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Recently, the problem of uncontrolled or resistant hypertension has gained enormous proportions (Daugherty *et al,* 2012). It may be very well accepted that mono-drug therapy is no longer effective due to the multifactorial etiology of hypertension and lack of proper lifestyle modifications by the patients. Several agents are employed in combination to manage the etiopathology and symptomatology of hypertension. Clinical practice has adopted the use of two or more classes of antihypertensive agents (Elliott, 2002; Paulis and Unger, 2010) for effective control of blood pressure in hypertensive patients. The basis behind such a decision is that since the etiology of hypertension is complex, it is prudent to employ a parallel control of more than one systems effecting increase in blood pressure. AT_1 and α_1 receptors are important targets in this regard and hence a simultaneous blockade of these targets might prove favorable. A superior therapeutic efficiency can be achieved through evenhanded modulation of multiple targets (Morphy *et al*, 2004). This may be achieved through polypharmacy, administration of fixed dose combinations or an agent directed to all the required targets, i.e. a multiple-targeted ligand. When compared to the other alternatives, the administration of a multiple-targeted ligand may offer certain advantages like more predictable pharmacokinetics, simple pharmacodynamic relationships, improved patient compliance and ease of therapeutic drug monitoring, if at all required (Morphy *et al*, 2004).

The compounds studied herewith belong to a series of 6,7-dimethoxyquinazolines with different substituents at 2^{nd} position based on structural modifications involving prazosin and losartan. These compounds were designed to show a balanced modulation of both the receptors in question i.e. AT_1 and α_1. This effect is supposed to be translated *in vivo* as the major mechanism involved in controlling the etiopathology of hypertension is targeted by these compounds.

Aim of this study involved screening of a series of 6,7-dimethoxyquinazolines for potential dual-antagonist activity on the AT_1 and α_1 receptors. Further to this, it was planned to evaluate the active compounds for toxicity and efficacy in the *in vivo* models of hypertension.

HYPOTHESIS

Structurally modified 6,7-dimethoxyquinazoline derivatives based on prazosin and losartan as respective parent compounds could be more effective in hypertension through simultaneous blockade of AT_1 and α_1 receptors

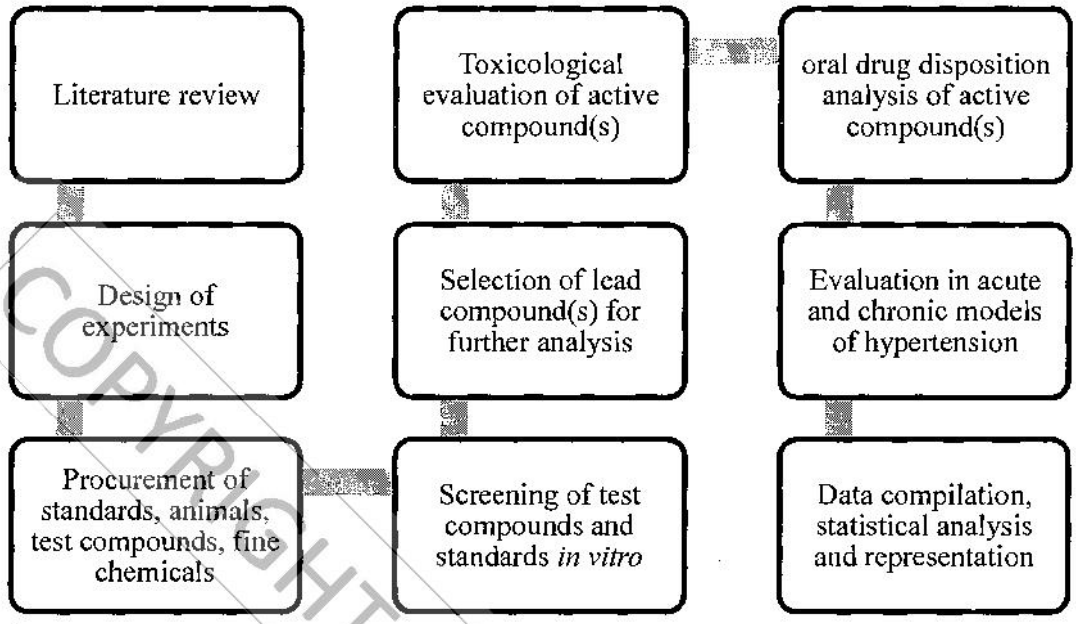
OBJECTIVES OF THE STUDY

1. To screen and identify the NCEs for potential dual-antagonist activity on the AT_1 and α_1 receptors using rat thoracic aorta preparation *in vitro*
2. To evaluate the active compounds for oral toxicity
3. To perform a pharmacokinetic evaluation of compounds showing positive activity on the rat thoracic aorta preparation
4. To evaluate the active compounds for their antihypertensive activity in acute and chronic models of hypertension in rodents

