Novobiocin

Katharina Decker, Carolin Dombrowsky, Belinda Escher, Sabrina Falco

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1. General information and discovery

Novobiocin belongs to the family of aminocoumarin antibiotics. In 1955 and 1956, it has been discovered by several industrial research centers (Wallick et al. in S. spheroides, Lin et al. in S. niveus and Pfizer Company)\(^1\) and published under different names. Cathomycin, Cathocin, Streptonivicin, Albamycin, Cardelmycin and Antibiotic P.A. were isolated in different strains of actinomycetes (bacteria) until Finland (1955), Welch and Wright\(^2\) ascertained that all these names were given to the same molecule. Therefore the name was changed to Novobiocin and Cathomycin, Albamycin, and Cardelmycin were kept as registered trade names.

Structure of Novobiocin

The molecular formula of Novobiocin is $C_{30}H_{36}O_{11}N_2$ and its molecular weight is 612.6 g/mol. The molecule is optical active in a solution of ethanol and in pyridine, soluble in metha-, etha- and butanol, acetic acid, dioxane and insoluble in ether, benzen, carbon tetrachloride, chloroform and water. Its structure has been examined by different research groups and is shown in figure 1. Novobiocin can be gained by fermentation and has a wide range of impact on different micro-organisms as shown in table 1. However, Novobiocin is not effective against acid-fast bacilli, fungi, rickettsiae and viruses. The intake of Novobiocin is orally or parenterally.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Minimum concentration for inhibition in $\mu$g/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micrococcus pyogenes var. aureus</td>
<td>0.19</td>
</tr>
<tr>
<td>Diplococcus pneumoniae</td>
<td>0.7 – 12.0</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>3.12</td>
</tr>
<tr>
<td>Corynebacterium diphtheria t. gravis</td>
<td>0.39</td>
</tr>
<tr>
<td>Corynebacterium diphtheria t. intermedius</td>
<td>0.39</td>
</tr>
<tr>
<td>Corynebacterium diphtheria t. mitis</td>
<td>0.39</td>
</tr>
<tr>
<td>Neisseria intercellularis</td>
<td>0.39</td>
</tr>
<tr>
<td>Pasteurella avicida</td>
<td>3.12</td>
</tr>
<tr>
<td>Proteus vulgaris M1</td>
<td>25.0</td>
</tr>
<tr>
<td>Escherichia coli W</td>
<td>&gt; 200.0</td>
</tr>
<tr>
<td>Salmonella typhi M</td>
<td>&gt; 200.0</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>&gt; 200.0</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>&gt; 200.0</td>
</tr>
</tbody>
</table>

2. The Novobiocin producing bacteria

The species *streptomyces* includes more than 600 types and belongs to the obligate aerobic, gram-positive, clubbed bacteria and *actinomycetes*. *Streptomyces spheroides* and *streptomyces niveus* produce Novobiocin. Both are found in ground. The filamentous growing bacteria possess a genome of $8.000 – 10.000$ bp with a high GC content (>70%).

6Grundlagen der Chemotherapie, Jürgen Drews, Springer-Verlag, 1979, S.15
S. spheroides and s. niveus are of interest because of their high variability in metabolism. They have substrate mycelia that differentiate in air mycelia when environmental conditions worsen. Afterwards the bacteria run trough sporulation. For these procedures different signaling molecules are needed. Their synthesis delivers approximately 10,000 of secondary metabolites. The organisation of the bacterial genome explains the coordination of differentiation and production of secondary metabolites: It is organised in clusters. Both of these procedures have the same regulatory elements and therefore are active or inactive together.

The secondary metabolites can be used in different ways: bactericide, fungicide, antiviral agent, insecticide, herbicide and cytostatic drug.

So far, researches have been „restricted to the investigation of Novobiocin resistance genes, especially gyrB’, and the production of novobiocin-deficient mutants.“ The discovery of genes responsible for the biosynthesis of Novobiocin is connected to the hope for the development of new drugs. The research for for genes linked to Novobiocin production in S. niveus has not been successful yet. In S.spheroides a 25,6 kb long dna sequence was found that is coding for the Novobiocin production pathway (figure 3). This could be used for recombinant Novobiocin production.

9Identification of the Novobiocin Biosynthesis Gene cluster of S. spheroides, Marion Steffensky et al., Antimicrobial agents and chemotherapy, May 2000, Vol. 44, No.5, S. 1214
10Identification of the Novobiocin Biosynthesis Gene cluster of S. spheroides, Marion Steffensky et al., Antimicrobial agents and chemotherapy, May 2000, Vol. 44, No.5, S. 1214
3. Synthesis

The molecule Novobiocin can be split into three different parts called ring A, B and C. In the first step of the synthesis each ring is modified by several enzymes. At the end the three rings are coupled together by enzymatic reactions and the molecule is furthermore modified.

Novobiocin is a natural molecule, produced by bacteria, this is the reason why nearly each part of the synthesis is catalysed by enzymes. A chemical synthesis strategy has yet to be developed.

**Ring A:**
The synthesis of the first ring (ring A) starts with the molecule Prephenate (a derivative of shikimic acid). In the first step a decarboxylase helps to remove a carboxyl group. Then a dimethylallylgroup is added to the benzene ring. The third and fourth step are catalysed by the same enzyme. It decarboxylates in presence of molecular oxygen two times.

