ORIGINAL ARTICLE

Effects of isoxsuprine hydrochloride on electrocardiographic and trace element status in sheeps

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Abstract

Objective: To study the effect of isoxsuprine hydrochloride on the ischaemic electrocardiographic change and trace element status in sheep.

Methods: This study was conducted from March 16 to 23, 2012, at Istanbul University, Turkey, and comprised sheep aged 6 months. The animals were divided into two equal groups. The control group was fed a standard diet and had free access to water. In the experimental group, isoxsuprine hydrochloride was injected at a dose of 0.6 mg/kg through the intramuscular route. Electrocardiographic changes, including creatine kinase and cardiac troponin-I, and serum levels of selenium, copper, calcium, magnesium, iron and zinc were investigated in healthy sheep. SPSS 15 was used for statistical analysis.

Results: The 14 sheep were divided into two groups of 7(50%) each. The overall mean weight of the study population was 35±10kg. Selenium, calcium, iron and zinc concentrations did not show any difference in serum samples ($p>0.05$). However, copper and magnesium concentrations decreased in serum after the administration of the drug ($p<0.05$). In the experimental group, ST segment depression and abnormal T-wave was found in 6(86%) animals within 60min.

Conclusion: Isoxsuprine hydrochloride increased cardiotoxicity risk in sheep.

Keywords: Isoxsuprine hydrochloride, Sheep, Cardiac troponin-I, cTnI, Creatine kinase-MB, CK-MB. (JPMA 66: 938; 2016)

Introduction

Isoxsuprine (IP) is a compound belonging to the β-phenylethylamine group and has a widespread medical background with its human and animal clinical use.[1] IP was first synthesised in 1956.[2] With regards to its molecular structure, this compound is similar to adrenalin and papaverin (Figure). It has a sympathomimetic effect and is 40 times more spasmolytic than papaverine.[3,4] It is used in the treatment of Raynaud's phenomenon in human medicine and its primary use is to prevent pre-term labour and tocolysis.[5] In 1961, the first beta adrenergic agonist drug to be used in tocolysis was IP.[6] In 1965, Devries and Wilson stated that IP could be used in cattle and sheep for uterus relaxation in various obstetric situations such as, simple dystocia, embryotomy and caesarean section (CS).[2] In veterinary practice today, it is used especially in the treatment of equine navicular disease and laminitis. It is also used as a tocolytic drug in horses, cows, pigs, sheep and goats. The recommended dose is 0.4-2mg/kg intramuscularly (IM) and tocolysis occurs within 10-15 minutes following administration.[2,4]

Sympathomimetics suppress uterus contractions by stimulating β-adrenergic receptors. IP is a β_1 and β_2-adrenomimetic drug and is used to relax the uterus, facilitate foetal mutations and exteriorise the uterus during CS.[7,8] Its smooth muscle-relaxing action is brought about through the increase in myometrial cyclic adenosine monophosphate (cAMP) due to the activation of adenylyl cyclase by the stimulation of β_2 receptors, leading to a decrease in uterus contractions.[9,10]

In clinical researches performed to investigate the activity between beta adrenergic agonists as tocolytic agents, it was found that IP is more active compared to ritodrine and isoproterenol.[11] However, its lack of activity towards specific β_2-receptors causes major side effects characterised by tachycardia and hypotension in human obstetrics. It has been reported that, pulmonary oedema may also occur with IP use in humans. Similar effects have been reported to occur in veterinary obstetrics.[12] Therefore, medical usage of this drug is possible with further research into drug activity and safety.

Laboratory testing plays a premier role in drug usage. Specific biochemical tests are used. For example, the

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cardiac-specific protein troponin is the most important cardiac enzyme at determining myocardial damage in the heart.[13] Regular monitoring of these enzymes constitutes the most important part of treatment protocol. IP belongs to the betamimetics group among tocolytic agents and reports state that maternal and foetal side effects may be observed during IP administration for tocolysis.[14]

Studies are scarce on the effect of IP on the cardiovascular system in animals. Additionally, no studies have been found regarding the evaluation according to cardiac enzymes. While studies in animals focus on the tocolytic activity of the drug, studies in humans concentrate on premature and excessive term labour. One reason for selecting sheep in this study was the rising need for large mammal species in cardiovascular research. Another reason was the similarity of sheep cardiac monocytes to those of humans due to possessing 1-4 nuclei.[15] The current study was planned to examine the effect of IP use on serum creatine kinase M and B (CK-MB) and cardiac troponin-I (cTnI) enzymes and on selenium, copper, calcium, magnesium, iron and zinc electrolytes. Electrocardiographic changes in healthy sheep were also examined.

