Borrelidin: A prospective drug

D V R N Bhikshapathi, Y Shravan Kumar, Y Madhusudan Rao and V Kishan*
University College of Pharmaceutical Sciences, Kakatiya University, Warangal 506 009, India

Received 23 December 2008; revised 20 April 2009; accepted 25 June 2009

Borrelidin, an antibiotic isolated from *Streptomyces* species and firstly isolated from *Streptomyces rochei*, is a possible drug candidate due to its recently reported antiangiogenic activity and other biological activities. Keeping in view the potential scope of the antibiotic, we performed computational studies like application of Lipinski’s rule for borrelidin, and found from the properties of the borrelidin that it possessed good absorption or permeability across the biological membrane. Influence of borrelidin on CYP3A enzyme was tested by pretreatment of the male Wistar rats at a concentration of 100 µg/mL/rat daily for 5 d by oral administration, buspirone was used as a substrate; the results indicated the role of borrelidin acting as an inhibitor of CYP3A. The effect of borrelidin on pretreated rat liver by micromorphological studies was also done and results showed the damage on liver with borrelidin pretreatment. Influence of borrelidin on platelets was studied at three different concentrations along with known antiplatelet aggregating agent, aspirin. Platelet aggregation was noticed for all the three tested concentrations in comparison to control. Influence of borrelidin on prothrombin time was also performed and compared with known anticoagulant, warfarin sodium, have little effect of borrelidin on the prothrombin time which indicated the anticoagulant activity.

**Keywords:** Borrelidin, Lipinski’s rule, CYP3A enzyme, platelets, prothrombin time

**Introduction**

Borrelidin, a 18 membered macrolide antibiotic (Fig. 1), was first isolated from *Streptomyces rochei* in 1949 by Berger and co-workers, and later from other species of *Streptomyces* including *S. griseus* BS 1325, *S. parvulus* Tu 4055, and *S. californicus* MTCC 4401, an Indian soil isolate.

The antibacterial activity of borrelidin has been found to be due to selective inhibition of threonyl tRNA synthetase and the activation of caspase-3 and caspase-8. Many researchers noticed a paradigm shift in biological activity of borrelidin. Recent reports indicated that borrelidin had potent antiangiogenesis activity and it also induced apoptosis of the capillary tube forming cells in dose dependent manner. The antiangiogenic effect of borrelidin was mediated by two mechanisms i.e., threonine-dependent and threonine-independent, and further apoptosis was also induced in capillary endothelial cells via Caspase-8/3 pathway.

Borrelidin was produced by fermenting the organism in medium containing 2% glucose, 2% soyabean flour, 0.5% NaCl, and 0.3% CaCO₃ at a pH of 6.5 for 72-96 h. *S. rochei* also produced borrelidin when grown in a medium containing glycerol, soyabean meal and yeast extract.

Borrelidin was demonstrated to have various biological activities such as antibacterial, antimitotic, antimicrobial, antiviral, herbicidal, insecticidal and antitumor. Borrelidin exhibited excellent antimalarial activity against both chloroquine sensitive and resistant strains in mice. In our search for antibiotics active against drug resistant bacteria, *S. californicus* was isolated from compost yard soil of Gundla Singaram village, Andhra Pradesh, India, which also produced borrelidin. In accompanying paper (Bhikshapathi et al., under preparation) we describe the antimicrobial effect of borrelidin on *Mycobacterium tuberculosis H₃₇Rv* (ATCC 27294) and multidrug resistant *M. tuberculosis*. Further, effects of borrelidin were studied for anti-HIV reverse transcriptase activity and Ames mutagenicity (Bhikshapathi et al., under preparation).

As borrelidin is speculated to be a possible drug candidate due to various reported biological activities and keeping in view the potential scope for further physiological activities associated with borrelidin, we applied the Lipinski’s rule for borrelidin to know the absorption or permeability across biological membrane. Studies were also conducted on the role of...
metabolizing enzymes like CYP3A during absorption and also the possible effect of borrelidin on rat liver parenchyma. Further, tests were also conducted to know the haematological status due to influence of borrelidin on blood platelets and prothrombin time.

