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Absence of detectable pharmacological effects after oral administration of isoxsuprine

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Summary

Isoxsuprine is reported to be a peripheral vasodilator used in human and veterinary medicine to treat ischaemic vascular disease. In horses, it is generally administered orally to treat navicular disease and other lower limb problems. To define the scope and duration of its pharmacological responses after oral administration, 6 horses were dosed with isoxsuprine HCl (1.2 mg/kg bwt) q. 12 h for 8 days and then tested to assess the duration and extent of pharmacological actions. There was no significant difference between isoxsuprine and control treatment values for heart rate, spontaneous activity, sweat production, anal muscle tone, core and skin temperatures, and cutaneous blood flow. The lack of pharmacological effect following oral administration was in sharp contrast to the marked response following i.v. dosing reported in earlier experiments.

Introduction

Isoxsuprine is a vasodilator drug used for cerebral vascular insufficiency and to control premature labour in man (Menard 1984). In veterinary medicine, it has been recommended for the treatment of navicular disease and other lower limb problems in horses (Turner and Tucker 1989; Wilson and Bolhuis 1996).

In a previous study, when isoxsuprine HCl was administered i.v. significant effects were observed on behavioural and physiological variables, including heart rate, spontaneous activity, sweat production, core and skin temperatures, and anal muscle tone (Harkins *et al.* 1996). These findings were consistent with those of another study measuring the effects of i.v. isoxsuprine which showed transient decreases in systemic blood pressure, vascular resistance, and stroke volume, along with transient increases in heart rate, cardiac output, and purposeful movement (Matthews *et al.* 1986). Although the observed effects were marked, the duration of pharmacological action was short and all measurable effects returned to control values within 4 h of administration of isoxsuprine.

Despite the relatively short duration of pharmacological action, isoxsuprine is one of the more frequently detected therapeutic agents in racing horses (R. Gown, personal communication) and has been detected 42 days after the last dose has been administered (Kellon and Tobin 1995). In equine medicine, isoxsuprine is commonly administered by the oral route rather than i.v.

Since the efficacy of a medication can be highly dependant on the route of administration, the objectives of this study were to assess the duration and extent of the pharmacological effects of oral isoxsuprine when administered at the dose and by the route currently used in equine therapeutics.

Materials and methods

Horses

Six mature Thoroughbred mares weighing 413–602 kg were used. The animals were maintained on grass hay and feed (12% protein: a 50:50 mixture of oats and an alfalfa-based protein pellet). They were vaccinated annually for tetanus and given ivermectin quarterly. A routine clinical examination was performed prior to the beginning of these experiments to assure that the animals were healthy and sound. During experimentation, horses were provided water and hay *ad libitum*. All horses were acclimated to their stalls 24 h prior to experimentation, and each horse was used as its own control.

Drug administration

Isoxsuprine HCl powder[1] 1.2 mg/kg bwt was administered in 0.5 cup of sweet feed (oats, corn and barley mixed with molasses) b.i.d. for 8 days. The horses were fed their usual ration of oats immediately after dosing. On the seventh day of dosing, experiments were conducted to determine the pharmacological effects of isoxsuprine. Heart and respiratory rate, sweat production, anal muscle tone, skin and body core temperatures, and cutaneous blood flow to the skin of the pastern were measured concurrently as described below. These experiments

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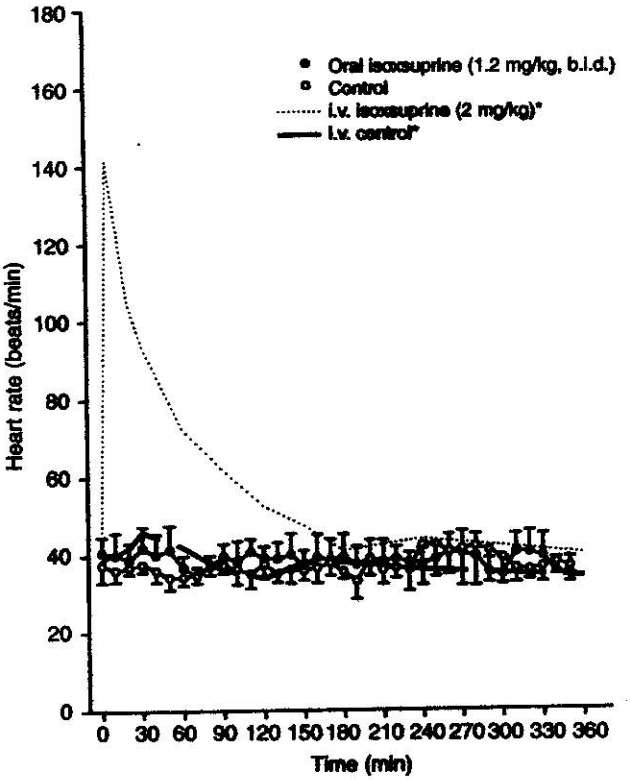

