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The pharmacologic effects of isoxsuprine

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Summary

Isoxsuprine is a therapeutic medication used to treat navicular disease and other lower limb problems in horses and is one of the more frequently detected therapeutic agents in racing horses. In crossover studies, horses were administered intravenous and oral isoxsuprine to determine the character and duration of pharmacological effects. Following intravenous administration, isoxsuprine significantly increased heart rate, spontaneous activity, and sweat production. There was an apparent, although statistically insignificant, increase in cutaneous blood flow. Skin temperature decreased below control values, and there was a significant decrease in core temperature. Isoxsuprine also reduced smooth muscle tone. In contrast, after oral dosing, there was no statistical difference between control and isoxsuprine-treated horses for any of the measured variables. It was concluded that the measurable physiologic effects of intravenous isoxsuprine are short-lived, since none of the above responses was apparent four hours or more after intravenous administration.

Keywords: isoxsuprine, sweat production, behavior chamber, muscle tone, thermography, horse

Die pharmakologische Wirkung von Isoxsuprin

Isoxsuprin besitzt als Vasodilatator die Eigenschaft, ischämische Zustände bestimmter Körperbereiche aufzuheben. In der Humanmedizin wird es bei der Therapie cerebraler Gefäßinsuffizienzen und bei peripheren vaskulären Erkrankungen verwendet. Der Einsatz von Isoxsuprin in der Pferdemedizin beschränkt sich auf Erkrankungen im distalen Gliedmaßenbereich, besonders der Strahlbeinregion. Isoxsuprin ist eine der häufigsten Drogen, welche bei Dopingproben der Rennpferde gefunden werden.

In mehreren Versuchen wurde sechs Pferden Isoxsuprin intravenös und oral verabreicht, um den Wirkungsmechanismus und die pharmakologischen Daten des Medikaments zu erforschen. Die Pferde bekamen eine Woche lang zweimal täglich 2,0 mg/kg Isoxsuprin i.v. bzw. 600 mg/Tier oral verabreicht. Als Kontrollbehandlung wurden den Pferden die Trägersubstanzen der Isoxsuprin-Präparation i.v. gegeben. Nach jeder Applikation wurden die Tiere klinisch untersucht.

Nach der intravenösen Applikation von Isoxsuprin zeigten die Pferde einen deutlichen Anstieg der Herzfrequenz, der Spontanaktivität und der Schweißproduktion. Die kutane Durchblutung stieg merklich an, die Veränderungen besaßen jedoch keine statistische Relevanz. Die Oberflächentemperatur sank unter die Kontrollwerte ab, und auch die rektal gemessene Körpertemperatur nahm subnormale Werte an. Isoxsuprin reduzierte weiterhin in geringem Umfang den Tonus des analen Schließmuskels der Pferde.

Die Untersuchungen nach oraler Isoxsupringabe erbrachten ganz andere Ergebnisse: keiner der gemessenen Parameter wich in signifikanter Weise von der Norm ab. Die Daten der Pferde unterschieden sich nach oraler Isoxsuprin-Therapie nicht von den Messungen bei dem Kontrolldurchgang. Weder nach intravenöser noch nach oraler Isoxsuprin-Applikation wurden Effekte auf die Atemfrequenz oder die Darmperistaltik bemerkt.

Die meßbaren Veränderungen bei intravenöser Isoxsupringabe waren nur von kurzer Dauer. Bereits 4 Stunden nach Verabreichung des Medikaments konnten keine Veränderungen mehr beobachtet werden.

Die therapeutische Dosis von Isoxsuprin wird in der Regel zweimal täglich gegeben. Die Studie zeigt, daß die Wirkung keinesfalls länger als 12 Stunden besteht. Daraus kann man schließen, daß es sinnvoll ist, Grenzwerte für Isoxsuprin beim Dopingtest anzusetzen, da der Stoff bis zu 30 Tagen nach seiner Verabreichung noch mittels eines ELISA nachweisbar ist.

Schlüsselwörter: Isoxsuprin, Schweißproduktion, körperliche Aktivität, Muskeltonus, Thermographie, Pferd

Introduction

Isoxsuprine is a vasodilator drug. In humans, it is used for cerebral vascular insufficiency, peripheral vascular disease, and to control premature labor (*Menard*, 1984). In equine medicine, isoxsuprine is recommended for the treatment of navicular disease and other lower limb problems (*Turner* and *Tucker,* 1989).

Isoxsuprine is frequently detected in racing horses (*Gowen*) for several reasons. The drug dose is relatively large, it is administered for several weeks, the drug may sequester in body fat, and the glucuronide conjugate of isoxsuprine is readily detectable by ELISA testing. It is not unusual to detect isoxsuprine in equine urine for seven days after the last dose of the agent, and the agent has been detected up to 37 days after the last dose (*Kellon* and *Tobin,* 1995).