In this paper, we describe certain physiological effects of borrelidin on CYP3A enzyme, on rat liver, drug likeliness for absorption or permeation across biological membrane by Lipinski’s rule, and the activity of borrelidin on prothrombin time and platelets.

Materials and Methods

Application of Lipinski’s Rule for Borrelidin to Predict Absorption through Biological Membranes

Christopher Lipinski’s rule of five analysis helped to raise awareness about properties and strategies that make molecules more or less drug-like. The guidelines were quickly adopted as it helped to apply absorption, distribution, metabolism and excretion considerations early in preclinical developments and could help to avoid preclinical and clinical failures13. Properties were calculated using software Cerius-2, Version 4.11, Accelrys Inc., San Diego, California, USA, 2005 and by Descriptor calculator in the QSAR module at GVK Bio Sciences, Hyderabad.

Effect of Borrelidin on CYP3A Enzyme

Buspirone was obtained from Sun Pharmaceuticals, Sodium chloride, Acetonitrile HPLC grade and glucose were obtained from Merck (India) Limited, Mumbai, and Dulbecco’s Phosphate buffer (pH 7.4) from Hi Media (India) Limited, Mumbai. Animals used for the study were male albino Wistar rats. Control animals were without pretreatment and test animals were pretreated with borrelidin in the concentration of 100 µg/mL/rat daily for 5 d by oral route. The solution was prepared using DMSO and distilled water (1:9).

Male Wistar rats were fasted overnight with free access to water before the experiments. The animals were anesthetized with pentobarbital (30 mg/kg/ip). The rats were exsanguinated, and abdomen was opened and small intestine was separated. Then whole small intestine was flushed with 50 mL of ice-cold saline and divided into 3 segments of equal length. Normal sacs with a length of 10 cm were prepared. Probe drug, buspirone, a known substrate for CYP3A, at a concentration of 10 µg/mL was prepared in pH 7.4 isotonic Dulbecco’s PBS (D-PBS) containing 25 mM glucose. The probe drug solution (1 mL) was introduced into the normal sac (mucosal side), and both ends of the sac were ligated tightly. The sac containing probe drug solution was immersed into 40 mL of D-PBS containing 25 mM glucose. The medium was pre-warmed to 37°C and aerated under bubbling condition with 5: 95% of CO2 : O2 for 15 min, the transport of the buspirone from mucosal to serosal surfaces across the intestine was measured by sampling the serosal medium periodically up to 120 min (15, 30, 45, 60 & 120 min)14. The samples were analyzed by HPLC under the following conditions:

- Mobile phase: Phosphate buffer (pH: 2.5) (50 mM): Acetonitrile (50:50)
- Flow Rate: 1 mL/min
- Column: C-18 RP analytical column (Phenomenex)
- λmax: 240 nm
- Range: 0.0050 AUFS
- pH was adjusted to 2.5 with ortho phosphoric acid.

The HPLC system consisted of an LC-10AT vp solvent delivery system (Shimadzu, Japan), a SPD-10A vp UV-visible variable wavelength detector (Shimadzu, Japan), and a 250 x 4.6 mm Luna 5μ C-18 reverse phase analytical column (Phenomenex, Netherlands).

Effect of Borrelidin on Rat Liver

To know the effect of borrelidin on rat liver after pretreatment with 100 µg/d for 5 d, liver from treated animals and control animals were sent for the biopsy. The slides were prepared and observed under microscope for micromorphological changes in liver after Leishman’s staining at a magnification of 10×100.

Effect of Borrelidin on Platelets

Platelet rich plasma was collected from Kakatiya Blood Bank, Warangal, aspirin from Merck (India) Limited, Mumbai. To 100 µL of platelet rich plasma,
100 µL of different concentrations (10, 30 & 100 µg/mL) of borrelidin and aspirin solutions were added and kept aside for about 30 min. Then, 10 µL of the mixture was transferred to the slide. Leishman smearing was made and examined under magnification of $10 \times 100$ for the platelet aggregations.

