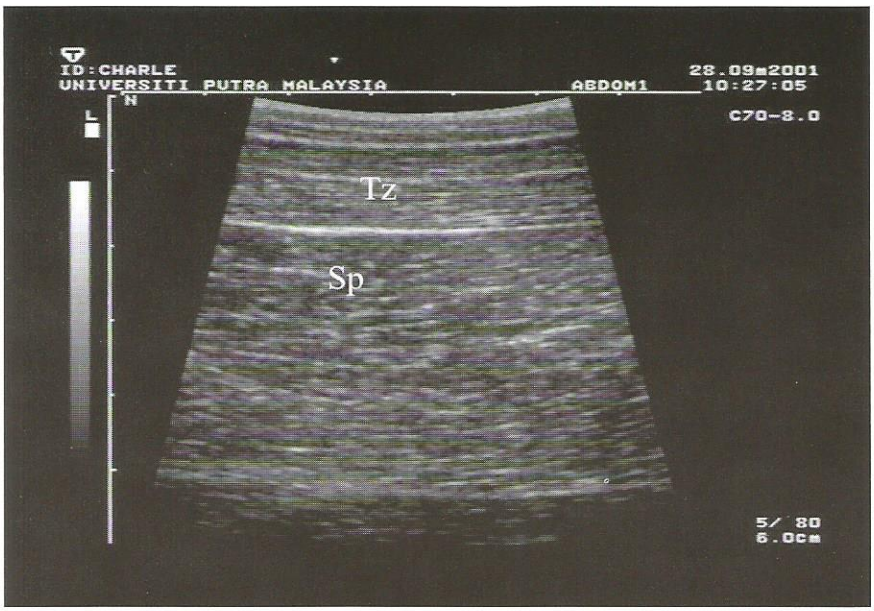

Fig. 1. An ultrasonograph of the normal muscle structures characterized by fine, linear, echogenic striae of connective tissue and epimysium. Tz, trapezius muscle, Sp, splenius muscle.

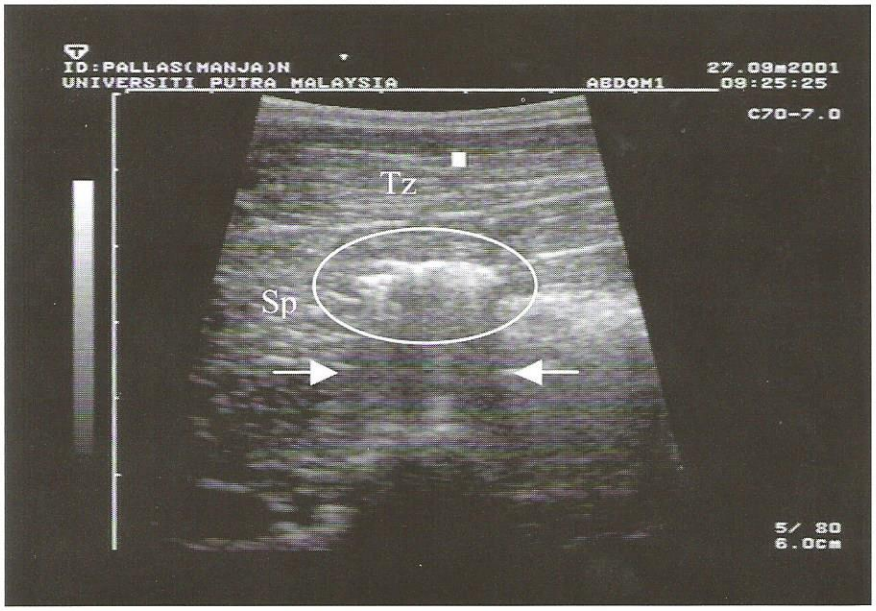

Fig. 2. An ultrasonograph of the injection site immediately after IM injection of antibiotic demonstrating an accumulation of the drug as shown by hyperechoic area (circle) with acoustic shadowing artifacts (arrows) within the splenius muscle (Sp). Tz, trapezius muscle.