Subjects and Methods

This study was conducted from March 16 to 23, 2012 at Istanbul University, Turkey, and comprised healthy Kivircik sheep aged 6 months that were 25-50kg in weight and were not pregnant. The animals were obtained from Istanbul University research farm. The animals were divided into two groups after approval from the Faculty of Veterinary Medicine at the university. The control group was fed a standard diet and had free access to water. In IP group isoxsuprine hydrochloride was injected at a dose of 0.6 mg/kg (Sanovel, Turkey).

Electrocardiographic (ECG) analyses was done after the administration of the drug within 60 minutes. ECG records were taken with the appropriate technique with Petas Kardiyopet 300 Electrocardiograph. Blood pressure (BP) and oxygen saturation (SpO_2) was measured with Edan M9B patient monitor. For this purpose, non-invasive BP (diastolic, systolic and mean) was taken from femoral or metatarsal arteria. SpO_2 was measuered from arterial blood.

Cardiac enzymes were investigated in serum after the administration of the drug within 24 hours. CK-MB levels were measured with the enzymatic method using the Abbott C8000 autoanalyser and identical commercial kits.

Cardiac troponin-I (cTnI) and total testosterone levels were determined using the IMMULITE® commercial kit using solid-phase, competitive chemiluminescent enzyme immunoassay with an immulite one immunoassay analyzer (DPC, USA). Due to the commercial kit properties, readings below 0.20 ng/ml could not be measured quantitatively and were considered to be negative. For cTn-I, values of 0.20 mg/ml and above were considered to be positive and measured quantitatively.

Trace element measurements were done in serum after the administration of the drug within 24 hours with Atomic Absorbtion Spectrophotometer (Schimadzu AA-6800). Selenium (Se) calculations were made with graphite oven (Schimadzu Graphite Furnace Atomizer EX7).

Data was analysed using SPSS 15. Normally distrubuted variables were assessed with student t test and non-normally distrubuted parameters with Mann-Whitney U test. Statistically significance was set at $p<0.05$ level.

Results

The 14 sheep were divided into two groups of 7(50%) each. The overall mean weight of the study population was 35±10kg.

Among the trace elements, decrease was noted only in the rates of Mg and Cu levels ($p>0.05$) (Table-1). Mean Mg and Cu levels of 2.34±0.65 and 80.45±12.41 in the control group, dropped 24 hours later to 1.05±0.83 and 63.0±23.76 in the experimental group.

While no statistical difference was determined between systolic and diastolic pressures in ECG analyses, heart rate (HR) exhibited a statistically significant increase ($p<0.05$)

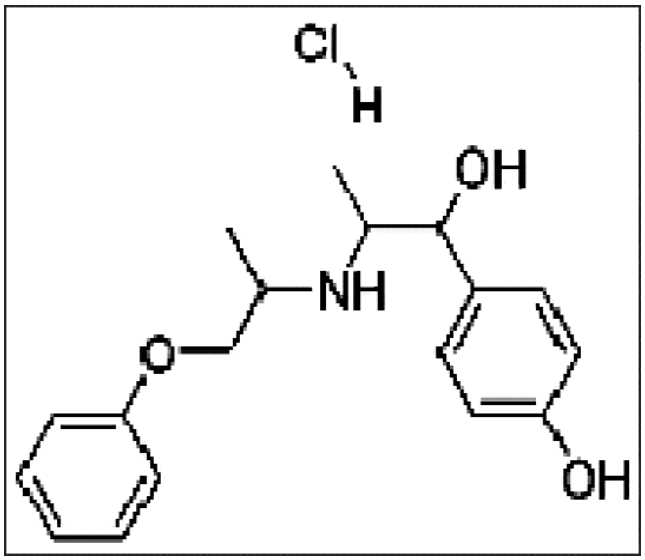

Figure: Chemical formula of isoxsuprine.