**Effect of Borrelidin on Prothrombin Time**

Warfarin sodium a free gift from Aventis Sanofi, Goa, sodium citrate, Merck (India) Limited, Mumbai, Thromborel-S, Erba, Germany and Erba Coag Uno Instruments, Germany were used. Citrated plasma was prepared by taking 1.8 mL of blood from healthy volunteers and adding 200 µL of sodium citrate (3.8%), centrifuging for 15 min at 3000 rpm. To 25 µL of citrated plasma, 100 µL of drug solutions were added and mixed properly and to that 50 µL of PT reagent (Thromborel-S) was added. Then prothrombin time was checked \(^{15}\). The experiments were conducted for different concentrations of borrelidin and warfarin sodium (25, 50, 100, 250 & 500 µM).

**Results and Discussion**

**Application of Lipinski’s Rule for Prediction of Absorption through Biological Membranes**

Lipinski’s rule is a computational approach developed to predict the solubility and permeability across biological membranes. According to Lipinski’s rule poor absorption or permeation is more likely when there are more than 5 H-bond donors, more than 10 H-bond acceptors, molecular weight greater than 500, C log P greater than 5 \(^{12}\). To evaluate drug likeness better, the rules have spawned many extensions, for example: Partition coefficient, log P in -0.4 to + 5.6 range, Molar refractivity from 40 to 130 \(^{16}\).

Properties of borrelidin with their calculated and desirable values as per Lipinski’s rule and its extensions are shown in Table 1. As per Lipinski’s rule of five, the drug borrelidin was expected to have good permeability. Even the extensions of Lipinski’s rule about molar refractivity supported this view since its molar refractivity was less than 130. All these desirable characters of borrelidin indicated good absorption or permeability across biological membrane.

**Effect of Borrelidin on CYP3A Enzyme during Absorption**

Buspirone, already known as a substrate for CYP3A metabolism \(^{17}\), was used as a marker compound to study the influence of borrelidin on CYP3A mediated metabolism. Results of control (without pretreatment) and pretreatment (with borrelidin, 100 µg/d for 5 d) of buspirone transport across gastro-intestinal membrane at three different regions, duodenum (Fig. 2a), jejunum (Fig. 2b) and ileum (Fig. 2c) are shown. The transport of buspirone from normal sac was increased by many

Table 1—Properties of borrelidin with their calculated and desirable values as per Lipinski’s rule and its extensions

<table>
<thead>
<tr>
<th>Properties</th>
<th>Calculated values</th>
<th>Desirable values</th>
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<tbody>
<tr>
<td>Molecular weight</td>
<td>489.5</td>
<td>&lt;500</td>
</tr>
<tr>
<td>H-bond acceptor</td>
<td>7</td>
<td>&lt;10</td>
</tr>
<tr>
<td>H-bond donor</td>
<td>3</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Log P (molecule based)</td>
<td>5.65</td>
<td>0.4 to + 5.6</td>
</tr>
<tr>
<td>Molar refractivity</td>
<td>120.58</td>
<td>40 - 130</td>
</tr>
</tbody>
</table>

![Fig. 2a-c—Transport of buspirone across rat intestine in non everted sac method for pretreated (100 µg/rat for 5 d with borrelidin) and control (without pretreatment): Influence of borrelidin on buspirone transport; a. in duodenum; b. in jejunum; & c. in ileum.](image)
folds at different regions of small intestine due to the pretreatment of borrelidin for 5 d. In the first 45 min, 1.5 to 4-fold increase was noticed in duodenum as compared to control rats. But from 60-120 min, no traces of buspirone were found transported in control animals. This indicated that the CYP3A metabolism played a big role in duodenum. The transport of buspirone was increased and depended on the time. Further, maximum transport of buspirone from intestinal sac was observed from jejunum and ileum when compared to that of duodenum.

