Leishmania spp. ile Doğal Enfekte Köpeklerin Artrosentezinde Ksilazin'in Elektrokardiyografik Etkisi

Electrocardiographic Effects of Xylazine in Arthrocentesis of Dogs Naturally Infected with *Leishmania* spp.

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ÖZ

Amaç: Bu çalışmanın, amacı *Leishmania* spp. ile doğal enfekte köpeklerde, genu eklemlerinin aseptik artrosentezi için preanestezik ajan olarak ksilazin ile sedasyon uygulanan köpeklerdeki elektrokardiyografik değişiklikleri araştırmaktı. **Gereç-Yöntem:** On üç enfekte yetişkin melez köpeğe (8 erkek, 5 dişi) premedikasyon olarak 1,5 mg/kg Xylazine HCl intramusküler olarak uygulandı. On iki derivasyonlu bilgisayarlı elektrokardiyografik muayeneler premedikasyondan önce ve indüksiyondan 30 dakika sonra yapıldı. Poliartritli köpeklerin artikulasiyo genu eklemlerinde amastigot tespiti için sinoviyal sıvı (SF) yaymaları mikroskop altında değerlendirildi. Kanin visseral leishmaniasis (CVL) tanısı *Leishmania* test kitleri (IDEXX SNAP® Leishmania Test) ve immünofloresan antikor testine (IFAT) dayanılarak konuldu. **Bulgular:** Mikroskopik olarak 11 köpeğin (%84,61) genu eklemlerinin SF'sinde amastigotlar tespit edildi. Ksilazin uygulamasından önce ve ilk uygulamada iki olguda ya hafif sinüs taşikardi ya da atriyal prematüre kompleks görüldü. Ksilazin uygulamasından 30 dakika sonra bradikardi (6/13), hafif sinüs taşikardi (2/13), atriyal prematüre kompleks (APC; 2/13), ventriküler prematüre kompleks (VPC; 2/13) ve birinci derece AV blok (1/13) görüldü. **Sonuç:** Ksilazin ile premedikasyon bradikardi, hafif sinüs taşikardi, APC/VPC veya birinci derece AV blok gibi istenmeyen yan etkilere neden olur ve bu da CVL'li köpeklerde ksilazin kullanımının dikkatle yapılması gerektiğini düşündürmektedir. Özellikle herhangi bir sedatif uygulamadan önce kardiyolojik muayene yapılmalıdır. Ayrıca, doğal enfekte köpeklerde *Leishmania* spp. enfeksiyonunun parazitolojik teşhisi için SF analizinin avantajlı olabileceği sonucuna varılmaktadır.

ABSTRACT

Objective: Aim of this study was to investigate the changes in electrocardiographic (ECG) parameters in naturally *Leishmania* infected dogs sedated with xylazine as a preanesthetic agent for aseptic arthrocentesis of the stifle joints. **Material and Methods:** Thirteen diseased adult mix dogs (8 male, 5 female) were administered 1.5 mg/kg Xylazine HCl intramuscularly as premedication. Twelve-lead computerized ECG examinations were made before premedication and at 30 min after induction. The synovial fluid (SF) smears were evaluated under microscopy for amastigotes detection in stifle joints of dogs with polyarthritis. A diagnosis of canine visceral leishmaniasis was made basing it on *Leishmania* test kits (IDEXX SNAP® Leishmania Test) and the immunofluorescence antibody test (IFAT). **Results:** The amastigotes were detected microscopically in the SF of stifle joints of 11 dogs (84.61%). Before xylazine administration and on initial administration, either mild sinus tachycardia or atrial premature complex (APC) was detected in two cases. Following 30 minutes of xylazine administration, bradycardia (6/13), mild sinus tachycardia (2/13), APC (2/13), ventricular premature complex (VPC; 2/13) and first degree AV block (1/13) were noticed. **Conclusion:** Premedication with xylazine induced undesirable side effects involving bradycardia, mild sinus tachycardia, APC/VPC or first degree AV block, which might suggest that the usage of xylazine in dog with CVL, must be done with cautions. Especially, prior to any sedative administration, cardiologic examination must be performed. It might also be concluded that SF analysis for the parasitological diagnosis of *Leishmania* spp. infection in naturally infected dogs might be advantageous.

