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Super Antibiotics, Part II. Hyperforin, Mass Spectroscopy (MS) and Gas Chromatography-Mass Spectrometry (GC-MS), Evidence of Permeability of the Blood-Testis Barrier (BTB) and the Blood-Brain Barrier (BBB) to Hyperforin

Ilia Brondz

Norwegian Drug Control and Drug Discovery Institute (NDCDDI), Ski, Norway
Email: ilia.brondz@gmail.com

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Abstract

In the first article of this series, we presented some evidence of hyperforin as an antibiotic, antiprotozoal, antiviral, anticancer, and immunomodulatory substance. In the present article, evidence of the permeability of the blood-testis barrier (BTB) and blood-brain barrier (BBB) to hyperforin and its distribution in other organs of the domestic pig (*Sus scrofa domesticus*) are revealed. Seven-month-old male boars with a body mass of 100 kg were fed a diet containing hyperforin. Organs were surgically removed under anesthesia. Organs were suitable prepared and extracted, and then analyzed using gas chromatography-mass spectrometry with supersonic molecular beams (GC-MS with SMB). The presence of hyperforin was recorded in all organs and body fluids. Special attention was paid to the evaluation of the presence of hyperforin in the brain and testes of experimental animals. The presence of hyperforin in the brain and testes of experimental animals was established by GC-MS with SMB. The results are of interest because penicillin and numerous other antibiotics cannot pass through the BTB or BBB if healthy or non-inflamed, which limits their use in patients with meningitis and gonorrhea. The findings are also of interest in cases of penicillin- and multi-antibiotic-resistant bacterial infections.

Keywords

Antibiotics, Hyperforin, Super antibiotic, Mass Spectroscopy, Gas Chromatography-Mass Spectrometry, Blood-Testis Barrier, Blood-Brain Barrier,

Methicillin-Resistant *Staphylococcus aureus* (MRSA)

1. Introduction

A previous publication [1] has explained the urgent necessity for the development and introduction of a new generation of antibiotics with the ability to act against methicillin-resistant *Staphylococcus aureus* (MRSA) and other antibiotic-resistant microorganisms, as well as against multi-antibiotic-resistant bacteria, into medical practice. Of especially high value are antibiotics such as hyperforin that exhibit positive immunomodulatory effects. In some cases, it is a primary necessity to have a tool that passes through the BTB or BBB at all stages of infectious diseases, as well as in generalized inflammatory stages, and in such as the healing and healthy (post inflammatory) stages, to allow the total clean-up of the organ from possible remaining infectious agents and the total eradication of infection from the organs, even after the disappearance of clinical symptoms.

Previous research [2] described some microorganisms affected by hyperforin, including those that are penicillin-resistant. Subsequently, the immunomodulatory action of hyperforin was presented in [3] [4] [5] [6]. After Brondz completed a graduate degree in the pharmaceutical sciences in 1979, he performed all research on the medical use of hyperforin at a private company, Jupiter, and later at Jupiter AS (Jupiter Ltd.) because of the unscientific, unethical approach of Docent Aasen (Pharmaceutical Department, University of Oslo, Norway), who aimed to commercialize the scientific work of Brondz without his knowledge.

The presented works are based on previous research [2] [3] [4] [5] [6] and studies performed in the former Soviet Union on the pharmaceutical form of Novoimanin [7] [8] [9], which contains a considerable percentage of hyperforin. Relatively new research has also revealed additional useful properties of hyperforin, such as anticancer [10], [11], anti-inflammatory [12], and antiprotozoal/antimalarial activities [13], as well as its role as an additional tuberculosis medication [14].

The 1972 thesis of Volosovets and Resnick, entitled "Novoimanin stimulant immunogenesis", provides some clues regarding the possible effect of Novoimanin on the immune system of experimental animals. However, Novoimanin is a complex mixture of a large number of natural products. It was therefore necessary to identify the exact substance that had an impact on the immune system: hyperforin [3] [4] [5] [6].