In contrast with its prolonged detection time in equine urine, isoxsuprine appears to have a relatively short duration of pharmacological action. There is little effect on blood chemistry parameters, heart rate, blood pressure, respiratory rate, and body temperature after oral administration of the agent (*Rose* et al. 1983). In similar work (*Deumer* et al. 1991), significant increases in lower limb temperatures were measured by thermography for only six hours after oral administration. Furthermore, the recommended treatment frequency of isoxsuprine for navicular disease is twice daily, suggesting a duration of action of less than 12 hours. These studies indicate it is unlikely that isoxsuprine exerts any significant pharmacological effect for longer than a period of 12 hours after administration of the last dose and also suggest there is no regulatory

need for the prolonged (30 day) detection times for isoxsuprine made possible by ELISA testing.

The overall goals of this research project were to establish the duration of pharmacological effects of isoxsuprine and to develop practical analytical guidelines (thresholds) for the detection of isoxsuprine or its metabolites in the plasma or urine of horses. Although isoxsuprine is administered orally in clinical situations, the physiologic effects of the drug are more easily characterized following intravenous administration. This study characterizes the pharmacological effects of isoxsuprine following acute intravenous administration and oral administration twice a day for seven days.

Materials and methods

Horses

Six mature Thoroughbred mares weighing 413–602 kg were used for this study. All horses were acclimated to their stalls 24 hr prior to experimentation. Except for measurement of skin thermography, a crossover experimental design was applied, and horses were used as their own controls.

Drug Administration

For intravenous administration, isoxsuprine HCl (Sigma Chemical Co, St. Louis, MO) was dissolved in 50 ml sterile water and 25 ml 95% ethanol. The solution was filtered through a sterile millipore filter (Cameo 25NS, Micron Separations, Inc, Westborough, MA), and injected intravenously at a dose of 2.0 mg/kg in less than one minute. Control horses were injected with 50 ml sterile water and 25 ml 95% ethanol which was also filtered through a millipore filter. For oral administration, horses were dosed with 600 mg isoxsuprine twice a day with a balling gun.

To determine the physiologic effects of isoxsuprine, four sets of experiments were conducted. During the first experiment, we measured skin thermography, heart rate, respiratory rate, and intestinal motility. The second set of experiments measured cutaneous blood flow to the left front pastern. The third set of experiments were also performed in the specially adapted stall and measured sweat production, anal tone, and skin and body core temperatures. The fourth set of experiments were performed in a motion chamber, which is a specially designed stall for measuring the movement of an unrestrained horse. Briefly, the stall contained four intersecting infrared beams for motion detection in any portion of the stall. The stall was sound-proofed, and the horse could not see out of the stall, both of which reduced external stimuli.

Skin thermography measurements

An Agema Thermovision 870 infrared scanner (Agema Infrared Systems, Secaucus, New Jersey) was used for infrared imaging of horses. The superficial skin temperatures of the front legs were recorded from a distance of 1.5 meters. Superficial skin temperatures were recorded at 1.75 sec intervals.

Cutaneous Blood Flow

One of the principle applications of the laser Doppler flowmeter (TSI model 403, St. Paul, MN) had been the measurement of cutaneous blood flow. The device noninvasively measures superficial blood flow (to a depth of 1.2–1.5 mm) with the use of a fiber optic cable that directs laser light into the tissue, illuminating a volume which contains both red blood cells and stationary tissue cells.

Although the flowmeter has not specifically been used to monitor cutaneous blood flow in the horse, there is no reason to suspect that measurements would be qualitatively different from those obtained in other species (e.g., human and rat). Since the superficial and middle vascular plexus of the cutaneous circulation of the horse are located within 1.0 mm of the surface of the skin (*Leach*, 1992), it is reasonable to assume that the flowmeter can be used to investigate blood flow responses in these vessels. Cutaneous blood flow was measured at the dorsal pastern. The hair was clipped and shaved, the skin was rubbed with isopropyl alcohol, a conducting gel (K-Y Jelly, Johnson & Johnson Medical Inc, Arlington, TX) was applied to the skin, and the fiber optic probe was secured with bandaging material.

Sweat production

A plastic sweat catchment device (SCD) was designed to collect all sweat produced following treatments. The SCD extended under the neck anteriorly, fit securely around both forearms, extended posteriorly to the front of the stifles, and was suspended about 20 cm below the ventrum of the horse by straps over the withers and lower back.

Anal tone

A bulb dynamometer (North Coast Medical, Inc., San Jose, CA) was used to measure changes in anal tone following isoxsuprine injection. By inserting the lubricated, 4 cm-diameter, cylindrical bulb into the anus of the horse, the maximal force of anal contraction was measured and stored on the dynamometer.

Skin and core body temperatures

Skin temperatures were measured with a surface thermistor (Model 409B, YSI Incorporated, Yellow Springs, OH) attached to the chest wall with a skin adhesive (Vetbond, 3M Animal Care Products, St. Paul, MN). Core body temperatures were measured with a general purpose thermistor (Model 401, YSI Incorporated, Yellow Springs, OH) placed 50 cm into the rectum and secured with adhesive tape to the tail. Temperatures were measured by a digital thermometer (Model 8402, Cole-Parmer Instruments Co., Niles, IL).