*Fig 1: Heart rate following control and oral isoxsuprine treatments. *Harkins* et al. *(1996).*

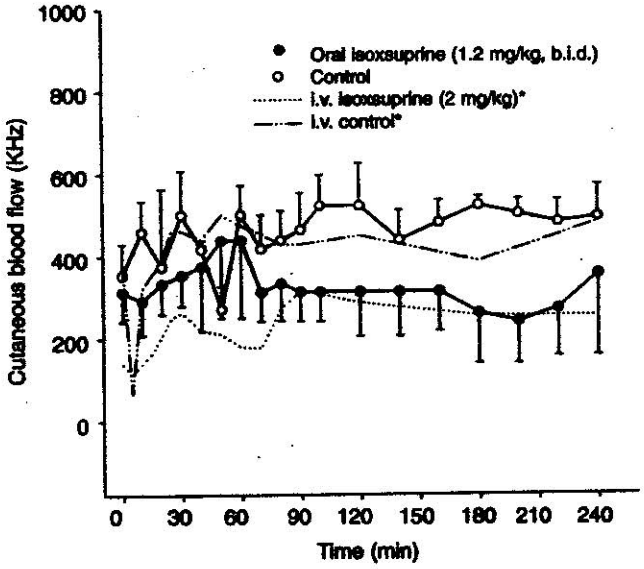

*Fig 2: Cutaneous blood flow following control and oral isoxsuprine treatments. *Harkins* et al. *(1996).*

were performed in a specially designed horse stall adapted for physiological measurements. On the eighth day of dosing, the horse was placed in a motion chamber to measure spontaneous movement. The ambient temperature varied between 6.7–21.6°C and relative humidity was 68–100%.

For control values, sweet feed alone was administered. A crossover design would have been preferable; however, the laser Doppler flow meter was designated for teaching purposes, and access to the instrument was limited. However, control flow meter values were obtained 6 weeks later, which should have given sufficient time for any effect of isoxsuprine to have been removed. It was not possible to schedule use of the flow meter more than one or 2 days in advance.

Respiratory and heart rates

Respiratory and heart rates were recorded by clinical observation every 10 min for 6 h after administration of the morning dose on the seventh day of treatment.

Cutaneous blood flow

Cutaneous blood flow was measured at the dorsal pastern with a laser Doppler flowmeter (TSI model 403)[2]. The flowmeter noninvasively measured superficial blood flow (to a depth of 1.2–1.5 mm) by detecting the Doppler frequency shift or laser light reflected by moving red blood cells in the skin. Detailed information on the particular laser Doppler system used has been described previously (Richardson *et al.* 1988). The hair on the pastern was clipped and shaved, the skin was rubbed with isopropyl alcohol, a conducting gel (K-Y Jelly)[3] was applied to the skin, and the fibre optic probe was secured with bandaging material. Flow was measured before administration and at 10 min intervals for the first 100 min, then at 20 min intervals until 4 h after administration of the morning dose on the seventh day of treatment.

