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A study on the effects of Borrelidin on cardiovascular and intestinal smooth muscles

D.V.R.N. Bhikshapathi[1*], N. Prasad[2], V. Kishan[2]

[1]Vijaya College of Pharmacy, Hayath nagar, Hyderabad
[2]University College of Pharmaceutical Sciences, Kakatiya, University, Warangal, India

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ABSTRACT

Borrelidin, an antibiotic isolated from Streptomyces californicus, is a potential drug candidate due to its recently reported antiangiogenic activity and other biological activities. Keeping in view the scope of the antibiotic, some biological studies were performed to know the effect of borrelidin on isolated frog heart at different concentrations (10, 30 & 100 µg / ml). Boreelidin resulted in considerable drop in heart beat, corresponding decreased cardiac output and finally cardiac arrest was noticed at 100µg/ml dose. Influence of borrelidin on peripheral blood vasculature was also tested and found to cause peripheral vasoconstriction at higher concentration, resulting in the reduction of the flow rate. Further, borrelidin could not show any effect on the motility of intestinal smooth muscle of guinea pig ileum.

Key words: Borrelidin, frog heart, guinea pig ileum, blood vessels.

INTRODUCTION

Borrelidin, a macrolide C18 non-glycosidic antibiotic, was isolated first time from *Streptomyces rochei* [1] and reported for antiborrelia activity. This antibiotic has now been produced from numerous organisms including *Streptomyces* C2989 [2], *S. griseus* BS 1325 [3], *S. parvulus Tü* 4055 [4], *S. candidus* (ATCC 202148) [5], and *S. californicus* [6]. This macrolide antibiotic presents a unique structural feature which is not described for any other known macrolide. The unique chemical feature of borrelidin is the presence of a single nitrile group at C12 of macrolide ring and natural products produced by microorganisms with nitrile group are presumed to be rare [7]. Keller-Schierlein in 1967 reported the structural elucidation of borrelidin and further confirmed by NMR and X- ray diffraction studies [8, 9]. The structure of borrelidin found to be planar and is nearly identical to antibiotic treponemycin [10].

The macrolide borrelidin possessed various biological activities including antibacterial, antimitotic, antiviral, herbicidal, insecticidal and antitumour activities [11]. The antibacterial activity of borrelidin was claimed due to the inhibition of threonyl tRNA synthetase and was not active against other aminoacyl transferases [12]. Borrelidin exhibited excellent antimalarial activity against both chloroquine sensitive and resistant strains in mice [13].

Further, borrelidin was also demonstrated to inhibit growth and proliferation of human cells via inhibiting threonyl tRNA synthetase which has been antagonized by the presence of elevated levels of threonine in Chinese hamster ovary cells [14]. The antiproliferative activity of borrelidin was attributed to its antiangiogenic activity by inhibiting

the capillary formation and also it caused the collapse of the formed capillary tubes in HUVEC cells. Further, the anti angiogenic activity was unfazed by the higher levels of threonine which highlighted the existence of alternative mechanism for capillary formation inhibitory activity. Borrelidin activated caspase-3 and caspase-8 which lead to apoptosis [15]. Due to vital biological activities exhibited by borrelidin, several organic chemistry groups attempted for its synthesis and biosynthetic pathway of borrelidin was also clearly understood. Subsequently, new congeners of borrelidin were reported with appropriate starting precursors [16-21].

Our group is involved in the isolation of antibiotics from microorganisms, first to report the isolation of borrelidin from *Streptomyces californicus* and also reported some of the unreported important activities including antitubercular activity of borrelidin [22, 23]. Literature sources revealed that some of macrolide antibiotics possessed cardio toxicity [24] and in a recent study involving azithromycin, five days therapy of azithromycin revealed a significant rise in cardiovascular deaths when compared with amoxicillin [25]. These reports encouraged us to know whether borrelidin possessed aforementioned activities as it also has a polyketide macrolide scaffold. This communication is aimed to disclose the effects of borrelidin on peripheral blood vasculature, using hind limb perfusion experiment, isolated frog heart smooth muscle and also on intestinal smooth muscle of guinea pig ileum.