Buspirone is a known substrate to study the effect of borrelidin on CYP3A metabolism from normal sacs. It is already known that rat enterocytes contain CYP3A, and this enzyme in general would reduce the intestinal absorption of drugs into systemic circulation because of metabolism. As CYP3A enzyme levels decreased from duodenum to ileum it was expected that the buspirone transport would increase. Following the same, a significant transport of buspirone from normal sacs (with pretreatment of borrelidin) was observed, which clearly indicated the role of borrelidin as an inhibitor of CYP3A. From the above results, it was concluded that borrelidin acted on CYP3A mediated drug metabolism either by direct inhibition or inhibiting the enzyme synthesis. It was earlier reported that the macrolide antibiotics like erythromycin, clarithromycin, etc., had the CYP3A inhibition only, since this drug also shared some of the structural features, it is expected that this also inhibited the activity of enzyme rather than the synthesis.

Effect of Borrelidin on liver-micromorphological Studies

Rat liver biopsy slides with and without pretreatment were examined under a microscope at a magnification of 10 x 100. Results are depicted in Fig 3a for control (without pretreatment), and Figs 3b & c with pretreatment of borrelidin. Due to pretreatment of rats with borrelidin, liver cells were swollen, dilatation of central and hepatic veins were noticed in comparison to normal cells and vein in control rat liver. This observation clearly indicated the damage to liver with the pretreatment of borrelidin; it also indicated that borrelidin has a toxic effect on liver, when treated with the concentration of 100 µg/d for 5 d.

Effect of Borrelidin on Platelets

Influence of borrelidin on platelet rich plasma was studied at three different concentrations (10, 30 & 100 µg/mL) along with that of a known antiplatelet

Fig. 3a-c—Photomicrographs of rat liver stained with Leishman stain (magnification 10 x100), showing: a. Control rat liver (without pretreatment) with normal central vein (Arrow) and normal liver cells without any damage.; b. Pretreated with borrelidin for 5 d (100 µg/rat) showing cellular damage and swollen cells; c. Pretreated with borrelidin for 5 d (100 µg/rat) showing liver damage with dilated central vein.
aggregating agent, aspirin. Results observed under microscope for borrelidin and aspirin on platelets are depicted in Figs 4a (control), 4b (borrelidin, 10 µg/mL), 4c (borrelidin, 100 µg/mL) and 4d (aspirin 100 µg/mL). Platelet aggregation was noticed for all the three tested concentrations in comparison to control. The magnitude of aggregates varied with the concentrations of borrelidin. However, at all the tested concentrations no effect of aspirin was seen on platelets i.e., platelets remained without any aggregation. The mechanism for platelet aggregation is not yet known for borrelidin. Fig 4d depicts the effect of highest concentration only.

Effect of Borrelidin on Prothrombin Time

Prothrombin time (in sec) was measured at different time intervals on citrated blood plasma from 6 healthy volunteers using Erba Coag Uno instrument. Normal range for prothrombin time is 10 to 13 sec and a calculated value INR (International normalized ratio) is 1.0 to 1.4. The INR is calculated by the following formula, INR = (Patient prothrombin time/mean normal PT). The calculated mean normal PT is 13 sec in a clinical setting (Jaya Hospital, Hanamkonda, AP). The normal PT range is 11-14 sec. All the observed and calculated values indicated that there was some effect on the prothrombin time in comparison with standard anti-coagulant drug i.e., warfarin (Fig. 5). This indicated the tendency for bleeding or otherwise blood clotting would be affected following the administration of borrelidin. However the mechanism for the anti-coagulant effect of borrelidin is not yet known.

Acknowledgement

The authors would like to thank the AICTE, New Delhi for providing research funds under MODROB scheme vide File No. 8020/RID/MODROB-5/2001-2002. DVRNB is thankful to AICTE for providing the scholarship under Quality Improvement programme for teachers and DAAD equipment grant, Germany. Authors thank Prof. Axel Zeeck of Goettingen University, Germany for gifting sample of borrelidin. Further, the authors are also thankful to Dr P V Beerbhadra Rao (VBR Diagnostics, Hanamkonda), for helping in platelet activity and micromorphological studies of rat liver and Mr B Kiran Kumar, GVK Biosciences, Hyderabad for calculating properties based on Lipinski’s rule for borrelidin and Mr Ranjit Kumar, Biochemist, Jaya Hospital, Warangal for helping in the activity of borrelidin on prothrombin time.

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