Sweat production

A plastic sweat catchment device (SCD) was designed to collect all sweat produced following treatments. The SCD extended under the neck anteriorly, fitted securely around both forelegs, 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. At the lowest point of the SCD, which was located between the front legs, a funnel collected the

TABLE 1: Comparison of mean ± s.e. peak pharmacological responses after i.v. and oral administration of isoxsuprine HCl. Control i.v. and isoxsuprine i.v. data are from a previous study and are reprinted by permission from Harkins *et al.* (1996)

Variable	Oral control	Oral isoxsuprine	I.v. control	I.v. isoxsuprine
Heart rate (beats/min)	43.1 ± 2.9	42.3 ± 4.1	44.3 ± 3.7	141.6 ± 5.7*
Cutaneous blood flow (Khz)	520.8 ± 75.1	437.5 ± 118.6	500.6 ± 105.9	315.6 ± 119.4
Sweat production (ml/5 min)	0.0	0.0	0.0	12.5 ± 5.6*
Anal muscle tone (psi)	2.2 ± 0.1	2.9 ± 0.1	2.3 ± 0.2	1.2 ± 0.3*
Skin temperature (°C)	31.5 ± 0.3	32.1 ± 0.5	31.1 ± 0.3	26.7 ± 0.5*
Core temperature (°C)	37.8 ± 0.2	37.7 ± 0.2	37.8 ± 0.1	37.2 ± 0.1*
Spontaneous activity (per 5 min)	67.0 ± 22.9	85.0 ± 19.6	124.0 ± 74.5	393.0 ± 59.0*

*Significant difference ($P<0.05$) between control and isoxsuprine treatments.

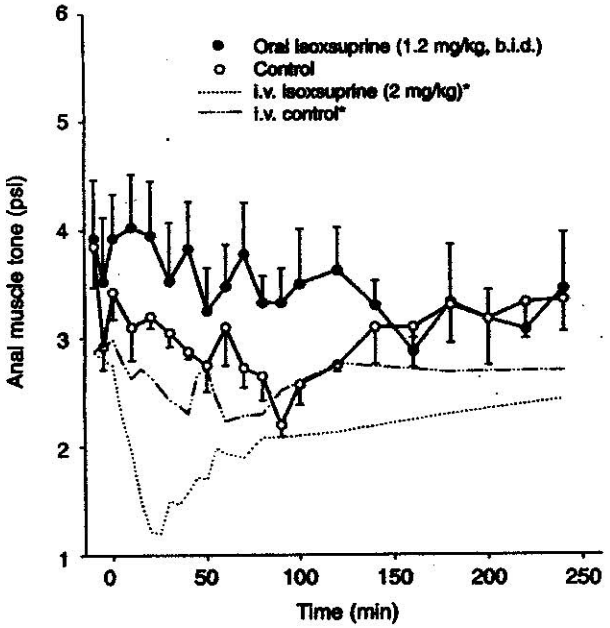

*Fig 3: Anal muscle tone following control and oral isoxsuprine treatments. *Harkins* et al. *(1996).*

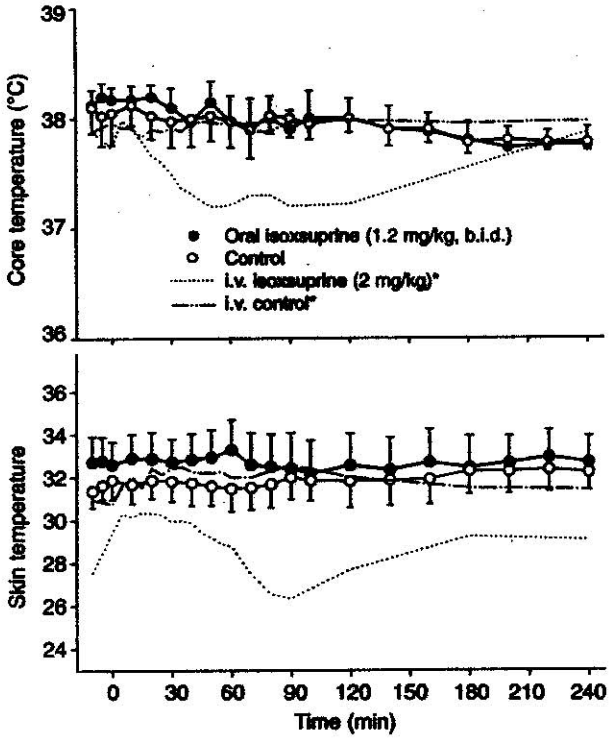

*Fig 4: Core and skin temperatures following control and oral isoxsuprine treatments. *Harkins* et al. *(1996).*

sweat into a graduated cylinder.

