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Research

Animal models of pulmonary arterial hypertension: A systematic review and meta-analysis of data from 6126 animals

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ABSTRACT

Numerous animal models of pulmonary hypertension are currently available. A systematic review and meta-analysis was performed of a number of experimental studies of disease induction based on several animal models. A meta-analysis was performed of 291 publications discussing the efficacy of 611 interventions to introduce disease pulmonary hypertension in 6126 animals. A meta-regression analysis was done to assess the effect of prolonged periods of disease induction on the outcomes. A random-effects meta-analysis was used to assess the impact of study characteristics and seek evidence of publication bias. A more pronounced worsening in hemodynamics or right ventricle hypertrophy was observed in animals exposed to Sugen combined with hypoxia, or left pneumonectomy followed by monocrotaline. Chronic hypoxia induced the poorest, but the most stable, response to disease induction with regard to elevated hemodynamic parameters, right ventricle hypertrophy and wall thickening. The greatest elevation of right ventricle systolic pressure was observed in animals exposed to isoflurane and the weakest to chloral hydrate. This result was true for different animal models and lengths of induction of pulmonary hypertension. Publication bias was found for all the crucial parameters. Development of pulmonary hypertension depends on the choice of animal model. Classic models, especially these related to chronic hypoxia, provoke a less severe response with regard to poorer hemodynamics and myocardial hypertrophy. The outcome of disease development can be strongly determined by the duration of induction, detailed experimental conditions and anesthesia procedure.

1. Introduction

Pulmonary hypertension (PH)) is a multi-factorial disease which leads to progressive right heart failure and death. It is defined by increased mean pulmonary artery pressure (mPAP) ≥ 25 mmHg at rest. PH can be associated with different clinical situations including wide spectrum of histological patterns and abnormalities, such as: remodeling of the pulmonary arterial vessels by the production of complex, multicellular vascular lesions that obstruct pulmonary arterioles and limit blood flow through the pulmonary arteries. As a consequence, right ventricular (RV) afterload increases significantly, thus contributing to the development of RV dysfunction and failure. PH has been divided into six categories with a complex clinical and potential etiological basis rather than a histological pattern. Of these, the most important advances in understanding and treatment have been seen for pulmonary arterial hypertension (PAH). Patients with PAH are characterized pathologically by a neointimal and plexogenic arteriopathy, and clinically by progressive deterioration with severe PH [1].

The greater understanding of the epidemiology, pathogenesis, and pathophysiology of PH has led to an explosion in research in the field of pulmonary vascular disease over the past few decades, especially PAH. Modern drug therapy provides a significant improvement in patient symptomatic status and a slower rate of clinical deterioration. Despite this, PH remains a chronic disease without a cure and there is still a need to identify and therapeutically target other significant pathogenic cellular and molecular signalling mechanisms. However, there is currently no perfect preclinical model that completely replicates human PH [2]. Monocrotaline (MCT) and chronic hypoxia (CH) are two well studied and published animal models of pulmonary hypertension. Monocrotaline is a toxic pyrrolizidine alkaloid present in the plant *Crotalaria spectabilis*. The exact mechanism by which MCT induces pulmonary hypertension is not well understood; however, it is known that the monocrotaline pyrrole (MCTP) has to be activated *in vivo* by mixed function oxidases in the liver (cytochrome P-450) to form the reactive compound MCTP, which leads to vascular injury. Such increases in pulmonary artery pressure and vascular remodeling are caused by early and often dramatic accumulation of

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mononuclear inflammatory cells in the adventitial sheath of small intra-acinar vessels [3]. A simple and hence, technically appealing, MCT model is available to a wide spectrum of investigators. Considering the similarity of the numerous pathological changes ('MCT syndrome') that are induced by monocrotaline, it should be stressed that some differences can also exist between this rat model of pulmonary vascular disease and human PH: the involvement of veins and effects on other organs, especially the heart and liver, for example. A recent report notes that monocrotaline or its metabolite reliably produce hepatic veno-occlusive disease in rats and can cause myocarditis of both the right and left ventricles, which complicates the study of RV hypertrophy/failure [4]. All these factors can contribute to increased animal mortality. Nevertheless, many agents including prostanoids, PDE-5i, statins, tyrosine kinase, ROCK and RAAS inhibitors have been evaluated with regard to their ability to prevent or reverse established monocrotaline-induced PH [2]. Another common procedure involves the use of hypoxic systems. Such a model allows vascular remodeling to be introduced, as well as hypertension and hypertrophy of the right ventricle. This is a useful method because it is very predictable and reproducible within a selected animal strain. However, the responses to chronic hypoxia vary between species and are also significantly affected by age. There is no indication that nonreversible intimal fibrosis or plexogenic lesions, similar to those in human PH, occur in chronically hypoxic models. The severity of the structural modification of hypoxic lungs is determined by factors other than just hypoxia. Hence, the chronic hypoxic models of PH in rodents may serve as models for less severe PH (not PAH) or human PH associated with hypoxia due to chronic obstructive pulmonary disease, pulmonary parenchymal disease or sleep disordered breathing [2,5].