MATERIALS AND METHODS

Solvents and reagents were purchased from E. Merck, Mumbai. Test animals were procured from institutional animal house. The animals maintained at a temperature of 25^{o}C and humidity 60%, were supplied with food and water. The study protocol was approved by the institutional animal ethics committee (IAEC), UCPSc, Kakatiya University, Warangal, A.P. Borrelidin was fermented by using Starch casein nutrient broth, isolated and purified from *Streptomyces californicus* broth in our lab using a mobile phase of chloroform:ethylacetate:methanol (68.3:29.3:2.4), which was developed to purify the crude extract by column chromatography using silica gel. Bioautography was carried out on silica gel precoated plates to detect the active fractions. The collected bioactive component was further purified by HPLC and spectral studies like LC-MS, NMR and FTIR were carried out to identify the antibiotic [6].

2.1. Effect of borrelidin on isolated frog heart smooth muscle (Straub's heart technique)

A big size frog was pithed to open the abdomen and pericardium was removed [26]. Sinus venosus, pulmonary arteries and veins, inferior vena cava and one of the branches of truncus arteriosus were ligated properly using thread. The branches of truncus arteriosus were cut off beyond the knot and Straub's cannula was inserted into the other branches of truncus arteriosus ensuring that the tip of the cannula reached the ventricle and then tied the heart in this position. Frog Ringer solution was flushed to eliminate blood and possible clot. The base of heart was hooked and connected it to a heart lever to record the heart responses. Solutions of borrelidin was prepared in sterile distilled water and DMSO (9:1) and diluted to afford 10, 30 & 100 μg/ml. The effects of borrelidin and control on heart were recorded.

2.2. Effect of borrelidin on peripheral blood vasculature

Effect of borrelidin on peripheral blood vessels of frog was performed by hind-limb perfusion method [26]. A big frog was dissected to expose heart and removed the pericardium carefully. The aorta was located and two loops were made. A cannula was inserted into aorta and tied the distal loop firmly; the cannula in turn was connected to frog Ringer solution reservoir. Blood from blood vessel was completely removed by flushing with Ringer solution and later the heart was cut off. Two threads were passed beneath the sinus venosus and made two loops. The sinus venosus was cannulated. The cannula was adjusted so that there was free flow of the fluid into the blood vessels and it could exit through the venous cannula and constant flow of Ringer solution was ensured. The fluid output per unit time was measured in drops till a constant output was obtained. Drug solutions were administered through the soft tube above the arterial cannula and the effect on blood vessels was studied.

2.3. Effect of borrelidin on guinea pig ileum

A 24 hour starved guinea pig was stunned on the neck and exsanguinated. The abdomen was opened to locate cecum and about 10 cm of the ileum proximity to the ileocecal junction was discarded. A thread was tied at the end of the remaining ileum which served as a marker and a piece of about 15 cm in length was separated. A small piece of ileum was placed in Kreb's solution and carbogen was bubbled constantly. The mesentery was removed and the ileum was cut into pieces of 2 cm long appropriately. The ileum pieces were always placed in physiological solution

and during experiment the tissue was equilibrated with 1 g tension for 45 minutes [26]. Drug concentrations of borrelidin in the range of 10, 30 and 100 µg/ml were added to tissue/ organ bath and recorded the responses.

RESULTS AND DISCUSSION

3.1. Effect of borrelidin on isolated frog heart smooth muscle

An attempt was made to know the cardiac effects of borrelidin in frogs. The heart of control frog could not demonstrate any effect on the heart i.e., heart rate (HR) and cardiac output (CO) and force of contraction were found to be intact as portrayed in **figure 1**. In comparison to control heart, the borrelidin treated heart remained unaltered with respect to CO and heart beats in the lower doses (10 and 30 µg/ml), but, high dose of borrelidin (100 µg/ml) showed a considerable drop in heart beat and corresponding decreased cardiac output was also observed as depicted in **figure 2**. In similar fashion, force of contraction was unaffected at lower doses, however cardiac arrest was noticed at 100 µg/ml dose. The aforementioned observations indicated that borrelidin had possessed a dose dependent cardiac depressant effect on frog's heart. Recent reports revealed that some of macrolide antibiotics demonstrated enhanced chances of risk of ventricular arrhythmia and cardiac arrest [27] and in particular erythromycin was demonstrated prolongation in QT interval [28]. The explicit reasons for macrolide antibiotics associated cardiac arrest are still unclear and require exhaustive study to reveal the unknown factor for such effects. Borrelidin possessed macrolide scaffold, and exhibited cardiac arrest at the higher dose. This is a serious note and extended studies with respect to electrolyte influence and effect on ECG may disclose reasons for cardiac arrest effect of borrelidin at the higher dose.