Anal tone

A bulb dynamometer[4] was used to measure changes in anal tone following isoxsuprine treatment. The instrument was originally designed to measure grip strength in arthritic patients, and the size of the bulb (8 cm long, 3.8 cm diameter) was suitable for insertion into the anus to measure maximal muscle contraction. Anal tone was measured before treatment and at 10 min intervals for the first 100 min, then at 20 min intervals until 4 h after administration of the morning dose on the seventh day of treatment.

Skin and core body temperature

Skin temperature was measured with a surface thermistor (Model 409B)[5] attached to the chest wall with a skin adhesive (Vetbond)[6]. Core body temperature was measured with a general purpose thermistor (Model 401)[5] placed 50 cm into the rectum and secured to the tail with adhesive tape. Temperatures were monitored by a digital thermometer (Model 8402)[7] before and at 10 min intervals for the first 100 min, then at 20 min intervals until 4 h after administration of the morning dose on the seventh day of treatment.

Spontaneous activity

The activity of the horse was detected by 4 Mini-beam sensors (SM31E and SM2A31R)[8] in a specially designed locomotor chamber described previously (Harkins *et al.* 1996). The outputs from the 4 sensors were summed and recorded on a data logger (CR10)[9] every 5 min. The total number of sensory activations was averaged over a 15 min period. Data were collected for 300 min after administration of the final dose on the eighth day of treatment. Data were expressed as mean step count per 5 min.

Statistical analysis

Data are presented as means ± s.e. Analysis of variance with repeated measures (Anon 1985) was used to compare values for each physiological variable at each measuring time obtained from the control animals with those from the isoxsuprine treated animals. Significance was set at $P<0.05$.

Results

Following oral administration of isoxsuprine, values for heart and respiratory rates, cutaneous blood flow, anal muscle tone, skin and core body temperatures, spontaneous activity and sweat production were similar to control values and are shown in Figures 1–5. Table 1 contrasts peak values following oral administration of isoxsuprine with corresponding values following i.v. administration (Harkins *et al.* 1996). There were significant differences between oral and i.v. values for all measured variables except cutaneous blood flow.

Discussion

Harkins *et al.* (1996) previously demonstrated that isoxsuprine (2 mg/kg bwt) administered i.v. significantly increased heart rate, locomotor activity, and sweat production. These changes were accompanied by decreased anal smooth muscle tone and decreased skin and core body temperatures consistent with increased heat loss through the skin (Table 1). The observed effects were consistent with an earlier study (Matthews *et al.* 1986) and yielded a coherent picture of the pharmacological actions of isoxsuprine in the horse after i.v. administration. Although the pharmacological effects were marked, they were relatively transient, with all variables returning to control values within 4 h of administration. While these experiments clearly demonstrated the pharmacological effects of isoxsuprine, they did not answer questions concerning the clinical and possible performance-altering effects after oral administration.

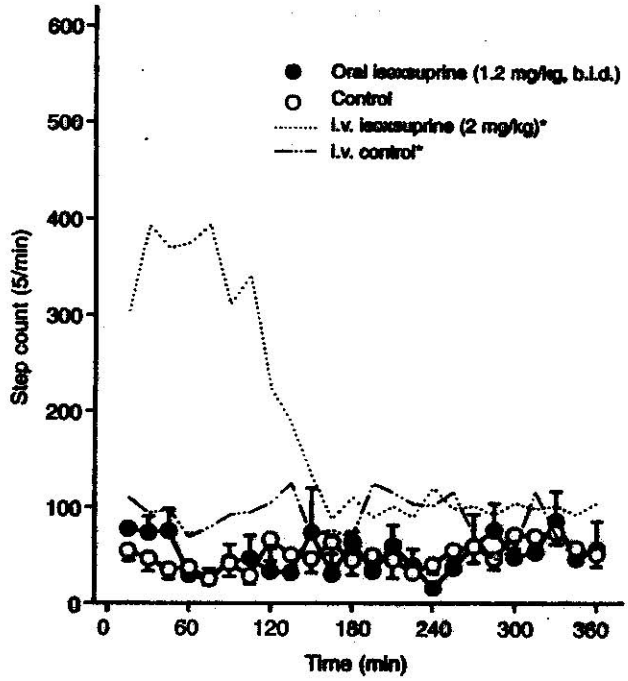

*Fig 5: Locomotor activity following control and oral isoxsuprine treatments. *Harkins* et al. *(1996).*

Although oral isoxsuprine is well established in clinical practice, its efficacy is poorly supported by objective data. In equine regulatory affairs, the question is even more crucial, since isoxsuprine or its metabolites have been detected in horse urine for up to 6 weeks after administration of the last oral dose, and identifications of isoxsuprine are common in post race urine samples.