INTRODUCTION

Canine joint diseases may be encountered in dogs of all age and breeds and evaluated by the cause as infectious and non-infectious. Inflammatory arthritis may result from Canine Visceral Leishmaniasis (CVL),[1-3] considered one of the most important protozoal diseases[4] and a common infection in the Mediterranean area.[5]

During CVL, arthritis may exist in two ways: first in existence of the parasite in the joint with granulomatous inflammatory response and second a type III hypersensitivity respond, with deposition of immune complexes within the joint.[3]

Arthrocentesis, synovial fluid aspiration from the joints via needle, is generally conducted under deep sedation or general anaesthesia. Frequently it is used for both diagnostic evaluation (synovial fluid analysis, microbiologic cultures, cytological evaluation, and injection of contrast material) and/or therapeutic applications (joint lavage to removal of fibrin and exudate, decompression of swelling, and drug injections).[6] Alpha-2 adrenoreceptor agonists (α_2-agonists) are attractive options in arthrocentesis because of their characteristics, making their usage popular as premedication for general anaesthesia. Their other pharmacological effects include analgesia, sedation, and anxiolysis.[7-9]

Xylazine has long been used in dogs for sedation and anaesthetic procedures.[9,10] Indeed, α_2-agonists, through stimulation of central and peripheral adrenoreceptors, significantly affect cardiovascular function, which becomes most significant in sick, unstable, or cardiovascular compromised patients.[7-9] From a point of view the cardiovascular effects of xylazine is quite important because CVL might affect cardiovascular system, in which the usage of xylazine remains unclear. Besides CVL might also affect cardiovascular system, foremostly causing myocarditis,[11-13] which might have influence or limit on the usage of drugs, such as xylazine, making alterations on the cardiologic parameters. The main negative cardiovascular effects of all α_2-agonists include bradycardia, and arrhythmias (1st and 2nd degree atrioventricular heart block), a dramatic reduction in cardiac output by up to 50% and an increase in systemic vascular resistance (SVR). [9,10]

Xylazine as a traditional agent and was the first α_2-adrenergic agonist to be used as a sedative and analgesic in veterinary practice.[14] Considering the arrhythmogenic and cardio-depressant effects of α_2-agonists, clinicians should be careful on the use of xylazine.[15-19]

Electrocardiography (ECG) is a valuable tool for interpretation of the possible arrhythmogenic effects of drugs on the cardiovascular system. It may provide valuable data regarding alterations in the electrophysiological function with being easy and cheaper method compared to other (none) invasive methods.[20] No previous work has been published on the effects of the xylazine on the ECG parameters in dogs with CVL subjected to arthrocentesis. These measurements are of value in ECG, suggesting possible safety usage of this compound. Therefore, the objectives of the present study were 1) to evaluate ECG parameters in naturally Leishmania-infected dogs under the sedation with xylazine for aseptic arthrocentesis of the stifle joints, and to evaluate diagnostic utility of microscopically SF analysis for leishmaniasis in dogs with polyarthritis.

MATERIAL and METHODS

Since the procedures used in the present study were classified as “routine clinical applications for diagnosis and treatment”, there was no need to get permission of local ethics committee, according to the Regulation on the Working Procedures and principles of Animal Experiments Ethics Committee published in the Official Gazette of Türkiye (15.02.2014/28914). Thirteen symptomatic dogs, 8 females and 5 males, were involved in this study. The dogs were referred to the University of Adnan Menderes, Faculty of Veterinary, Departments of Internal Medicine and Surgery due to CVL. The dogs presented polyarthritis, stiffness on hind legs, exfoliative dermatitis, onychogryphosis, lymphadenopathy, and/or loss of weight were screened for the presence of CVL due to CVL-related clinical signs.[21-23] In these dogs, CVL was diagnosed by a SNAP test, and then confirmed by IFAT, as described below. On the other hand to comparatively evaluate the efficacy of Xylazine HCl, control group was designed with Leishmania negative dogs which were presented for arthrocentesis. These dogs were sedated with the same protocol described for Leishmania positive dogs. The dogs were diagnosed with immune-mediated (non-infectious) nonerosive polyarthritis (IMPA).[24,25]

At initial administration, a tentative diagnosis of CVL was made by rapid diagnostic test kits (IDEXX SNAP® Leishmania Test) and by IFAT technique, with the cut-off titer of 1:40 as confirmatory.[26] The dogs underwent to sedation with Xylazine HCl (1.5 mg/kg IV) prior to arthrocentesis.

Afterwards, the samples of SF were obtained from both stifle joints of each animal by aseptic arthrosynthesis as described by Clements.[6] The feasible amount of SF, summing up 26 arthrocentesis, were collected[27] via a fine needle (25x7mm), and a 5 ml syringe containing ethylenediamine tetraacetic acid (EDTA) to prevent clot formation. ECG signals of each animal were obtained by 12-channels and 10 - lead computerized ECG device (BTL®-08 MT Plus), simultaneously. ECG was performed by bi-polar extremity derivations with augmented leads, and derivation II was used to evaluate ECG parameters including heart rate and rhythms, durations and amplitudes of ECG waves and segments.

Afterwards, smears were prepared on glass slides without centrifugation, following arthrocentesis and then were stained using Wright-Giemsa. At least 5 glass slides were prepared from the SF obtained from each stifle joint. The stained smears were examined

under a microscope (1000X) and classified as positive or negative for the presence of *Leishmania* spp.