EXPERIMENTS PLANNED TO ACHIEVE THE OBJECTIVES

1. Functional antagonism assay on rat aortic strips using phenylephrine and angiotensin II
2. Single dose and repeat-dose toxicity evaluation of the selected NCE(s) by the procedures mentioned in OECD guidelines 423 and 407 respectively
3. Oral pharmacokinetics of the selected compound(s) in rats by HPLC-UV method
4. Inhibition of *in vivo* pressor response following intravenous injection of the selected compound(s) through invasive recording of arterial blood pressure
5. DOCA-salt induced hypertension in rats to determine the effect of selected NCE(s) upon chronic renal hypertension

FLOW OF WORK



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Two isoforms of ACAT are known: ACAT1 and ACAT2. Apart from the physiological aspect, production of cholesteryl esters by ACAT isoforms makes a noteworthy contribution to the pathogenesis of atherosclerosis. ACAT1 aids the progression of atherosclerosis via accumulation of cholesteryl esters in macrophages leading to conversion of smooth muscle cells to foam cells, ultimately leading to plaque initiation and subsequent events (Fazio et al. 2001; Linton and Fazio 2003). ACAT2 has been found to be responsible for dietary cholesterol absorption through intestinal microvilli by converting the polar cholesterol to non polar esters. Pan-specific or non-specific ACAT inhibition has been tried by different researchers with varying levels of success. Several studies identifying potential ACAT inhibitors for the management of hyperlipidemia and atherosclerosis have been reviewed (Pal *et al*, 2012). The past 2 decades have seen a generous number of publications on the subject of ACAT inhibitors. More than 150 patents have been filed suggesting a keen interest amongst researchers and in the commercial arena regarding ACAT inhibition as a potential therapeutic strategy for atherosclerosis. The development of several synthetic, herbal or microbial origin ACAT inhibitors has allowed the researchers to understand the role of ACAT in cholesterol turnover. Between the day when the first ACAT inhibitor was discovered and to this day, lot of progress has been made in the understanding of structure, function, localization and inhibition of ACAT isoforms. However, there is yet a lacuna in the therapeutic class of ACAT inhibitors. Despite several discouraging attempts, the search for a safe and effective ACAT inhibitor for the management of atherosclerosis persists.

The present study deals with development of a screening method for ACAT inhibitors and then screening a series of potential compounds for ACAT-inhibitory activity. These compounds belong to a class of urea derivatives and are assumed to show inhibition of ACAT catalytic activity. This inhibitory activity is supposed to be effective in controlling hyperlipidemia and the hallmark features of atherosclerosis.

Primary aim of this study involved development and validation of a simple assay method which can be used to assess the ACAT-inhibition potential of the small molecules in question without the use of radiometric facilities which are more commonly utilised for the same purpose. Succeeding this, it was aimed to screen a

series of test compounds using this novel method and thus identify potential active compound(s) which could be studied for their efficacy in an animal model of atherogenesis.

HYPOTHESES

1. Cholesteryl esters formed as catalytic products of ACAT reaction can be quantified by planar chromatography
2. Urea-based derivatives can be effective in the prevention of atherosclerosis through ACAT-inhibitory mechanisms

OBJECTIVES OF THE STUDY

1. To develop a method for the screening of ACAT inhibitors
2. To screen new chemical entities for potential ACAT-inhibition activity
3. To evaluate the active compounds for oral toxicity
4. To evaluate the active compounds for anti-hyperlipidemic and anti-atherosclerotic activity

EXPERIMENTS PERFORMED TO ACHIEVE THE OBJECTIVES

1. Development and validation of novel HPTLC-based method for screening of cholesteryl esters
2. Screening and identification of NCEs for potential inhibition of microsomal ACAT activity using rat liver microsomes-*ACAT Assay*
3. Evaluation of selected NCEs for their potential to affect triglyceride turnover *in vivo* using the Poloxamer-407 induced lipoprotein lipase inhibition model in rats
4. Single dose and repeat-dose toxicity evaluation of the selected NCE(s) by the procedures mentioned in OECD guidelines 423 and 407 respectively.
5. Determination of the efficacy of the selected NCE(s) in a model of diet-induced atherogenesis

FLOW OF WORK