European Journal of Medicinal Chemistry 46 (2011) 3581–3589

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

In-vitro metabolic inhibition and antifertility effect facilitated by membrane alteration: Search for novel antifertility agent using nifedipine analogues

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ARTICLE INFO

Article history:
Received 20 December 2010
Received in revised form
10 May 2011
Accepted 10 May 2011
Available online 19 May 2011

Keywords:
Antifertility
Nifedipine
Spermatozoa
Motility
Metabolism
Model membrane

ABSTRACT

In search of non-hormonal male contraceptives, analogues of nifedipine, which causes reversible infertility, have been synthesized and their interaction at molecular level with model membrane has been probed. Analogues act differently with respect to their antifertility action. This is achieved by altering the cell metabolism thereby directly affecting the motility which is responsible for fertility. Secondly, these drugs bind differently to the interior of the cell-membrane affecting the membrane fluidity, architecture and dynamics. Sulfasalazine and D_4 interact to a larger extent and alter the lipid bilayer phase to a hexagonal. D_1, D_2 and D_3 do not have considerable effect. D_4 is the most promising candidate as a lead compound for the development of novel non-hormonal male antifertility agents.

1. Introduction

Considering the fact that several steroid and peptide hormones play an essential role in the process of spermatogenesis, use of hormonal methods as contraceptives has been the choice for the development of oral contraceptives. However, looking at its adverse side effects and undesired pathological circumstances [1] such as increase in the incidence of cancer and hormonal imbalance, there is a growing interest in the development of non-hormonal oral contraceptives [2]. This focuses our research towards exploration of non-hormonal male contraceptives which might elicit a more rapid onset of infertility as compared to hormonal approaches [3]. Antifertility drugs such as gossypol and other compounds isolated from the plant *Tripterygium wilffordi* have irreversible effects and toxicity issues [4]. Thus, there is a need to develop reversible, non-hormonal antifertility agents with minimal side effects towards safe and effective contraceptive approach.

Sperm motility plays an important role in normal fertilization process. Ionic fluxes through ion channels are crucial in sperm metabolism, maturation, capacitation and in initiating the process of gamete interaction. Acrosomal reaction of spermatozoa is highly associated with L-type calcium channels [5]. The importance of Ca^{+2} ions in regulating diverse processes in sperm, including motility and acrosome reaction has been reported [6]. Attention to ion channels as drug targets for contraception has grown with the realization that there are sperm specific ion channels located on the sperm tail [7].

Nifedipine (N) (Fig. 1(A)), L-type Ca^{+2} channel blocker antihypertensive drug, apart from its well known cardiotherapeutic activity, is known to have effect on reproductive functions in male rats and is known to cause reversible infertility by altering sperm lipid metabolism [8,9]. However, if used as antifertility agent, its antihypertensive effect will be undesirable [2]. In view of this, we have selected nifedipine as a prototype lead and synthesized its four analogues (D_1–D_4) (Fig. 1(C)) to carry out comparative analysis at molecular level. The results have also been compared with the anti-inflammatory drug sulfasalazine (S) (Fig. 1(B)), which is also known to affect acrosome reaction [10]. Thus the side effects of nifedipine and sulfasalazine, primarily known for different therapeutic action have been optimized. Also this provides a model of comparison in order to develop a newer series of antifertility agents with greater promise. Analogues of nifedipine have been synthesized by modifying the carboxyl ester chain on dihydropyridyl nucleus along with substitution on aryl nucleus. Structure activity relationship (SAR) suggests that para substitution of 1, 4-dihydropyridine hinders cardio therapeutic activity [11]. To support this hypothesis we have

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doi:10.1016/j.ejmech.2011.05.022