**Ring B:**
To synthesise ring B the amino acid L-tyrosine is needed. First an adenosine monophosphate (AMP) is added to the carboxyl group. Then the molecule is coupled to PCP (peptidyl carrier protein). After this the β-position of the amino acid is oxygenised resulting in a hydroxyl group in β-position, which is oxygenised again into its keto form. Due to tautomerism the molecule also exists in its enol form. In the next step the
benzene is oxygenised in ortho-position to the former amino acid group. Now the molecule will be immediately lactonize and lose AMP

![Chemical structure](image1.png)

**Ring C:**
The starting molecule for the synthesis of ring C is glucose-1-phosphate which is a derivative of alpha-glucose. In the first step the phosphate group is substituted by desoxythymidindiphosphate (dTDP). Then the hydroxyl group at position four is oxygenised. In the next two steps the enzymes converts the sugar in its C3-epimer and methylate the fifth position. In the last step the fourth position is reduced to a hydroxyl group in the inverse configuration compared to the starting molecule, resulting in the molecule L-Novobiose.

![Chemical structure](image2.png)

After the synthesis of the three rings they have to be coupled together and is furthermore modified. In the first step ring A and ring B are linked together by a peptide bond. Second the benzene from ring B is methylated by a methylase. After this the L-Novobiose is added to ring B. Then the hydroxyl group at carbon atom four is methylated. The last step to get the complete molecule Novobiocin is to carbamylate the hydroxyl group at carbon atom three.

**4. Clinical use:**

Novobiocin is an aminocoumarin antibiotic against infections of gram positive bacteria like *Staphylococcus*. In addition it is active against *Staphylococcus epidermidis* and may be used to differentiate it from the other coagulate-negative *Staphylococcus saprophyticus*, which is resistant to Novobiocin, in culture.
Novobiocin has many side effects, so it is just used in case of grave infections like MRSA (methicillin-resistant Staphylococcus aureus), which is mostly seen in hospitals, because of an high amount of contacts with antibiotics.

5. Mechanism of acting:

Novobiocin acts as a competitive inhibitor by blocking the ATP binding site of the Gyrase B subunit. Gyrase is a tetramer consisting of two A and two B subunits, subunit A binds with its tyrosine rest to the DNA and is responsible for cutting and reassembling the DNA double-strand and subunit B for ATP binding, so that the ATP gate between the two subunits closes and the conformation changes.

The Gyrase reaction mechanism is similar to the eukaryotic Topoisomerase II. Gyrase catalyses the relaxation of right-handed coiled plasmid DNA so that it can be read more easily by DNA polymerases. This reaction happens in the absence of ATP, so it is unaffected by the intake of Novobiocin. The difference between Topoisomerase II and Gyrase is that it can add negative supercoils to the relaxed DNA to save space in the cell. This part of the reaction is depending on the presence of ATP so it can be manipulated by the intake of Novobiocin.

Novobiocin binds to the N-Terminus of the B subunit. In conclusion the Gate closes and the binding site is blocked so that ATP is not able to bind anymore. The enzyme Gyrase can not change its conformation like it would do, if ATP binds, so it is not able to add negative supercoils to the relaxed DNA after replication and as a result the DNA expands and the cell bursts. As a consequence the bacteria die.

In addition some parts of the replication depend on different types of the Topoisomerase II like the last step of termination, where the two rings of DNA double-strand have to be divided so that the cell division can start. If this mechanism is inhibited by Novobiocin too, the cell can not replicate completely and it would burst too.
Important for the mechanism of inhibition is aminocoumarin and without the carbamic acid of the sugar Novobiose the inhibition would be much weaker. Novobiocin has a high affinity for Gyrase but it also inhibits a few of the eukaryotic Topoisomerases too, which may be the reason why it causes heavy side effects.

6. Side effects

The known side effects of Novobiocin are gastrointestinal disturbances (e.g., irritant bowel), allergic reactions, disturbances of the blood count, yellow coloring of the skin and scleras as well as liver malfunctions.

On account of the high sensitization rate of Novobiocin it often comes to allergic reactions in the form of an urticaria and/or an exanthem accompanied with fever mostly. The disturbance of the blood picture can be apparent by a removal of erythrocytes or leukocytes (anemia or leukocytopenia) and by a removal of all three blood cell types (pancytopenia). Jaundice (yellow coloring of the skin and the scleras) can arise from the decomposition product of the novobiocin and/or from an enrichment of bilirubin (hyperbilirubinemia) which is a decomposition product of hemoglobin.

In “normal” bilirubin metabolism (without the intake of Novobiocin) the lipophilic bilirubin binds via loose non-covalent bond to the protein albumin. The so-called unconjugated bilirubin arises which is transported over the blood to the liver. The reduction of bilirubin is catalyzed by an enzymatic reaction in the liver in which bilirubin is coupled to glucuronic acid by UDP-glucuronosyltransferase. The arisen molecule is called "conjugated" bilirubin and is hydrophilic. It can be eliminated over the gall and the intestines. By the intake of Novobiocin, the bond formation between bilirubin and albumin is inhibited which leads to a transportation disturbance to the liver. Furthermore, the enzyme UDP-glucuronosyltransferase is inhibited, therefore, the reduction and the elimination of bilirubin is not possible.

This side effect has a special effect strongly within newborn children. Because of the incompletely developed blood-brain barrier the bilirubin can dispost in the basal ganglia of the cerebrum. This syndrome is called kernicterus. If not treated, this can lead to developmental disorders up to death, why the medication is immediately finished with Novobiocin at the appearance of jaundice.