DOES MORPHINE USE INCREASE RISK OF ATHEROSCLEROSIS IN ANIMALS ON NORMAL OR HIGH-CHOLESTEROL DIET?

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Abstract

INTRODUCTION: Opioid peptides and exogenous opioids such as morphine have important effects on the cardiovascular system. Today, the opioid system is being considered as a therapeutic target receptor for reducing myocardial ischemia through inhibiting the G protein. Opioid addiction, on the other hand, is one of the major challenges facing humanity and the truth about the effects of opium use on the cardiovascular system is often misted by wrong beliefs. The effect of an exogenous opioid (morphine) on the development and progression of fatty streaks in hypercholesterolemic rabbits was investigated in this study.

METHODS: The rabbits were randomly divided into four groups (five in each group): normal, normal + morphine, high-cholesterol, and high-cholesterol + morphine. Biochemical parameters including total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), malondialdehyde, triglyceride (TG), fasting blood sugar (FBS), quantitative chronic reactive protein (CRP), coagulation factor VII, fibrinogen, platelet count, RBC count, WBC count and hemoglobin were measured at the start and end of the study. Pathological studies were conducted on the right and left coronary arteries of the animals to look for evidence of fatty streak formation.

RESULTS: The results showed that morphine administration along with a normal diet led to a significant increase in levels of cholesterol, coagulation factor VII, and fibrinogen, while enhancing fatty streak formation in the right and left coronary arteries ($P<0.05$); it also significantly increased levels of coagulation factor VII, platelets, and weight of rabbits ($P<0.05$). However, it had no effect on fatty streak formation in the right and left coronary arteries.

CONCLUSION: This study demonstrates that morphine use with both normal and hypercholesterolemic diet increases the risk factors of cardiovascular diseases and atherosclerosis, although it accelerates the development of early atherosclerotic lesions only when administered with normal diet.

Keywords: Atherosclerosis, Morphine, High Cholesterol Diet, Animal Study.

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Introduction

Morphine is an alkaloid found in opium which exists endogenously in bodies of humans and animals.[1] As an endogenous opioid molecule, morphine, act as a hormone or a neurotransmitter-like substance in humans or animals.[2] Exogenous morphine bond with receptors resembling endogenous opioids in the brains of mammals.[3] Thus far, a number of opioid receptors have been found in the bodies of humans

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and animals. It has been found that opioid alkaloids may bond with more than one receptor type.[4-7] Opioid receptors are non-existent on the surface of atrial and ventricular myocardial cells, but they are present on nerve cells supplying the heart and coronary endothelial cells. Delta and kappa receptors are the most predominant cardiac opioid receptors, respectively.[8] The heart muscle is capable of synthesizing and storing opioid peptides. Opioid peptides may be secreted from nerve cells supplying the heart.[9-10] Studies have shown that production and secretion of opioid peptides and their serum levels increase in stressful conditions, disease, and cardiac ischemia. Otherwise stated, opioid receptors offer protection against damage from ischemia and arrhythmias.[11-12] Addiction constitutes a major crisis in human communities. Opium use and its effects on the cardiovascular system are often misted by wrong beliefs. The effect of morphine use coupled with normal and high-cholesterol diets on cardiovascular disease risk factors and fatty streak formation was investigated in rabbits in this study.

Materials and Methods

Twenty adult male New Zealand White rabbits were supplied from Iran Pasteur Institute and kept in special animal hutches of Isfahan University of Medical Sciences on Super Fosskorn standard rabbit chow basic diet. After 12 hours of fasting, blood samples were obtained from the animals to measure biochemical parameters, including total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), malondialdehyde, triglyceride (TG), fasting blood sugar (FBS), quantitative C reactive protein (CRP), coagulation factor VII, fibrinogen, platelet count, RBC count, WBC count and hemoglobin. Measurement of FBS and serum lipids was performed with an ELAN autoanalyzer using the enzymatic method. CRP was measured via the turbidimetric method. The antioxidant capacity and malondialdehyde level were determined using the spectrophotometric method.[14,15] Fibrinogen was measured based on clotting time. Coagulation factor VII was measured based on the percentage of its activity in plasma and plasma clotting time compared to standard samples.[16]