Mass Spectral Elucidation of the Structure of Hyperforin

The study of hyperforin was started in the former Soviet Union. Because of the thermolability of hyperforin, it was difficult to record its gram mol (molecular weight) by MS using electron impact (EI). In the former Soviet Union the gram mol of hyperforin was elucidated by element analysis. In Norway in 1978, we experienced the same problems. To correct this, the fraction containing hyperforin was used for biological detec-

tion via element analysis and MS with EI, even though MS with EI did not give conclusive results. To obtain conclusive results for M^+ and support the fragmentation of the hyperforin molecule as an isoprene-contacting substance, MS with chemical ionization by NH_3 was performed [2].

The results supported the element analysis of the gram mol (536 gr/mol) of hyperforin as M^+ *m/z* 537 and demonstrated the presence of isoprene chains by fragmentation [2] (**Figure 1**).

Subsequently, in all MS investigations, M^+ *m/z* 537 was used as a reference point and the specificity of molecular fragmentation as *m/z* 55, *m/z* 56, *m/z* 67, *m/z* 68, *m/z* 69, and *m/z* 83, as well as the presence of a characteristic ion with *m/z* 277, *m/z* 401, *m/z* 411, *m/z* 413 and *m/z* 469 used as a support.

2. Materials and Methods

2.1. Biological Materials

Hyperforin was donated by Jupiter Ltd., Norway. Sample was analyzed by MS for authenticity.

Three domestic pigs (*Sus scrofa domesticus*) were used in the experiments. Male boars aged 7 - 8 months with body masses of 100 - 110 kg were fed a diet containing hyperforin (10 mg/kg of body weight) for 14 days, three times/24 h. Several organs (including the brain, testis, heart, lungs, kidneys, liver, and blood) were surgically removed under anesthesia, and after suitable preparation, extraction, and clean-up, were analyzed by gas chromatography-mass spectrometry with supersonic molecular beams (GC-MS with SMB). Extracts were also kindly provided by Jupiter Ltd., Norway.

2.2. Gas chromatography-Mass Spectrometry with Supersonic Molecular Beams (GC-MS with SMB) of *Sus scrofa domesticus* Testes Extracts

One milligram of vacuum-frozen dry extract from testes was dissolved immediately before the GC-MS analysis in 1 mL of chromatography-grade *n*-hexane (quality for liquid chromatography LiChrosolv® (Merck Millipore)), which was purchased from Sigma-Aldrich, Norway.

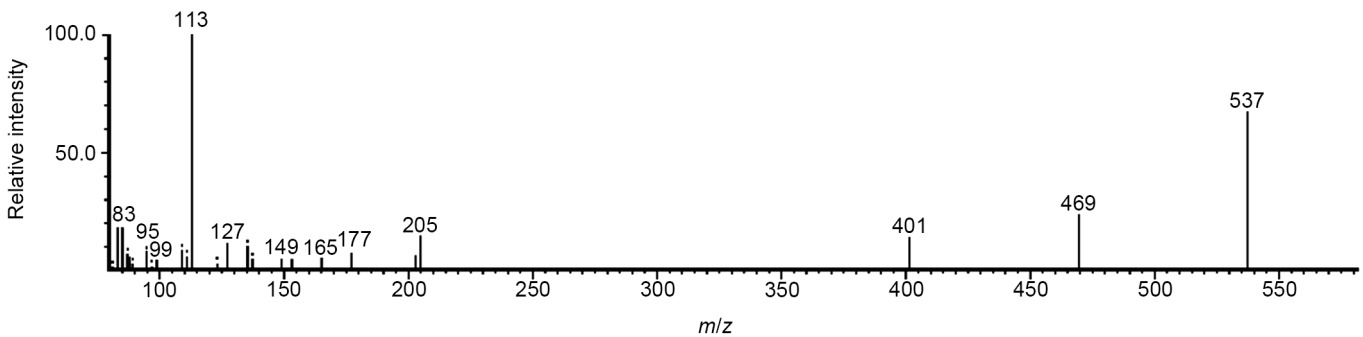

Figure 1. The mass spectrum of hyperforin shows an M^+ of *m/z* 537, which implies that the gram/mol should be 536. The details are given in [2].

