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Review

The calcium chloride-induced rodent model of abdominal aortic aneurysm

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ABSTRACT

Abdominal aortic aneurysm (AAA) affects ~5% men aged over 65 years and is an important cause of death in this population. Research into AAA pathogenesis has been fuelled by the need to identify new diagnostic biomarkers and therapeutic targets for this disease. One animal model of AAA involves perivascular application of calcium chloride ($CaCl_2$) onto the infra-renal aorta of mice and rats to induce extracellular matrix remodelling. Twenty-three studies assessing $CaCl_2$-induced AAA and six studies assessing AAA induced by a modified $CaCl_2$ method were identified. In the current report the preparation and pathological features of this AAA model are discussed. We also compared this animal model to human AAA. $CaCl_2$-induced AAA shows the following pathological characteristics typically found in human AAA: calcification, inflammatory cell infiltration, oxidative stress, neovascularisation, elastin degradation and vascular smooth muscle cell apoptosis. A number of mechanisms involved in $CaCl_2$-induced AAA have been identified which may be relevant to the pathogenesis of human AAA. Key molecules include c-Jun N-terminal kinase, peroxisome proliferator-activated receptor-γ, chemokine (C–C motif) receptor 2, group x secretory phospholipase A2 and plasminogen. $CaCl_2$-induced AAA does not display aortic thrombus, atherosclerosis and rupture which are classical features of human AAA. Advantages of the $CaCl_2$-induced AAA technique include (1) it can be applied to wild type mice making assessment of transgenic rodent models more straight forward and rapid; and (2) $CaCl_2$-induced AAAs are usually developed in the infra-renal abdominal aorta, which is the most common location of human AAA. Currently findings obtained from the $CaCl_2$-induced AAA model or other animal models of AAA have not been translated into the human situation. It is hoped that this deficiency will be corrected over the next decade with a number of clinical trials currently examining novel treatment options for AAA patients.

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1. Introduction

Aortic aneurysm is the general term for any dilation (aneurysm) of the aorta to greater than 1.5 times normal size [1]. Abdominal aortic aneurysm (AAA) represents a weakened and dilated region of the abdominal aorta usually affecting the infra-renal segment [2]. AAA has previously been reported to affect ~5% men aged over 65 years [3,4]. Recent studies suggest that AAA prevalence is decreasing. A recent large screening study in Sweden reported an AAA prevalence of 1.7% in 65-year-old men [5]. A recent epidemiological study in England and Wales suggests that AAA mortality is decreasing and AAA presentation is shifting to an older population of ≥75 years [6]. The pathological features of AAA include increased elastin degradation, vascular smooth muscle cell (VSMC) apoptosis, oxidative stress and inflammation [7,8]. The risk of AAA rupture increases with increasing aortic diameter [9]. Mortality after AAA rupture is about 80% for those who reach hospital and ~50% for those who undergo surgery [10,11]. There is no established therapy for small AAAs [12]. Larger (greater than 5.5 cm in diameter) or symptomatic AAAs usually undergo open surgical or endovascular repair [13]. Both surgical repair methods have some limitations. Open surgery is associated with a significant peri-operative mortality and morbidity; while up to 20% patients who undergo endovascular repair require reintervention within 5 years [14].

AAA diagnosis is problematic because most aneurysms are asymptomatic until rupture. While ultrasound screening has been introduced in older males in some countries, the feasibility of continuing such programs is in question with the evidence suggesting that AAA incidence is decreasing [6]. There is therefore great current interest in identifying novel diagnostic markers and appropriate molecular targets for drug development [15]. The mechanisms underlying AAA initiation and progression remain incompletely understood, contributing to significant shortfalls in the diagnosis and management of AAA. Experiments using human biopsies and cells derived from these biopsies are helpful in providing information on end-stage AAA pathogenesis. Such studies are limited by the decreasing availability of samples from open AAA repair and the inherent difficulties in modelling *in vivo* pathology. Animal models enable the study of AAA pathogenesis *in vivo*.