The results in this paper show no evidence of pharmacological effects in the horse following 7 days treatment with oral isoxsuprine (1.2 mg/kg bwt b.i.d.; total dose of about 1.2 g/day). This dosage regimen was chosen to reproduce clinical use and the dose was continued for 7 days to ensure that any pharmacological effect was achieved in the test animals. The same evaluation procedures were used as described previously (Harkins *et al.* 1996); however, orally administered isoxsuprine had no effect on heart rate, cutaneous blood flow, sweat production, anal muscle tone, skin and core body temperatures, and spontaneous locomotor activity. The lack of a pharmacological response was markedly different from the responses seen after i.v. administration, when substantial effects were seen on most of the variables measured.

These results are in good agreement with Matthews *et al.* (1986), who reported significant pharmacological and behavioural effects of i.v. isoxsuprine (0.6 mg/kg bwt). In particular, they reported substantial decreases in blood pressure, vascular resistance, and stroke volume, accompanied by increases in heart rate and cardiac output. However, there were no measurable effects after oral administration of this dose b.i.d. for 4 days. Our study, which also showed no pharmacological effects after oral administration of a substantially higher dose for 7 days, both confirms and extends the findings of Matthews *et al.* (1986) concerning the lack of pharmacological effect of oral isoxsuprine. Furthermore, Matthews *et al.* (1986) were unable to detect plasma concentrations of isoxsuprine after oral dosing and concluded that plasma concentrations were insufficient to produce pharmacological responses in the cardiovascular system.

Comparison of the analytical results reported by Matthews *et al.* (1986) with their kinetic data suggests that plasma isoxsuprine concentrations of at least 30 ng/ml are required for pharmacological effects. Although the lower detection limit of their method was not defined, inspection of the data suggests that the peak plasma concentration of isoxsuprine obtained after oral dosing was less than 5 ng/ml.

More recent pharmacokinetic experiments by Joujou-Sisic *et al.* (1996) further support the hypothesis that plasma isoxsuprine concentrations following oral administration are inadequate to produce pharmacological effects. Furthermore, they showed that orally administered isoxsuprine (0.25 mg/kg b.i.d. for 3 days and 0.6 mg/kg b.i.d. on the fourth day) is rapidly conjugated, and that conjugated isoxsuprine was always present in plasma at a higher concentration (up to 500 times greater) than parent isoxsuprine.

A review of the data from Joujou-Sisic *et al.* (1996) suggests that isoxsuprine concentrations of about 50 ng/ml are required for pharmacological effects following i.v. administration. However, orally administered isoxsuprine had an apparent bioavailability of only 2.2%. Peak plasma isoxsuprine concentrations were never higher than 5 ng/ml and declined to less than 1 ng/ml by 1 h after dosing. These findings are consistent with the lack of pharmacological effects of oral isoxsuprine reported in the present study and the earlier experiments of Matthews *et al.* (1986).

The study by Joujou-Sisic *et al.* (1996) also explained how isoxsuprine could be detected for very long periods after its last administration. Since the glucuronide metabolite is cleared very slowly from plasma, isoxsuprine was detected in urine from orally dosed horses for up to 6 weeks after the last administration. This finding is consistent with the well known persistence of residues of isoxsuprine in post race urine samples.

In a recent study, Ingle-Fehr and Baxter (1996) used laser Doppler flowmetry to measure digital and laminar blood flow in normal horses. After the horses were treated orally with 1.2 mg/kg bwt of isoxsuprine, b.i.d. for 10 days, there was no effect on digital and laminar blood flows when measured on Days 2, 4, 7, and 10 of treatment. The authors concluded that the benefits of isoxsuprine treatment for ischaemic conditions of the foot were questionable. Intravenous isoxsuprine (0.6 mg/kg bwt) also failed to increase blood flow in the hoof wall laminae of horses when measured by laser Doppler flowmetry[1] (H.S. Adair, personal communication).