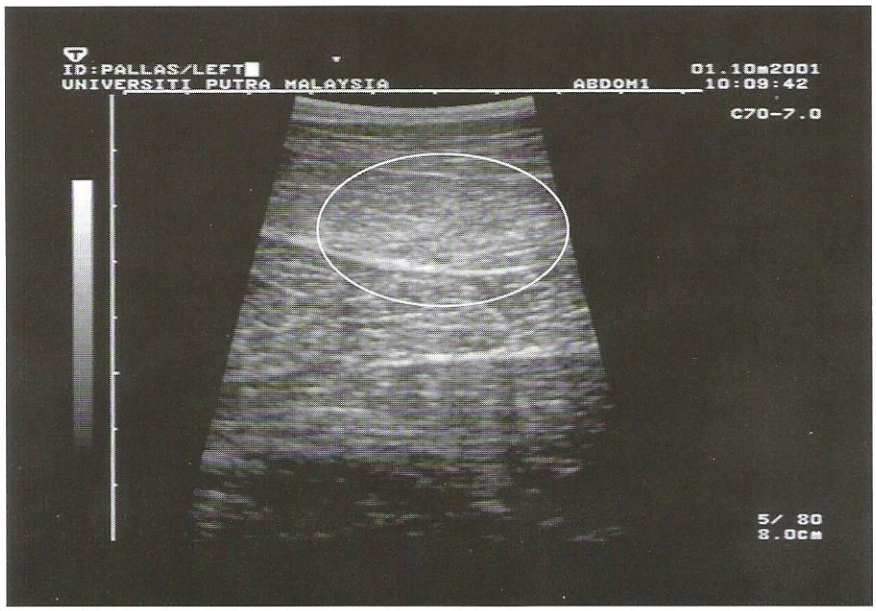

Fig. 3. An ultrasonograph of the antibiotic injection site 96 hours post-IM injection demonstrating a hyperechoic area (circle) indicating mild reactions still occurred within the splenius muscle (Sp).

the muscle disappeared within three days post-IM injection and muscle reaction could be detected at the injection site while the antibiotic deposited intermuscularly disappeared within approximately 24 hours post-IM injection with the muscle reaction almost negligible. This may be due to the fact that intramuscular injection of an antibiotic allows slow absorption and has more surface contact with muscle parenchyma, thus producing more reactions compared to intermuscularly deposited antibiotic which is easily dispersed through the blood vessels.

The serum CK concentrations showed a slight increased in all horses. This may be due to the non-irritant effect of the antibiotic used and the small sample size. The increase in serum CK concentration after the injection of antibiotic was prominent individually approximately 24 hours after the injection followed by slow decline to the normal level. In this study, the muscle was injured only once. Since CK has short half-life of less than 6 hours in plasma serum and reaching maximum serum concentration 6-12 hours post-injury (Wayne, 1988), the lack of a significant response is expected. To obtain a clearer definition of the usefulness of ultrasonography in detecting muscle tissue reactions to intramuscularly administered drugs, the number of samples and drug concentrations should be increased. Although penicillin injections have been reported to cause the highest incidence of reactions and considered to be one of the most common cause of drug allergy (Huber, 1988), ultrasonographically none of the horses in the study exhibited a reaction to the antibiotic. There is a possibility that individual variations in hypersensitivity or reactions to drug may be the contributing factor. The small volume of antibiotic injected (10 ml) have also contributed to the poor muscle reaction to the drug.

The muscle reactions to IM injections of antibiotic and its progress can be defined ultrasonographically. A non-significant increase in serum CK concentrations in this study indicated the mild tissue reactions. Ultrasonography provided a clear definition of the drug deposition within muscle and muscle reaction to IM injection.

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RINGKASAN

KAEDAH NON-INVASIF BAGI MENGKAJI TINDAKBALAS SETEMPAT OTOT SELEPAS SUNTIKAN INTRAOTOT ANTIBIOTIK YANG LAZIM DIGUNAKAN PADA KUDA

Kajian ini dijalankan bagi menghuraikan tindakbalas otot dengan kaedah ultrasonografi selepas suntikan antibiotik bersama pengukuran aras kreatine kinase serum pada kuda. Tiga ekor kuda dari Pusat Ekuin, UPM telah digunakan. Sebuah mesin ultrasound Mod-B dihubungkan kepada penerima gelombang aturan datar berbilang frekuensi berukuran 5-8 MHz telah digunakan sepanjang kajian. Pengimejan ultrasonografi dilakukan sebelum suntikan intraotot dan selepas suntikan kira-kira 6, 24, 48, 72 jam dan selepas setiap 3 hari sehingga otot kembali normal. Sampel darah telah diambil sebelum suntikan intraotot dan kira-kira 6, 24, 48 dan 72 jam selepas suntikan dijalankan bagi mengukur aras kreatine kinase serum. Terdapat peningkatan setempat ekogenisiti di lokasi dimana drug disuntik dan otot kembali normal selepas kira-kira 2 minggu suntikan. Pada salah seekor kuda, suntikan sebenarnya adalah interotot. Analisis kreatine kinase serum merekodkan peningkatanyang rendah dalam setiap individu kuda selepas 24 jam suntikan. Tindakbalas otot selepas suntikan intraotot boleh dihuraikan dengan kaedah ultrasonografi dan perkembangan tindakbalas otot sehingga pengembalian stuktur normal boleh diawasi dengan kaedah ultrasonografi. Pengimejan ultrasonografi terhadap tindakbalas otot dan kembalinya struktur normal dan ekogenisitinya bersama peningkatan pengukuran aras kreatine kinase selepas suntikan menunjukkan drug yang digunakan dalam kajian ini tidak menyebabkan kerosakan bererti pada otot kuda. Kaedah ultrasonografi memberi peninjauan jelas lokasi perletakan drug diantara otot dan pengawasan perkembangan tindakbalas kawasan itu selepas suntikan.