The NeBrasCa ERA-IB project: biotechnological development of brasilicardin analogs as new immunosuppressant drugs

The European research consortium NeBrasCa provided access to the promising immunosuppressive compound brasilicardin using a combination of biotechnology and semi-synthesis

Immunosuppressive drugs represent valuable tools to treat a wide range of autoimmune diseases and have been available since the 1970s when they were first used in successful organ transplantations. Despite the subsequent development and progress in this field and an increasing number of treatment options, there are still two major unmet clinical needs that need to be addressed by an intensified development of new immunosuppressive drugs: firstly, most organ transplants continue to have low rates of long-term graft and patient survival and even today almost 50% of all lung transplant recipients die in the first five years after transplantation.

The second issue is the fact that the currently available immunosuppressive drugs possess serious side effects. Nonselective antiproliferative drugs, such as azathioprine, cyclophosphamide or methotrexate affect various fast proliferating cells and cause serious bone marrow suppression, impair host resistance, and increase the incidence of infections. Typically, they also have a slow onset of action and a moderate efficacy that declines after several years of treatment.

However, even the more selective calcineurin-inhibitors cyclosporine A and tacrolimus as well as the mTOR-(mammalian Target Of Rapamycin)-inhibitors rapamycin (*syn.* sirolimus) and everolimus, which all together act on different stages of the T- and B-lymphocyte activation, possess serious side effects, including acute neurological toxicity, chronic nephrotoxicity, arterial hypertension, biphasic effects to bone structure, and hypertriglyceridemia.

A further specific small molecule represents the inosine monophosphate dehydrogenase-inhibitor mycophenolate mofetil. It causes adverse events, such as anaemia, teratogenicity and an increased risk of opportunistic infections, such as activation of latent viral infections (e.g. *herpes* virus, cytomegalovirus and BK virus). Biologicals, such as antibody-based drugs, have a faster onset of action and exhibit a higher specificity than the existing small molecule-based therapies but they can also cause severe side effects such as the development of idiopathic thrombocytopenic purpura, progressive multifocal leucoencephalopathy, and lupus-like syndromes or vasculitis.

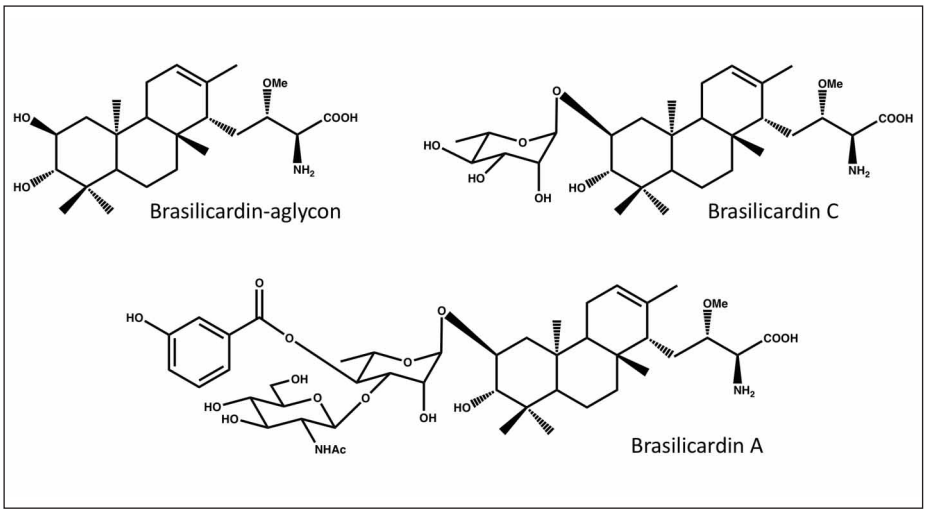

Fig. 1 Chemical structures of brasilicardins

Fig. 2 The European NeBrasCa Team

Moreover, they are very expensive and therefore only applied in the induction therapy (i.e. the first six weeks after transplantation) and not in the subsequent life-long maintenance therapy. Since the side effects of a single immunosuppressant is often too high, commonly an individual combination therapy consisting of two to four immunosuppressants, each with a reduced dose, is applied to balance out the side effects.

The development of new immunosuppressants that show an improved long-term patient's survival and a lack of undesirable side effects thus remains a high priority.