One of the animal models used to investigate AAA is that induced by calcium chloride ($CaCl_2$) [16,17], and research employing this model has provided insight into the pathogenesis of AAA. The current systematic review describes the technical aspects, cellular and molecular features of this animal model and comments on the relevance of this model to human AAA.

2. The development of the $CaCl_2$ model

Calcification of the human aorta is common in older adults [18,19]. A study in which 582 aortic specimens from the proximal portion of the aortic arch were examined demonstrated that calcification of the aortic media starts in people younger than 19 years and that the presence and severity of calcification increases with age [19]. Intimal calcification is present in most patients who have atherosclerosis and more severe aortic calcification has been shown to predict worse long term outcome [20]. Calcification is a feature of human AAA and about 80% of AAAs show considerable aortic wall calcification [21].

To investigate the relation between calcification and AAA Gertz et al. did some pioneering research in 1988 using male New Zealand rabbits [22] and identified that $CaCl_2$ applied to the adventitia of a carotid artery induced aneurysm formation. They applied 0.5 M $CaCl_2$ solution to the adventitial surface of the right common carotid artery and kept the entire vessel segment immersed in the solution for 15 min. Three weeks later the luminal diameter of the arterial segment treated with $CaCl_2$ increased by a mean of 61% compared with the contralateral carotid artery treated with sodium chloride (NaCl). Scanning electron microscopy showed endothelial cell fragmentation at the site of $CaCl_2$ administration. Histological examination demonstrated that the arterial dilatation was accompanied by elastin calcification, loss of VSMCs, and marked infiltration of inflammatory cells including neutrophils, lymphocytes, monocytes and multinucleated giant cells [22]. This was the first report of a novel model of human aneurysm.

This approach to inducing aneurysm formation was first applied to the abdominal aorta by Freestone et al. in a studied published in 1997 using New Zealand rabbits [23]. Applying 0.25 M $CaCl_2$ alone to the abdominal aorta did not induce AAA formation up to 12 weeks after surgery. Applying this solution caused endothelial damage, evidenced by the loss of endothelium-dependent relaxation, but minimal inflammatory cell infiltration was induced. Addition of 0.05 M thioglycollate to the $CaCl_2$ solution along with exposing the rabbits to a high cholesterol diet however was sufficient to induce AAA formation after three weeks [23].

Over the last decade rodent models have been developed for many diseases and a large range of research tools have been developed to enable elegant studies to be performed in these animals including knockout and transgenic technologies. In 2001 Chiou et al. reported performing AAA induction in mice by the $CaCl_2$ method [24]. The investigators applied 0.68 M $CaCl_2$ to the abdominal aorta of C57BL/6 mice for 10 min. An AAA formed three weeks after surgery. Histological examination demonstrated that aortic dilatation was accompanied by VSMC depletion, elastin degradation and infiltration of lymphocytes and macrophages. High concentrations of pro-inflammatory cytokines and matrix metalloproteinases (MMPs) were also noted within the dilated aortas [24–26].

3. Technical aspects of the $CaCl_2$-induced AAA model

By searching the PUBMED database and hand searching of the reference lists of relevant articles we identified 23 articles which reported employing the $CaCl_2$-induced AAA model in rodents and 6 articles which reported employing modifications of the $CaCl_2$ method in rodents. The technical aspects of the $CaCl_2$-induced AAA model employed in these 23 studies were similar. Briefly, after the animals were anesthetized, the fur on the abdomen was removed and a midline abdominal incision was performed. The abdominal aortic region located distal to the renal arteries and proximal to the iliac bifurcation was exposed. A gauze pre-soaked in $CaCl_2$ was then directly applied to the adventitia of the infra-renal abdominal aorta for a period of time and then removed. Alternatively, a swab soaked with $CaCl_2$ solution was employed to “paint” the abdominal aorta