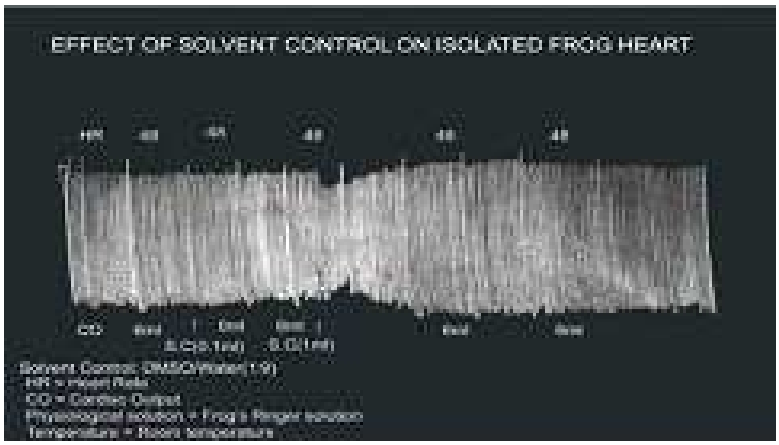

Figure 1: Influence of solvent control (DMSO/W 1:9) on frog heart

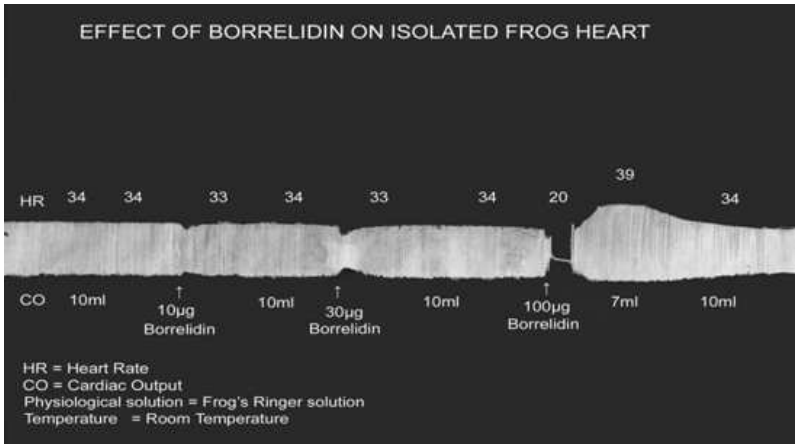

Figure 2: Dose dependent cardiac depressant effect of borrelidin on frog's heart

3.2. Effect of borrelidin on peripheral blood vasculature

Effect of borrelidin on the blood vasculature was studied using frog hind limb perfusion experiment in which the volume of output was measured at different levels. Perfusate flow rate was found by counting the no of drops per minute for consecutive 3 minutes and an average number of drops/min was calculated. An average of 7 drops/min and 8 drops/min was observed for untreated and solvent control respectively.

In borrelidin treated frogs, which were administered with 10, 30 and 100 µg/ml borrelidin, and average number of drops/ min were found to be 5, 4 and 3 respectively. Effect on the peripheral vasculature was observed at relatively high concentration of borrelidin (100 µg/ml) when compared to control animals and at low dose (10 µg/ml) practically no influence was observed and shown in **figure 3**. Based on the observation we presumed that the decreased peripheral blood flow could be attributed to the cardiac arrest of borrelidin at the similar dose.