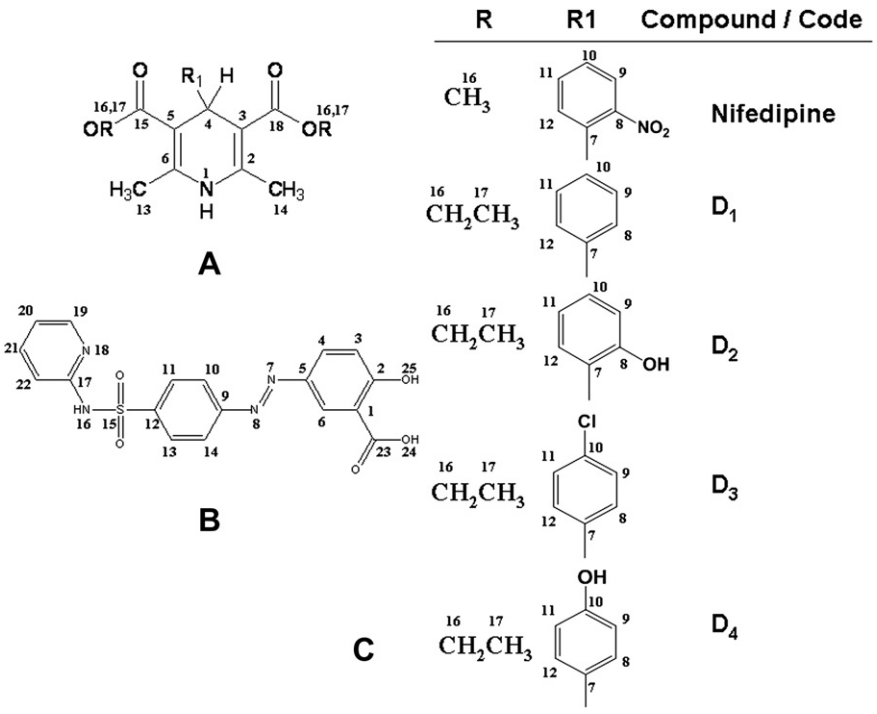

Fig. 1. Molecular structure of (A) nifedipine, (B) sulfasalazine and (C) nifedipine analogues.

synthesized para and ortho analogues to optimize their antifertility action. This allows probing the optimum requirement of the groups for maximum antifertility action.

Insertion of lipophilic calcium ion antagonists into the sperm plasma membrane has been shown to have an inhibitory effect on sperm fertilization [12]. In view of this, we have probed the insertion/interaction of these molecules into model membrane prepared from dipalmitoyl phosphatidyl choline (DPPC). The polymorphism, dynamics and thermotropic behavior of the system has been analyzed at molecular level using multinuclear NMR and differential scanning calorimetric (DSC) techniques. The antifertility effect of nifedipine, its analogues and sulfasalazine, has been evaluated by monitoring anaerobic glycolysis (lactate signal, the end product) of intact sperm cells with time using ^{13}C NMR spectroscopy [13]. These combined results are expected to provide basis for selecting the best analogue which might act as lead molecule in the development of anti-fertility agent which is non-hormonal, reversible and may act like a lipophilic calcium ion antagonist.

2. Results

2.1. AlogP calculation

Hydrophobicity/hydrophilicity of the drug influences the behavior of a molecule in a living organism, affecting its bioavailability, transport, reactivity, toxicity, metabolic stability and many other properties. We have therefore calculated the AlogP values for the molecules used in our study using VG method in Marrin Sketch Version 5.2 of ChemAxon software. The AlogP values are nifedipine 1.766, D_1 2.569, D_2 2.327, D_3 3.234,D_4 2.327 and sulfasalazine 3.463.

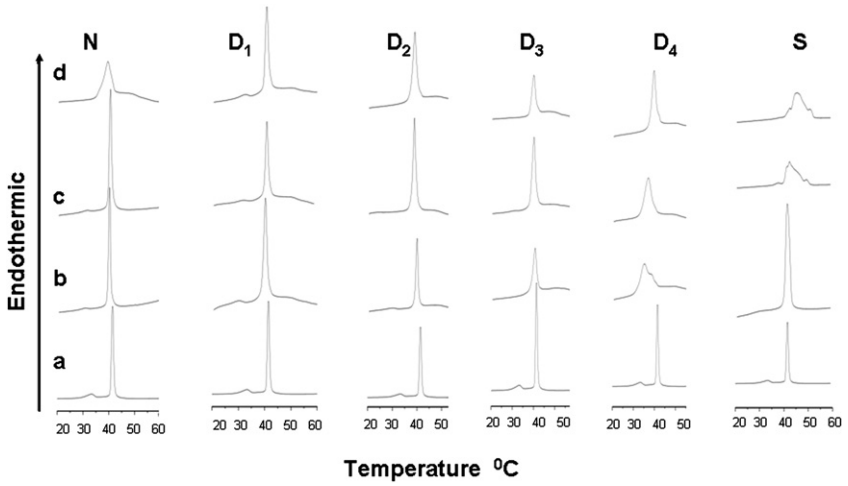

Fig. 2. DSC plots of DPPC (50 mM) incorporated with (N) nifedipine, D_1, D_2, D_3, D_4 and (S) sulfasalazine. The additives: lipid molar ratios are (a) 0:100, (b) 1:5, (c) 1:2 and (d) 1:1.