An Experimental Model for Studying Atherosclerosis

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ABSTRACT

The aim of the present study was to clarify if old N-MRI rat, a strain which is easily available in Iran and cheap to maintain, is a suitable alternative to those previously reported. In this model, we compared three quantifiable parameters between old and young rats: biochemically, involving measurement of differences in the lipid profile. Histologically, the differences of the thickness of the wall of the aorta, the apparent degree of splitting within the aortic media, and the formation of foamy lipid layers on the outermost layer of the aorta. Pharmacologically, *in vitro* contractile responses/mg wet tissue weight to submaximal concentration of phenylephrine (1 µM) and the rate of relaxation min^{-1} mg^{-1} tissue weight following administration of 0.1 mM of acetycholine. The proposed model was validated using lovastatin as test drug known for its lipid lowering and anti-atherosclerosis actions. The results showed that this model to be reliable, quantifiable and capable of detecting the effect of orally administered lovastatin. We recommend this model as an easy and accessible experimental model for various atheroscleorosis investigations. *Iran. Biomed. J. 7 (2) 65-71, 2003*

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INTRODUCTION

Experimental models to investigate the process of atherosclerosis has been described in both man [1] and experimental animals [2]. The aims of such studies were twofold. They attempted to gain an understanding of atherosclerosis process and to develop models in which the effectiveness of various interventions could be evaluated for their potential effects on this process. In addition, experimental atherosclerosis studies in man by necessity and ethics, are limited. On the other hand, it is recognized that the extrapolation of results from animals to man should be guarded.

Most investigations carried out for studying atherosclerosis involve the use of a classical administration of high fat, high cholesterol diet, and an agent that produce artherosclerotic plaques in the arteries over a period of 3 to 6 months [2]. On the other hand, investigations involving experimentally induced hyperlipidaemia may use the same methodology can commence after a few weeks rather months [2]. Still a more rapid method for studying hyperlipidaemia is the triton-induced model [3]. In this model, an intraperitoneal administration of 400 mg/kg of isooctylpolyoxyethlenephenol (triton) produced within 48 h, an established and a measurable hypercholesterolaemia in rats [2]. These methods carry an inherent weakness in that they do not truly represent the natural process of atherosclerosis. Since the process of atherosclerosis is characterized by its silent and slow progress of deposition of lipids in the major arteries, including the aorta, over a prolonged period. In order to overcome this limitation, other researchers [4], have used genetically modified strains of animals that have an inherent tendency to develop atherosclerosis. Still, more recent introduction is diabetes-accelerated atherosclerosis porcine [5] and rodent models [6]. In these models, the effect of diabetes on the development of atherosclerosis and hyperliproteinaemia are investigated. All these studies clearly reflect that there is a lack of a consensus on the animal model to use. With these limitations in mind, other researchers

considered more natural approach. They used old animals from different stains not available in Iran, and are proposed to reflect more the natural phenomenon of this disease [7].

Once an experimental model was found, the next stage was to evaluate the effectiveness of various treatment protocols. Different parameters have been measured as an indication of the degree of atherosclerosis progression or regression. These included the extent of lipid lowering, rate of blood flow [8], reduced thickness of the arteries [7], relaxation of major arteries following acetycholine [1], or on follow up treatment for the rate of mortality in human studies [9]. These various studies clearly reflect the fact that a universally accepted consensus on what to measure as an indication of extent of progression or regression of atherosclerosis is also lacking.

It has been known that aged animals from various strains develop the characteristic features of atherosclerosis [7]. It was therefore of interest to investigate if old N-MRI rats are suitable alternatives to others proposed for such studies. In this study, we provide evidences that *old* N-MRI rats, a strain of rats which is widely breed in Iran and cheap to keep under normal laboratory conditions, is also a suitable alternative to other not easily available strains. The model has an additional advantage that it simultaneously takes into account of three quantifiable parameters that are thought to reflect the measure of the severity of atherosclerosis.

The studies were divided into two phases: first, to determine and evaluate the parameters which are related to atherosclerosis and show the differences between young and aged rats from the biochemical, histological and pharmacological viewpoints, and second, to validate the usefulness of the chosen model by its response to lovastatin, a cholesterol-lowering drug known to be effective in treatment of atherosclerosis [10].

MATERIALS AND METHODS

Animals. Young and aged rats of N-MRI strain (2 and 11 months old) were utilized. These were purchased from Tehran Animal Centre. The animals were housed in groups of three in PVC cages, had free access to water and standard laboratory chew, and were maintained at 27 ± 5°C and 60 ±10% humidity. The lighting cycle was on 12 hourly day/night bases and lighting conditions were between 7 a.m. to 7 p.m.

In the initial phase of investigation, 16 rats (8 young, less than 2 months old, and 8 aged, 11 months old) were used. The average weight of the old and young rats used was 230 and 50 g, respectively .