RESULTS

Leishmania snap test kits revealed that all 13 cases were positive. Besides, IFAT showed that all cases had seropositive with titters ranging from 1:40 to 1:640. The SF specimens were examined under microscopy and amastigotes were noticed in the SF of both stifle joints of 11 dogs (84.61%). Before xylazine administration and on initial administration, mild sinus tachycardia and APC were observed in 1 dog amongst cases. Following 30 minutes of xylazine administration, bradycardia (6/13), mild sinus tachycardia (2/13), APC (2/13), VPC (2/13), and first-degree of AV block (1/13) were noticed (Table 1).

Table 1. Electrocardiographic changes during arthrocentesis procedure in dogs with CVL. Control group involved Leishmania negative dogs with IMPA submitted for arthrocentesis and sedated according to the protocol described.

	Before and After Xylazine Administration			
	Diseased dogs		Control dogs	
ECG abnormality	**Before**	**After**	**Before**	**After**
Mild sinus tachycardia	1/13	2/13	-	-
Bradycardia	-	6/13	-	4/7
Atrial premature complex	1/13	2/13	-	-
Ventricular premature complex	-	2/13	-	-
First degree AV block	-	1/13	-	1/7

DISCUSSION

The present study showed the ECG changes in naturally infected Leishmaniasis in dogs, before and after intramuscular xylazine injections, underwent arthrocentesis. In a previous experimental study evaluating cardiovascular and respiratory effects of romifidine and/or xylazine in ketamine anaesthesia in dogs,[28] ECG showed arrhythmias. In that study, the vast majority of cardiovascular alterations induced by xylazine or romifidine involved bradycardia. Second-degree atrioventricular (AV) heart block was also noticed in some of the dogs, whereas there was no evidence of first-degree and third-degree AV heart block.[28] In the present study, in good accordance with the literature information,[15-20] bradycardia most seen arrhythmia in 8 out of 13 dogs. Cardiac and respiratory depression caused by xylazine has long been recognized in the literature,[17-19,28] whereas their details are lacking in animals subjected to arthrocentesis, an important procedure in the diagnosis of polyarthritis in dogs. In parallel line with the purpose of this study, before the arthrocentesis which was performed to collect SF samples for the diagnosis of CVL, xylazine was administered to all cases. As aforementioned above, xylazine administration lead to the changes in cardiac electrophysiology, such as bradycardia, mild sinus tachycardia, APC/VPC or first-degree AV-block in dogs involved in the present study. The results were comparable to those of xylazine administration in other animal species.[29-32]

Xylazine caused bradycardia and AV-block in goats injected intravenous xylazine HCl at a dose of 0.1 mg/kg.[29] This should be interpreted cautiously because of animals episodes of missed[30,31], and irregular heart-beats along with bradycardia after xylazine administration.[32] In ponies, intravenous administration of xylazine caused sinoatrial (SA) blocks and transient AV-blocks within the 1st minute of its injection.[33] Similarly ECG alterations were observed in the dogs involved in the present study. Besides, in control group, 4 out of 7 *Leishmania* negative dogs presented and sedated for arthrocentesis suffered from bradycardia. Although only a limited portion of control cases were enrolled, due to ethical concerns, it may be comparatively and safely suggested that xylazine side effects were more often detected in dogs with Leishmaniasis, in contrast to dogs with IMPA.

Serological examinations, mostly Enzyme-Linked Immunosorbent Assays (ELISAs) and IFATs are strongly suggested by the World Organization for Animal Health[34] for the diagnosis of CVL. Contrarily, serological studies may cause false-positive results (i.e. cross reactions with other etiological agents).[27] Besides, serological tests are more expensive than the other techniques of parasitological diagnosis.[35] Parasitological analyses provide 100% specificity, and are reasonably priced with variable sensitivity.[36] Samples of skin lesions, lymph node, spleen and bone marrow might be used for parasitological diagnosis of CVL in naturally infected dogs, whereas the use of SF for this propose has been rarely reported.[36,37] Arthritis might be a sequel of CVL, due to the presence of the *Leishmania* spp. or immune complex deposition in the joint.[27] Relatively very few studies have already stated the identification of amastigotes on canine SF.[22,27,38] In the present study, SF analysis for the parasitological diagnosis of *Leishmania* spp. infection in naturally infected dogs revealed that 11 specimen out of 13 were detected to have amastigotes, suggesting the advantage of this technique in clinical settings.

CONCLUSION

Premedication with xylazine induced undesirable side effects involving bradycardia, mild sinus tachycardia, APC/VPC or first-degree AV block which might

suggest that the usage of xylazine in dog with CVL must be done with cautions. Especially prior to any sedative administration, cardiologic examination should be performed. Thus, based on these findings, it may be recommended to administer anti-arrhythmic drugs such as atropine to prevent xylazine-induced arrhythmias before xylazine administration.

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