Spontaneous Activity

Horse activity was detected by 4 Mini-beam sensors (SM31E and SM2A31R, Banner Engineering, Minneapolis, MN). The output from the four sensors was summed and recorded on a data logger (CR10, Campbell Scientific, Inc, Logan, UT) every 5 min. The total number of times the sensors were activated was averaged over a 15 min period. Data was collected for 60 min before and 300 min after administration. The data recorded before injection was the baseline data and was used to calculate the mean baseline activity. Data was expressed as percent of baseline step count per 5 min.

Statistical analysis

Data are presented as means ± SEM. Analysis of variance with repeated measures was used to compare control and isoxsuprine values for each physiologic variable at each measuring time. Because of the large individual variability in sweat production following intravenous administration, the sign test was used to compare the effects of control and isoxsuprine treatments on that variable at each measuring time. Significance was set at $P<0.05$.

Results

Heart rate

Following intravenous injection, there was a dramatic increase in heart rate. Heart rate was significantly greater than control values 5 to 150 min and returned to control values 180 min after treatment. A maximal mean heart rate of 143.0 bpm was measured 5 min after isoxsuprine administration. Following oral dosing, heart rate was not significantly different from control values.

Skin thermography measurements
There was a rapid decrease in thermographic temperature of the front legs following intravenous administration. There was a significant decrease from control values of foreleg skin temperature 30–180 min after administration. The lowest mean temperature (27.0° C) was measured 60 min after treatment.
Cutaneous Blood Flow
For intravenous administration, cutaneous blood flow to the pastern appeared to increase dramatically then decrease to baseline during the first 60 min following injection. However, large variations precluded a significant difference in blood flow between control and isoxsuprine-treated horses. For oral dosing, there was no significant difference in blood flow between control and treated horses.
Sweat production
There was a dramatic increase in sweat production following intravenous isoxsuprine administration. We were able to show significant differences in sweat production between control and isoxsuprine treatments at 15, 20, 25, 30, 35, and 40 min after injection. Measurable sweat production continued through 60 min, and total sweat produced varied from 3–198 ml/horse. There was no sweat production following oral dosing.
Anal muscle tone
Following intravenous administration, anal muscle tone gradually decreased in control horses for 60 minutes after treatment and gradually returned to near-baseline values. Anal muscle tone of isoxsuprine-treated horses decreased at a much greater rate than anal tone of control horses. Following oral dosing, there was no significant difference in anal tone between control and isoxsuprine-treated horses.
Core temperature
For intravenous administration, there was no significant change in core temperature following control treatment (25 ml ethanol and 50 ml sterile water). However, there was a significant decrease from control values after isoxsuprine treatment that persisted through 120 min. For oral dosing, there was no difference between control and isoxsuprine treatment.
Skin temperature
There was an increase in skin temperature (vs. baseline values) following control treatment (25 ml ethanol and 50 ml sterile water). There was also a significant increase in skin temperature (vs. baseline values) 5–25 min after isoxsuprine treatment; however, isoxsuprine-treatment values were not significantly differently from controls until 45 minutes after treatment, and the significant difference persisted until 120 min post treatment. For oral dosing, there was no difference between control and isoxsuprine treatment.
Spontaneous Activity
For intravenous administration, there was a significant increase in activity from control values 15 to 105 min after injection of isoxsuprine. Peak activity occurred 30 to 75 min following administration. For control horses, there was a significant decrease in activity 60–90 min following administration, when compared with baseline values. For oral dosing, there was no difference between control and isoxsuprine treatments.
Respiratory rate and intestinal sounds
For intravenous and oral dosings, there was no significant difference in respiratory rates or intestinal sounds between control and isoxsuprine-treated horses.

Discussion

Within minutes of intravenous injection, the heart rate rose rapidly. Peak HR was reached 5 min after injection and declined exponentially thereafter. Spontaneous activity also increased immediately after intravenous injection and decreased to control values within 120 min after administration. Similarly, sweat production, smooth muscle tone, skin temperature, and core temperature were significantly affected following intravenous administration.
A drug's influence on spontaneous activity is an important quality of any agent when assessing the effect of that agent on performance horses. The increased activity following intravenous administration was significant but relatively short lived. Possibly, the increased activity seen in isoxsuprine-treated horses would have been even greater without the ethanol vehicle.
All measured physiologic effects of intravenous isoxsuprine returned to control values within 4 hr of administration. Following oral administration, there were no measurable effects. In light of the relatively short duration of effects and prolonged detection of isoxsuprine, it seems reasonable to establish threshold limits for parent isoxsuprine or its metabolites in urine or blood. This would enable performance horses with navicular disease or other similar conditions to perform after the effects of the drug have subsided but before the long clearance time now required because of sensitive ELIZA testing.

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