In the second stage of work, another 16 rats were used (9 months old when started the experiments). The animals were divided into two groups of 8. The first group was used as controls to which 1 ml of normal saline was administered orally, daily for 8 weeks. The second group was administered orally, via a blunt-head feeding syringe, 4 mg/Kg lovastatin (Sermic, Mexico) using 10 mg/ml suspension in normal saline, daily for 8 weeks [11].

Experimental Procedure. Following the injection of an anaesthetizing dose of pentobarbital sodium, a blood sample was withdrawn from the tail of each animal for biochemical evaluation of serum lipids. The animals were then sacrificed by exsanguination. The thoracic aorta was removed, and sections were used for both pharmacological and histological tests.

Biochemical investigations. A 2-ml blood sample was withdrawn from the tail of the anaesthetised rats and centrifuged at 600 ×g and then the serum was separated for the measurement of the lipid profile: LDL, HDL, VLDL, total cholesterol and triglyceride levels, using standard enzymatic based laboratory kits (Man, Iran).

Pharmacological investigations. Utilizing the classical endothelium-mediated vasomotor relaxation response to acetycholine inphenylephrine -induced pre-contracted aorta [12]. In order to perform the pharmacological evaluations, approximately a 4-mm long aortic ring was removed from the thoracic part, weighed and placed in an oxygenated Tyrode solution maintained at 37°C. The Tyrode solution had the following composition (% w/v): NaCl 0.9, KCl 0.02, $MgCl_2$ 0.01, $CaCl_2$ 0.02, NaH_2PO_4 0.005, $NaHCO_3$ 0.01, Glucose 0.1 [13].

The aortic rings were then connected to an isometric transducer (model F60, USA) and to a 4-pen Darco chart recorder. The aortic rings were suspended under 1 g tension, by a stainless steel triangular wire, which was slowly and carefully inserted into the lumen without damaging the endothelial layer. The following protocol was adopted: following the equilibration period of 1 h and washing with fresh Tyrode solution every 15min, the aortic rings were exposed to a dose of 1 µM final bath concentration of phenylephrine hydrochloride. The plateau level of contraction produced was recorded for all groups, from which the level of contraction/mg wet tissue weight was calculated and statistically compared. This concentration was the

submaximal concentration that produced 75% of maximum contraction in the

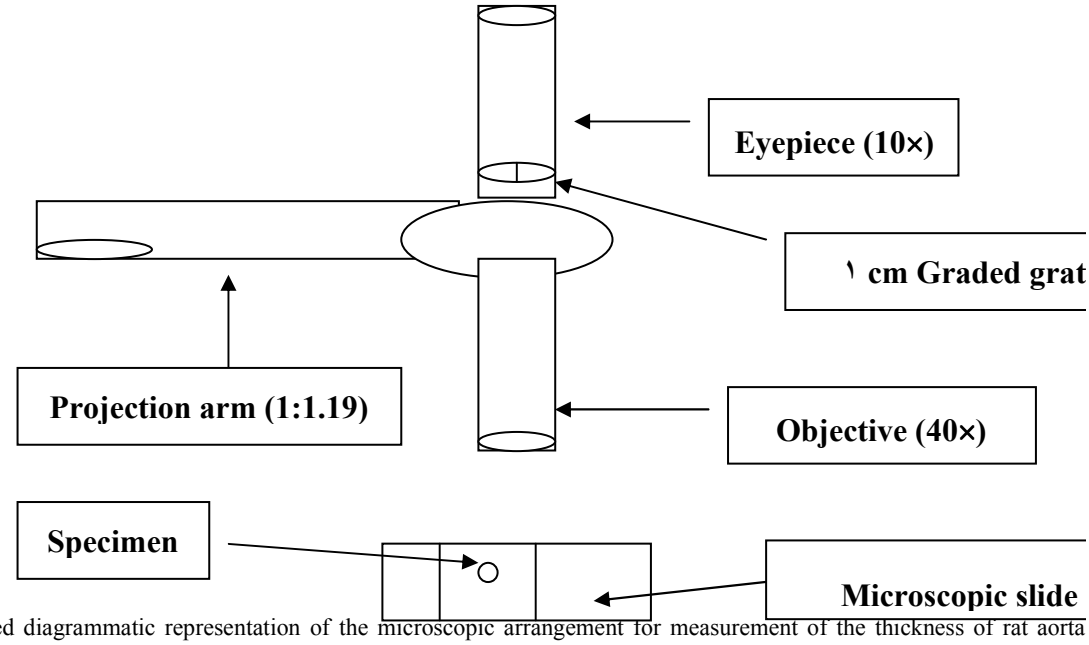

Fig. 1. A simplified diagrammatic representation of the microscopic arrangement for measurement of the thickness of rat aorta samples. Note that the magnification for the sample projected to the eye piece is 400×, which is reduced by the projection arm by a factor of 1: 1.19, providing a final magnification at the projection end 336× that of the actual thickness of the sample.