Although the above results show a clear and consistent picture of the pharmacology and pharmacokinetics of isoxsuprine in the horse, they are in apparent conflict with reports of therapeutic efficacy of oral isoxsuprine. Isoxsuprine is used in human medicine and is also used in equine medicine to improve blood flow and circulation in the foot. Clinical studies (Rose *et al.* 1983; Turner and Tucker 1989; Deumer *et al.* 1991; Wilson and Bolhuis 1996) have reported significant therapeutic responses in horses placed on oral isoxsuprine. If oral isoxsuprine is clinically effective and useful in the treatment of navicular disease and similar conditions of the foot, then such effects are subtle, are not identifiable by the methods of pharmacological analysis at our disposal and occur at very low plasma concentrations.

These apparent inconsistencies have been noted by others. Deumer *et al* (1991) noted that no free isoxsuprine could be detected in the plasma of horses following oral administration, which contrasted with their reported pharmacological activity.

The apparent lack of a pharmacological effect following oral isoxsuprine in horses prompted a careful review of the therapeutic efficacy and pharmacokinetics of oral isoxsuprine in

man. In both man and animals, vasodilation is widely reported following i.v. intra-arterial, and intramuscular administration of isoxsuprine. However, when administered orally, isoxsuprine failed to increase blood flow in the calf, foot, or hand of human subjects. Furthermore, blood pressure was unchanged and there was no difference in heart rate (Zsoter and Baird 1974).

In another study the use of isoxsuprine in man with Raynaud's phenomenon, was assessed using colour thermovision to visualise the degree of circulation in the hands. Following i.v., administration (8 mg), isoxsuprine positively affected various cardiovascular parameters (Wesseling and Wouda 1975). On the other hand, there was no difference in heat emission from the hands and fingers between subjects given control and oral isoxsuprine (20 mg) treatments (Wesseling *et al.* 1981).

Since isoxsuprine stimulates β-adrenergic receptors and causes vasodilation by direct relaxation of vascular smooth muscle, it was used in a study of vasculogenic impotence in men (Knoll *et al.* 1996). After 2 months oral medication (10 mg t.i.d.), the study concluded that oral isoxsuprine was not effective in the treatment of patients with vasculogenic erectile dysfunction.

This present study is the second step in a 3 phase approach to determining the relationship between analytical findings and pharmacological effects of isoxsuprine. The third phase will be the design of kinetic experiments to answer questions concerning the relationship between analytical findings and pharmacological effects (Tobin 1981).

As a practical matter for equine veterinarians, this study and other recent reports raise concerns that clinicians should: 1) rely on their clinical experiences to determine the circumstances for which isoxsuprine appears to be effective, 2) be aware that isoxsuprine metabolites may remain detectable in urine for at least 6 weeks and 3) warn clients about cross-contamination of feed or water to preclude chemical identifications of isoxsuprine in the urine of untreated horses.

In summary, there is limited objective evidence to suggest pharmacological responses to oral isoxsuprine at or below the dose levels used in these experiments in horses. Furthermore, there is compelling pharmacokinetic evidence to suggest that isoxsuprine is subjected to almost complete first-pass metabolism when administered to horses by the oral route. The high extent of first-pass metabolism means that orally administered isoxsuprine is unlikely to attain pharmacologically effective plasma concentrations. In this regard, review of the available pharmacokinetic data suggests that plasma concentrations of at least 30 ng/ml are required for pharmacological effect whereas to date concentrations, reported in the literature, following oral dosing experiments have not risen above 5 ng/ml.

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Manufacturers' addresses

[1]Sigma Chemical Co, St Louis, Missouri, USA.
[2]St Paul, Minnesota, USA.
[3]Johnson and Johnson Medical Inc, Arlington, Texas, USA.
[4]North Coast Medical Inc, San Jose, California, USA.
[5]YSI Incorporated Yellow Springs, Ohio, USA.
[6]3M Animal Care Products, St Paul, Minnesota, USA.
[7]Cole-Parmer Instruments Co, Niles, Illinois, USA.
[8]Banner Engineering, Minneapolis, Minnesota, USA.
[9]Campbell Scientific, Inc, Logan, Utah, USA

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