The experimental GC-MS with SMB based on a Varian 1200 GC-MS system was performed as described in [15] [16]. Separation of the compounds was carried out using a VF-5HT column (0.25 mm I.D., 0.1 μm film thickness, and 4 m length; Varian, Middleburg, The Netherlands). Reduction of the column's length was performed in the laboratory. The helium column flow rate was 16 mL/min. One μL of the extract in *n*-hexane at an approximate concentration of 100 ppm was injected with a split ratio of 10:1 using the Varian 1079 injector at 240˚C. The GC oven was programmed from 120˚C - 280˚C at 20˚C/min, and the drug hyperforin was eluted at about 5.69 min without any degradation, because of the use of a short column and a high column flow rate [17] [18]. Ion source degradation was prevented using a contact-free, fly-through EI ion source [17] [19]. The separation and detection of hyperforin in the mixture are shown as a TIC chromatogram in **Figure 2**.

3. Results and Discussion

The GC-MS analyses shown in **Figure 2** demonstrated the presence of hyperforin in the testes of healthy animals, which had been administered hyperforin through food. The presence of hyperforin was recorded in the brain and other organs such as the heart, lungs, kidneys, liver, and blood. The presence of hyperforin in the testis and brain of *Sus scrofa domesticus* suggests that the drug can pass through a healthy BTB and BBB. Because *Sus scrofa domesticus* serves as a physiological and pharmacological model in many medical experiments [20] [21] [22] [23] and has been accepted as a good approximation to physiological and pharmacological processes in humans, based on the results described above, it is possible to propose that hyperforin passes freely through the BTB and BBB in humans. The presence of hyperforin was recorded in the kidney, which is also important in cases of venereal illnesses such as gonorrhea and syphilis.

Hyperforin was recorded in the lungs, which is advantageous regarding its use in patients with pneumonia and tuberculosis [14]. In the past year, a rise in the number of malaria cases was observed in India, and the reappearance of malaria in Southern Europe was reported. In this regard, it is important to develop an additional tool to fight malaria [13].

4. Conclusions

The qualitative presence of hyperforin in the testes and brain, and in other organs of *Sus scrofa domesticus* was studied by GC-MS with SMB. Despite the observation that the peak with a retention time (R_t) between 5.6 and 5.8 min also contained other lipophilic molecules in the background, the presence of hyperforin in this peak was undisputable. M^+ *m/z* 537 was recorded, and fragments from isoprenoid chains were observed as *m/z* 55, *m/z* 56, *m/z* 67, *m/z* 68, *m/z* 69, and *m/z* 83, as well as the presence of a characteristic ion with *m/z* 277, *m/z* 411, *m/z* 413 and *m/z* 469. The standard hyperforin had the same R_t (chromatogram not shown) and characteristic M^+ *m/z* 537 and the characteristic series of fragment ions with *m/z* 55, *m/z* 56, *m/z* 67, *m/z* 68, *m/z* 69, *m/z* 83, and with *m/z* 277, *m/z* 401, *m/z* 411, *m/z* 413 and *m/z* 469 [24] [25] [26].