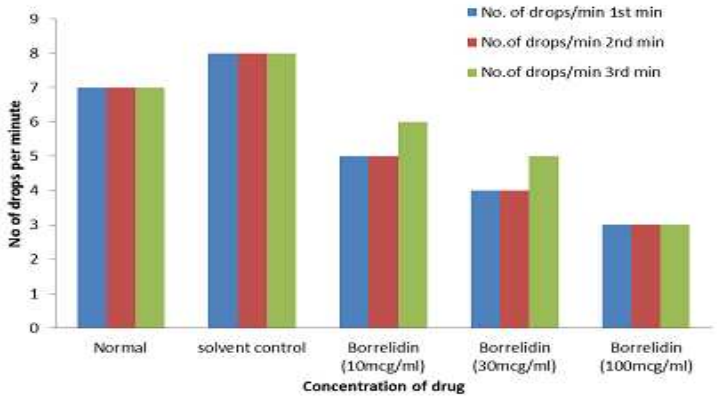

Figure 3: Effect of Borrelidin on peripheral vasculature of frog's heart

3.3. Effect of borrelidin on guinea pig ileum

Different doses of borrelidin viz., 10, 30 and 100 µg/ml were employed to understand the effect on the motility of ileum. Motility of guinea pig ileum was not observed at any study dose of borrelidin. The effect of solvent control was also shown in **figure 4**, which inferred that guinea pig smooth muscle had not shown sign of motility even at the higher concentration of borrelidin. Numerous clinical antibiotics exhibited gut motility and which would lead to colitis [29], however, borrelidin could not produce any intestinal motility and hence its use at higher dose may not cause any adverse effect on intestinal smooth muscle.

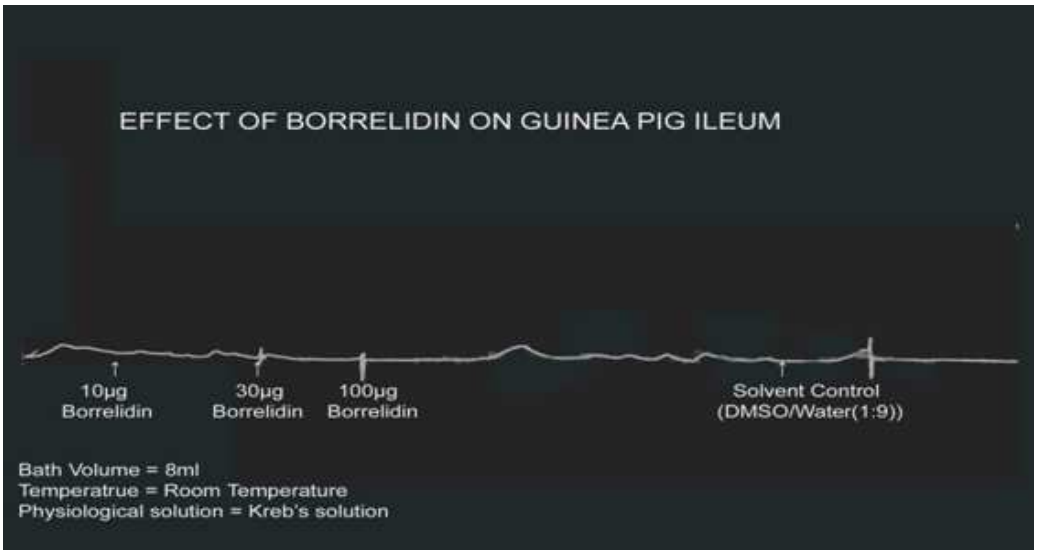

Figure 4: Effect of borrelidin on guinea pig ileum

CONCLUSION

Effects of borrelidin on amphibian heart and peripheral vasculature and further on guinea pig ileum were studied by following reported experimental protocols.

The Straub's technique was used to know the influence of borrelidin on cardiac output and heart rate and both were observed unaltered at the lower doses of borrelidin (10 µg / ml and 30 µg / ml). However, at higher concentration of borrelidin, considerable drop in heart beat and reduced cardiac output were noticed. Influence on the peripheral vasculature was observed at relatively high concentration (100 µg / ml) of borrelidin due to peripheral vasoconstriction.

Borrelidin was tested on intestinal smooth muscle of guinea pig ileum to know effect on the motility. No influence of borrelidin was observed on motility of guinea pig ileum even at the highest test dose 100 µg/ml.

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