The rabbits were randomly divided into four groups (five in each group): normal, normal + morphine, high-cholesterol, and high-cholesterol + morphine. The rabbits were weighed at the start and end of the experiment. High-cholesterol diet was prepared using pure cholesterol powder (Merck ®) (1% supplemented to normal diet). Each rabbit was injected with 10 mg morphine intravenously every other day. The animals were studied for two months. At the end of the experiment, blood samples were again obtained from the rabbits. The animals were then anesthetized using 120 mg/kg pantobarbital and their right and left coronary arteries were excised and rinsed in normal saline. The specimens were kept in 10% formalin. Samples were taken from the right and left coronary arteries of each rabbit. Three consecutive sections were mounted on one slide, with a total of 30 slides collected from one animal. Of 120 microscopic slides prepared, each containing 3 sections, three stained with hematoxylin and eosin were observed by optical microscope at 40×,100×, and 200× magnitude and the presence and extent of fatty streak formation were scored from 0 to 5.[13] Measurement was conducted using the Longitudinal Scale Micrometer method. Scoring was performed at 100× after fixing the micrometer at the position of the ocular lens of the microscope.

Data analysis was performed with SPSS. Biochemical parameters were analyzed with t-test and pathology data were evaluated with independent sample t-test. P values less than 0.05 were considered significant.

Results

Statistical analysis revealed no significant difference between body weight and biochemical factors measured at the start of the study. Meanwhile only data significantly different between the groups are shown in tables. High-cholesterol diet led to a significant increase in TC, TG, CRP, and LDL. It also significantly enhanced fatty streak formation in right and left coronary arteries ($P<0.05$) (Table 1). Comparison of normal diet with normal diet + morphine group showed that morphine significantly increased TC, coagulation factor VII and fibrinogen. It also significantly increased fatty streak formation in the right and left coronary arteries ($P<0.05$) (Table 2). Morphine administration with high-cholesterol diet significantly increased coagulation factor VII, platelet count, and weight of rabbits while decreasing their RBC count, nonetheless, no significant change in fatty streak formation in the right and left coronary arteries was observed ($P<0.05$) (Table 3).

Discussion

The results of this study demonstrate that morphine administration along with a normal diet increases some biochemical parameters (CVD risk factors) and enhances fatty streak formation in the coronaries. Morphine administration coupled with high-

cholesterol diet had no effect on fatty streak formation and only increased some of the risk factors. It was found in a study that injecting hypercholesterolemic rats with 75 mg/day morphine for five days increases TC, LDL, and very low-density lipoprotein (VLDL) and decreases HDL, and that these effects are reversible by administering naltrexone. Morphine can also increase LDL and VLDL in rats on a normal diet.[17]

TABLE 1. Comparison of biochemical parameters and pathological findings at the end of the study between the normal and high-cholesterol groups (only parameters with significant difference are shown).

Group	Cholesterol (mg/dl)	Triglyceride (mg/dl)	1CRP (mg/dl)	LDL (mg/dl)	Right coronary (score)	Left coronary (score)
Normal	127.20±9.31	89.60±16.67	0.620±0.130	76.00±15.36	0.80±0.84	0.84±1.00
High-cholesterol	705.60±70.3	107.60±11.17	1.340±0.666	614.20±60.3	2.20±0.84	1.14±0.71

Data are shown as Mean± SD
1- C reactive protein

TABLE 2. Comparison of biochemical parameters and pathological findings at the end of the study between the normal and normal + morphine groups (only parameters with significant difference are shown).

Group	Cholesterol (mg/dl)	FVII % Activity	Fibrinogen mg/100	Right coronary (score)	Left coronary (score)
Normal	127.2±9.31	378.80±27.16	327.94±68.60	0.8±.84	0.84±1.00
Normal + morphine	155.80±15.8	495.20±65.68	334.80±53.13	2.00±1.00	2.80±0.84

Data are shown as Mean± SD

TABLE 3. Comparison of biochemical parameters and at the end of the study between the high-cholesterol and high-cholesterol + morphine groups (only parameters with significant difference are shown).