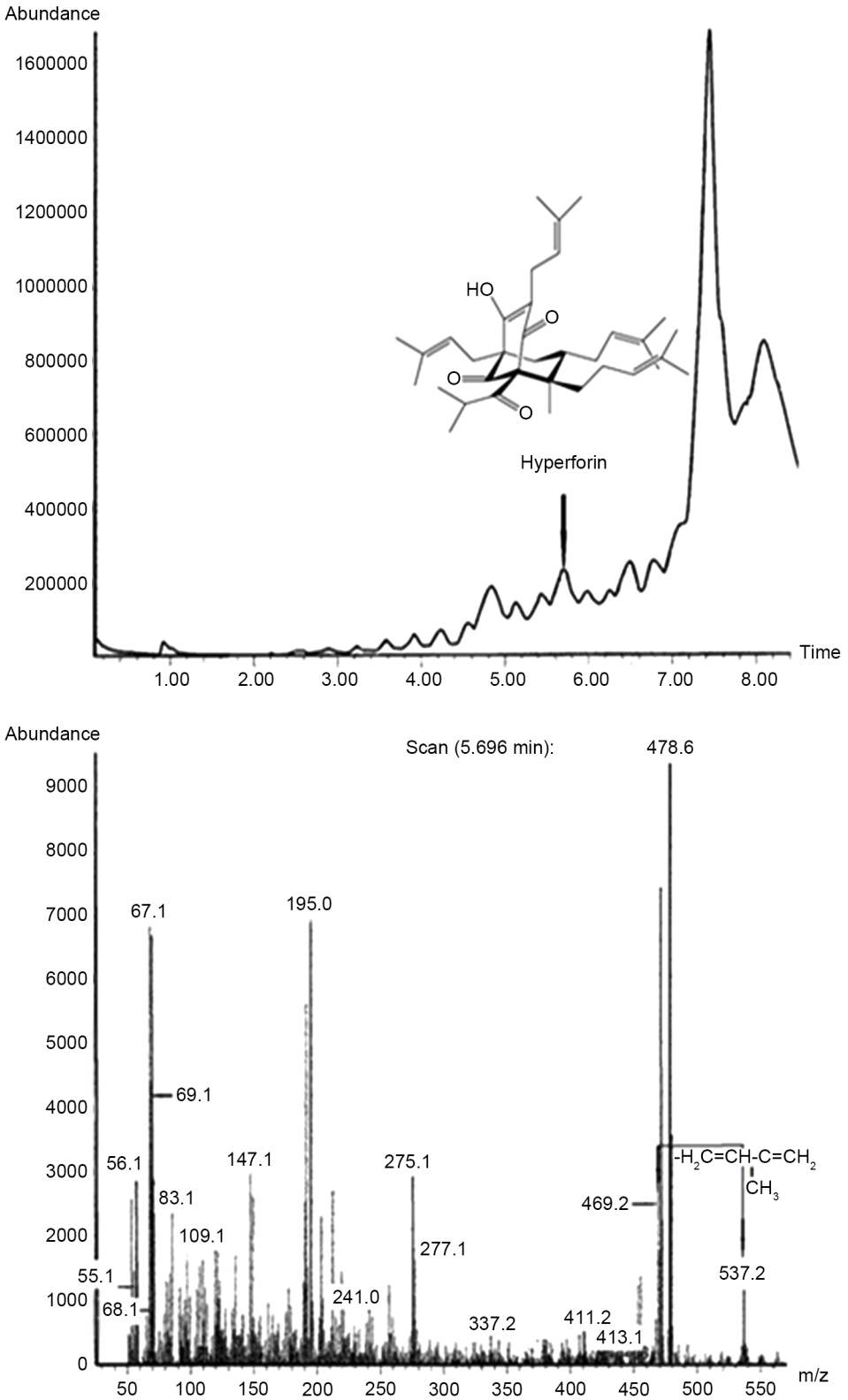

Figure 2. The upper trail is the TIC of MS of 1 μL of the *Sus scrofa domesticus* testis extract in *n*-hexane. The peak containing hyperforin appears between 5.6 and 5.8 min, at 5.696 min. The lower trail is the mass spectrum taken at 5.696 min.

However, the quantitative presence of hyperforin in different organs and body fluids can be analyzed most suitably via supercritical fluid chromatography (SFC) and supercritical fluid chromatography–mass spectrometry (SFC-MS), because these can be performed at low temperature and by pre-isolation of the peak containing hyperforin, followed by re-chromatography or two-dimensional chromatography. The advantages of using SFC, SFC-MS compared with other chromatographic methods are presented in [27] [28] [29] [30]. There is no doubt that hyperforin passes through the BTB and BBB, and its antibacterial action against *N. meningitidis* and *N. gonorrheae* is clear. Based on its multifunctionality, hyperforin could become a useful tool in the struggle against a broad spectrum of inflammatory, infectious, and cancerous diseases. It could also become a supporting medication in cases of tuberculosis and malaria. The presence of hyperforin was recorded in the testis, brain, hart, kidney, lungs, skin, and blood of *Sus scrofa domesticus* after administration through the diet.

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