Table-1: Levels of trace elements.

	Control	IP	p
Ca (mg/dl)	8.1±1.3	9.3±0.7	0.06
Se (µg/dl)	123.0±11.4	117.0±8.6	0.32
Fe (µg/dl)	196.5±21.4	175. 4±2.3	0.16
Zn (µg/dl)	39.1±7.3	33.5±4.7	0.21
Mg (mg/dl)	2.34±0.6	1.05±0.8	0.07
Cu (µg/dl)	80.45±12.4	63.0±23.7	0.11

*P < .05
IP: Isoxsuprine
Ca: calcium
Se: selenium
Fe: Iron
Zn: Zinc
Mg: Magnesium
Cu: Copper.

Table-2: Mean values and standard deviations of blood pressure parameters and heart rate in sheeps.

	Control	IP	p
Systolic (mmHg)	93.7±4.8	100.6±11.1	0.09
Ortalama (mmHg)	74.3±6.6	80.1±8.4	0.80
Diastolic (mmHg)	63.7±7	69.0±9.2	0.98
SpO_2 (%)	84.3±4.5	94.1±2.5	0.84
HR (bpm)	72.1±6.4	105.2±8.8	0.021*

*P < .05
IP: Isoxsuprine
SpO_2: Oxygen saturation
HR: Heart rate.

Table-3: Levels of cardiac enzymes and lipid profiles.

	Control	IP	p
Troponin (cTnI) (ng/ml)	0.18 0.01	0.27 0.02	0.03*
CK-MB (U/l)	82.3 5.3	105.2 6.2	0.72
Triglyceride (mg/dl)	23.1 6.7	33.2 4.2	0.98
HDL (mg/dl)	34.2 8.3	39.1 5.1	0.65
LDL (mg/dl)	37.4 3.1	45.1 2.3	0.67
VLDL (mg/dl)	4.0 0.34	3.3 1.4	0.78

*P < .05
IP: Isoxsuprine
CK-MB: Creatine kinase-MB
HDL: High-density lipoprotein
LDL: Low-density lipoprotein
VLDL: Very low-density lipoprotein.

(Table-2). HR was 72.1±6.4 in the control group, while in the IP group it was 105.2±8.8, 24 hours later.

Also, cTnI displayed a statistically significant increase in the IP group compared to the control group ($p<0.05$) (Table-3). The level of cTnI in the control group was 0.18±0.01 ng/ml, but 24 hours later, it was 0.27±0.02 ng/ml in the IP group.

Discussion

Today, the most suitable animal species for cardiac human clinical setting is sheep.[16] Sheep are being used more widely due to the advantages of their easy handling, docility and slow growth rate.[17] Also, despite the fact that human cardiac disease research is carried out based on mice and rat models, the morphological and physiological features of the heart exhibit differences rather than similarities. For example, the human cardiomyocyte cell cycle and cardiomyocyte morphology is similar to large animals, particularly sheep. Sheep are preferred in acute myocardial infarction (AMI) and heart failure studies due to the resemblance between sheep and human cardiac myocytes.[18]

Cardiac damage assessment criteria focus on clinical history, ECG, serum enzymes and biochemical markers. ECG is a useful test for the diagnosis of heart disorders. Among the most important findings in the present study is the ST-segment depression and abnormal T-wave in 6 of the 7 sheep administered IP. ST-segment depression and an abnormal T-wave are usually ECG abnormalities. ECG ST-segment depression has long been recognised as a pattern of ischaemia. However, the explanation of mechanisms responsible for this is controversial. In order to make a timely diagnosis of high-risk myocardial ischaemia and electrolyte or drug-related abnormalities, it is extremely important to know the different ischaemic and non-ischaemic morphological features. ST-segment depression and changes in the T-wave may be secondary to abnormalities of depolarisation ie, pre-excitation or abnormalities of QRS voltage or duration. However, there was no QRS abnormality in this study. On the other hand, ST-segment depression and abnormalities in the T-wave may also occur without QRS abnormality. This situation is called primary repolarisation abnormalities. Pericarditis, myocarditis, drugs (digitalis, anti-arrhythmic drugs) and electrolyte disorders may be listed among the reasons for this kind of ischaemia.[19]