Group	Factor VII % Activity	PLatelets×103/ml	Weigh of rabbits(Kg)	RBC×106/ml
High-cholesterol	364.8±74.21	332.2±91.36	2.32±.77	6.038±.69
High-cholesterol + morphine	520.2±124.84	435.6±151.64	3.17±.86	3.79±0.53

Data are shown as Mean± SD

Another study on rats has demonstrated that stimulation by stressors which trigger the release of endogenous opioid increases serum cholesterol and that administering morphine to these animals for five days doubles their LDL, VLDL, TC, and TG levels. The latter study shows that the endogenous opioid system plays an important role in causing stress-related hypercholesterolemia.[18]

Studies on the effects of narcotic alkaloids including morphine and cocaine on lipid peroxidation in the mitochondria of rat brain cells have shown that morphine triggers the production of malondialdehyde, an atherosclerosis risk factor.[19] It was found in 1986 that shortly before complete obstruction of the coronaries and development of long-lasting and irreversible ischemia, certain protective mechanisms known as ischemic preconditioning (IPC) in the heart provide some protection against MI. This protective phenomenon is seen in most animal species (pigs, dogs, rats, rabbits, etc.) and probably humans.[20] It has recently been shown that opioids are one of the regulators and underlying mechanisms of IPC[21] and opioid receptors generally play a protective role against damage from ischemia and cardiac arrhythmias.[22-24] It has been well recognized that production and release of opioid peptides and their serum levels increase under stressful conditions, such as cardiac disease and/or ischemia. Although animal and cellular studies conducted so far suggest that activation of opioid receptors have protective effects on the cardiovascular system, any generalization of the results of this study to humans must be preceded by evidence of similarity between human and animal mechanisms. Some studies have demonstrated that narcotics increase the risk of CVD in laboratory animals; others however, have shown that opioid use may have protective effects on the heart and lessen ischemia-related damage. It has been stated that activation of delta-opioid receptors exerts cardi-

oprotective effects though mechanisms similar to IPC, and that activation of these receptors mediates the acute protective effect of morphine in the heart. However, the effects of narcotics on fatty streak formation have been addressed by few studies. It has been confirmed that the loss of vascular endothelial cell viability following the treatment with morphine is due to apoptosis, not necrosis. Together, these data suggest that in the drug abuser, morphine may initiate the apoptotic process in vascular endothelial cell resulting in an increase in vascular endothelial cell permeability, and thus, a heightened susceptibility to infection and inflammation that is known as the most important risk factor for atherosclerosis.[25] The present study showed that morphine administration significantly accelerates atherosclerosis in the coronaries. In light of these findings, further cellular, molecular, clinical and animal studies are warranted to fully explore the effects of narcotics on the human body.