In recent years both MCT- and CH-based models have been used less frequently in favor of new ones. Such alternative animal models can be combinations of several procedures, such as exposure to monocrotaline with left pneumonectomy or injection of Sugen (SU5416) [6] followed by hypoxia. Other potential approaches are based on genetic manipulation, e.g. overexpression of calcium binding protein (S100A4/Mts1) [7] and interleukin-6 (IL-6) [8], or bone morphogenetic protein receptor type 2 (BMPR2) deletion [9]. Despite this, the animal models still do not model human disease with sufficient validity to guide drug development, and the complexity of the pathophysiological profile of PH excludes such animal models from research into novel potential treatment options. However, recent studies provide a solid 'qualitative' discussion on progress that has been made in animal modeling with regard to its value for further clinical assessments in PH [2,10,11]. Our research is intended to describe and compare the efficacy of the most popular animal models, i.e. CH, MCT and their novel modifications, from a quantitative point of view. The present study reviews papers which assess the potential therapeutic or preventive efficacy of unregistered drugs in animal pulmonary hypertension.

In particular, the questions addressed by the study are as follows: (a) Do the particular animal models of pulmonary hypertension differ in their impact on pH development? (b) If so, how can such influence be quantified, and is there any summary 'ranking' to obtain? (c) Are there any additional factors that can determine the outcomes? (d) If these factors exist, how can such influence be quantified? (e) Are there any relationships between the particular animal models and such additional determinants.

2. Methods and materials

2.1. Search strategy

The search corpus comprised Medline (from 1991), and ISI Web of Science (from 1991). The search was performed on December 31, 2015, with the aim of identifying animal experiments modelling pulmonary hypertension in the past 25 years. The databases were searched using the following search terms in titles and abstracts (also in combination with MESH terms): (mice OR rat OR mammals) AND (pulmonary hypertension) AND (RVP OR PAP OR RVSP OR RVH OR wall thickness OR RV/LV + S OR pulmonary artery pressure OR remodeling). There were no language restrictions.

2.2. Inclusion and exclusion criteria

The results included experiments where animals had been exposed to several interventions to develop PH, which was manifested with significant haemodynamic deterioration in the pulmonary circulation: i.e. elevations in right ventricle mean/systolic pressure (RVP/RVSP), in mean pulmonary artery pressure (mPAP) and/or right ventricle hypertrophy (RVH), usually expressed as RV/LV + S ratio. Other haemodynamic parameters included mean arterial blood pressure (MABP), cardiac output (CO), stroke volume (SV), pulmonary vascular resistance (PVR) and total peripheral resistance (TPR); however, these were not mandatory. The histological examinations were desirable but not obligatory, and were expressed as percentage medial wall thickness; this was selected on the basis of its great repeatability, which allowed for comparison studies to be identified. The percentage medial wall thickness of the pulmonary arteries was measured using the formula:

$$(2x \text{ medial wall thickness})/(\text{external diameter}) \times 100(\%) \quad (1)$$

or

$$(\text{external diameter} - \text{internal diameter})/(\text{external diameter}) \times 100(\%). \quad (2)$$

The results included experiments in which the outcome of an intervention in a cohort of animals was compared with that of a cohort of healthy animals (Sham) and an untreated group exposed to one or more procedures to introduce PH (Vehicle). The examined studies included those examining the preventive efficacy of potential therapeutic options, where the exposure to tested substances began with PH induction (preventive model), and those examining the reversal of PH, where drugs were administered after the development of PH (therapeutic model). The following studies were excluded: transgenic studies, experiments on newborn or pregnant animals, those where the intervention was given with the expressed intention of worsening rather than improving outcome, and in which acute PH was induced. Detailed information is provided in Supplementary Data (Fig. S1).

2.3. Data extraction

The following data was recorded: author, year of publication, type of regimen (preventive or therapeutic), type of intervention used to induce PH, with the dose of 'inducer' if needed, time for PH induction before the administration of tested substances was commenced, type of animal (species, strain, sex, age and initial weight), anaesthetic(s) used during haemodynamic measurements (agent and dose), and outcome assessment. The outcome measure was based on mean outcome, SD or SEM and number of animals per group. Quality assessments of the identified papers (one point per item) were performed according to checklist items provided by prior investigators: (1) publication in a peer-reviewed journal with its impact factor (year of publication) (2); (3) randomized allocation to experimental group; (4) blinding of the group allocation during the experiment; (5) blinded assessment of outcome; (6) a