There are studies reporting that ST-segment changes are an indicator of myocardial ischaemia. ST-segment depression is the most common finding of ischaemia. It has been stated that the T-wave changes accompanying this finding may be due to myocardial hypoxia.[20] In a study in sheep[21] to highlight the relationship between ST-segment depression and ischaemia, attention is drawn to epicardial signs measured at various degrees of transmural ischaemia. In that experimental study, ST-depression occurred at one of the lateral borders of the

epicardium or side zones, in healthy and ischaemic heart tissue. Depression occurred in the ischaemic region either with or without ST-elevation. The ST-segment depression and T-wave abnormality, determined in 6 of the 7 sheep in this study, create a risk regarding cardiac safety during clinical use of the drug.

Cardiac enzymes investigated in this study included the myocardial isoenzyme of CK-MB and cTnI. Cardiac troponin is known as the gold standard for most animal species in determining myocardial injury. In the 2000s, the American College of Cardiology and the European Society of Cardiology reported cTnI, in particular, to be a highly sensitive and specific biomarker of myocardial injury. This is also important regarding risk stratification and an increase in this biomarker gives information about the clinical severity of the disease and life expectancy. Furthermore, it can be used as a clinical biomarker in cardiotoxicities related to sympathomimetics and anticancer drugs and diseases including; cardiac injury, unstable angina, minimal infarct, left ventricular hypertrophy, congestive heart failure, pulmonary embolism, blunt trauma, sepsis, moderate renal disease, renal failure and diabetes mellitus.[13] In this study, a significant increase was determined in serum levels of cTnI and CK-MB in the 24 hours after IP administration. One study[22] reported a significant cTnI increase within 1 day following ischaemia in sheep. Also, it has been stated that, cTnI exhibits a significant increase within 1 day after myocardial injury in humans.

All of the betamimetics used for the purpose of tocolysis cause an increase in HR and stroke volume generating a substantial increase in cardiac output. This is frequently 50% more than the existing increased cardiac output brought about by normal pregnancy. In a study[23] in which uterine artery blood flow (UtBF) and umbilical vein blood flow (UmBF) of IP and salbutamol in non-labouring chronic sheep preparations were investigated, it was reported that during the administration of IP and salbutamol to lambs, UtBF and maternal arterial pressure decreased significantly and that tachycardia, hyperglycaemia and acidaemia developed.

While studies are present indicating that phenylethylamine group drugs should not be used for the purpose of premature labour arrest due to their sympathomimetic and cardiovascular side effects, other studies also state that the cardiovascular side effects of IP are minimal. In a study[3] where the uterus activity of IP was pharmacologically investigated, uterus activity was reported to have decreased in 66% patients. Furthermore, the presence of maternal side effects of the drug accompanied by tachycardia and hypotension was reported. In a study performed in pregnant sheep,[12] similar maternal and foetal side effects were reported. Similarly, it has been reported that, following IP administration to horses, a significant tachycardia developed alongside decreased systemic BP. The additional intense sweating has been thought to be due to either the direct effect of the drug on the CNS or the increased peripheral blood flow.[24] Tachycardia developing in relation to the stimulation of β-adrenergic receptors is a serious side effect and in some cases may require cessation of the treatment. Some sources recommend co-administrative use of cardio-selective β-adrenergic blockers in order to reduce this side effect.[25] In the present study, the tachycardia and hypotension findings in relation to IP use in healthy and non-pregnant sheep were consistent with these studies.

In the light of information[26] tocolytic agent use may increase myocardial infarct incidence as well as the findings in the present study, it has been determined that use of the drug may cause a significant risk regarding cardiotoxicity in sheep. Also, due to similarities between cardiac myocytes, care should be taken with its use in humans. Some authors[27] propose that nifedipine is the most effective and reliable tocolytic in the specific treatment of premature labour. Although this information is contradictory to the present European first-line approach in which betamimetics are used, safer new agents are needed that will end some contradicting approaches regarding maternal and foetal toxicities.

Conclusion

Isoxsuprine hydrochloride increased cardiotoxicity risk in sheep.

Disclosure: No

Conflict of Interest: No

Funding Sources: No

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