Reference

1. Stefano GB, Scharrer B, Smith EM, Hughes TK JR, Magazine HI, Bilfinger TV, Hartman AR, Fricchione GL, Liu Y, Makman MH. Opioid and opiate immunoregulatory processes. Crit Rev Immunol 1996;16:109-144.
2. Beckett A, Casy A. Synthetic analgesics: stereochemica1 considerations. J Pharm Pharmcol 1954;6:986-1001.
3. Lord JAH, Waterfield A, Hughes J, Hans W. Endogenous opioid peptides: multiple agonists and receptors. Nature 1977; 267:495-499.
4. Traynor JR, Elliott J. δ-Opioid receptor subtypes and cross-talk with μ-receptors. Trends Pharmacol Sci 1993; 14: 84-86.
5. Clark J, Liu L, Price M, Hersh B, Edelson M, Pasternak M. Kappa opiate receptor multiplicity: evidence for two U50, 488-sensitive K1 subtypes and a novel K3 subtype. J Pharmacol Exp Ther 1989; 251: 461-468.
6. Rothman RB, Bykov V, de Costa BR, Jacobson AE, Rice KC, Brady LS. Interaction of endogenous opioid peptides and other drugs with four kappa binding sites in guinea pig brain. Peptides 1990; 11: 311-331.
7. Stefano GB, Melchiorri P, Negri L, Hughes T K, Scharrer B. (D-Ala2)-Deltorphon I binding and pharmacological evidence for a special subtype of delta opioid receptor on human and invertebrate immune cells. Proc Natl Acad Sci USA 1992; 89: 9316-9320.
8. Wittert G, Hope P, Py1e D. Tissue distribution of opioid receptor gene expression in the rat. Biochem Biophys Res Commun 1996;218:877-881.
9. Debold AJ. Atrial natriuretic factor: a hormone produced by the heart. Science 1985; 230:767-770.
10. Barron A, Jones CE, Cafftey JL. Pericardial repair depresses canine cardiac catecholamines and met-enkephalin. Regul Pept 1995;59:313-320.
11. Kevelaitis E, Peynet J, Mouas C, Launay JM, Menasche P. Opening of potassium channels: the common cardioprotective link between preconditioning and natural hibernation? Circulation 1999; 99:3079-3085.
12. Eliasson T, Mannheimer C, Waagstein F, Andersson B, Bergh CH, Augustinsson LE, Hedner T, Larson G. Myocardial turnover of endogenous opioids and calcitonin-gene-related peptide in the human heart and the effects of spinal cord stimulation on pacing-induced angina pectoris. Cardiology 1998;89:170-177.
13. Van Vlijmen BJM, Van den Maagdenberg AMJM, Gijbels MJJ, Van den Boom H, Hogen Esch H, Frants RR, Hofker MH, Havekes LM. Diet-induced hyperlipoproteinemia and atherosclerosis in apolipoprotein E3-leiden transgenic mice. J Clin Invest 1994;93: 1403.
14. Miki M, Tamai H, Mino M, Yamomoto Y. Free radical chain oxidation of rat red blood cells by molecular oxygen and its inhibition by α-tocopherol.Arch Biochem Biophys 1987;258(2):373-80.
15. Kostner K, Yang P, Neunteufl T, Golgar D. Is oxidative stress causally linked to unstable angina pectoris? A study in 100 CAD patients and matched controls. Cardiovasc Res 1997;36:330-6.
16. Mutanen M, Krusivs T. Habitual Diet, platelet function, fibrinogen and factor VII coagulant activity in young Finns. Journal of Internal Medicine 1998;237:577-83.
17. Brayant HU, Story JA, Yim GK. Morphine-induced alteration in plasma and tissue cholesterol levels. Life Sci 1987; 41(5): 545-54.
18. Bryant HU, Kuta CC, Story JA, Yim GK. Stress-and morphine-induced elevations of plasma and tissue cholesterol in mice: reversal by naltrexone. Biochem Pharmacol 1988; 37(19):3777-80.
19. Dasne P, Ratty AK. Studies on the effects of Narcotic alkaloid cocaine and code in on non enzymatic lipid peroxidation in rat brain mitochondria l987;37(C):258-264.
20. Liy GS, Thomton J, Van Wink1e DM, Stan1ey AWH, Olsson RA, Downey JM. Protection against receptors in rabbit heart. Circulation 1991;84:350-356.
21. Schultz JJ, Hsu AK, Gross GJ. Ischemic preconditioning in the intact rat heart is mediated by δ1 but not μ or K Opioid receptors. Circulation 1998;97:1282-1289.
22. Schultz JE, Garret J. Opioids and cardioprotection. Pharmacol Ther 2001;89:123-137.
23. Jason NP, Eric RG, Garret JG. Opioid-induced preconditioning: Recent advances and future perspectives. Vascular Pharmacology 2005;42:211-218.
24. Olivier WV, Lea MD, Franklin LR, Daniel P, Donald E. Endogenous cardiac opioids: Endogenous Cardiac opioids: Enkephalins in adaptation and protection of the heart. Heart, Lung and Circulation 2003;12:178-187.
25. Liu HC, Anday JK, House SD, Chang SL. Dual effects of morphine on permeability and apoptosis of vascular endothelial cells: morphine potentiates lipopolysaccharide-induced permeability and apoptosis of vascular endothelial cells . J Neuroimmunol 2004;146:13-21.