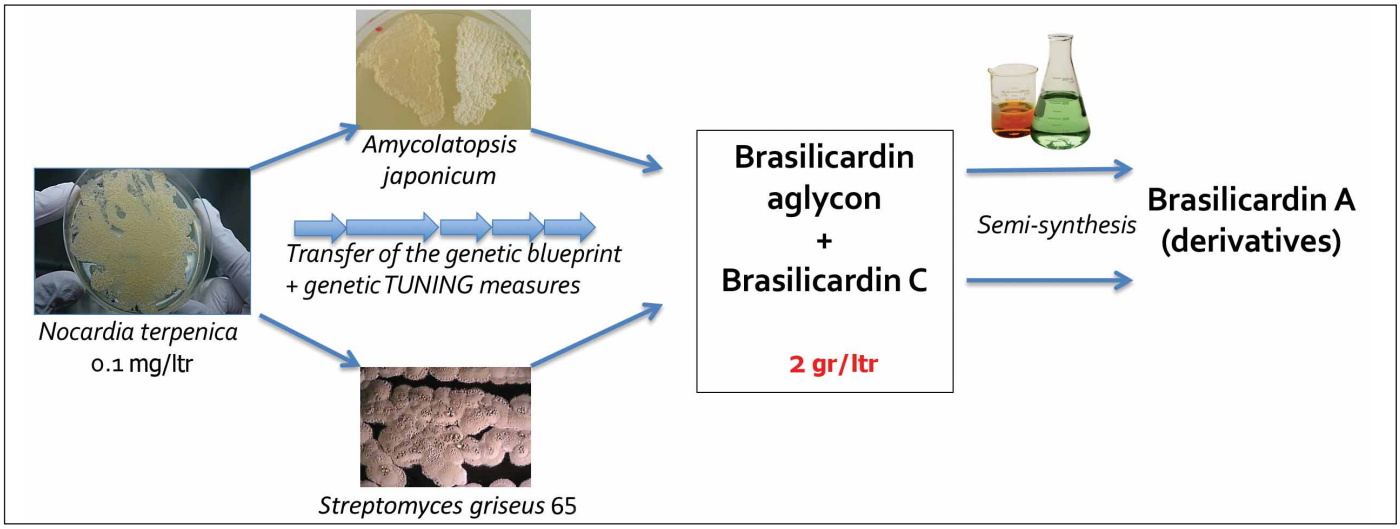

Fig. 3 Concept of the combined approach employing biotechnology and semi-synthesis

Brasilicardin – a promising novel immunosuppressant

Brasilicardin A (Fig. 1) is an immunosuppressive natural product isolated from the human pathogenic bacterium *Nocardia terpenica*. It was shown to be highly potent in a mouse mixed-lymphocyte reaction (MLR) assay and surpassed with an IC_{50} value of 0.057µg/ml indeed the potency of standard drugs such as cyclosporine and tacrolimus.

Brasilicardin mediates its activity by inhibition of the amino acid transporter system L. This system is the major transporter for essential amino acid uptake in activated human T cells. More precisely, it represents an obligatory 1:1 amino acid exchanger, which can couple the cellular uptake of branched-chained and aromatic amino acids with the efflux of cytoplasmic amino acids such as glutamine. An inhibition leads to a cellular depletion of essential amino acids within activated lymphocytes and causes GCN2-dependent integrated stress responses which in turn results in the inhibition or retardation of cell proliferation.

With this novel mode of action, brasilicardin A is significantly less toxic than cyclosporine and tacrolimus. Owing to this underexploited target and the reduced toxicity, brasilicardin was expected to be a promising new immunosuppressive drug.

However, its development was hampered due to the scarcity of available material. This is given since the original producer is classified as a human pathogenic strain (biosafety level BSL-2) and shows a low production yield. Very recently, the technical feasibility of the total synthesis of brasilicardin A was demonstrated, however, due to a low overall yield, this approach has to be regarded as well as non-economical.

The solution of the NeBrasCa consortium to overcome the production bottleneck to push forward the drug development

Encouraged by the promising preliminary data and the given production bottleneck, we, the five-strong international NeBrasCa-team (Fig. 2) embarked on the development of a safe (BSL-1) and economical biotechnological production platform. In a first step, we were able to transfer the genetic blueprint for brasilicardin into over 70 non-pathogenic host bacteria, which were closely related to the original producer strain.

Two strains thereof proved to be high yield production strains; *Amycolatopsis japonicum* and *Streptomyces griseus* 65. Thus, in the second phase of the project, these two strains were improved by genetic manipulations such as overexpression of the involved structural genes, which encode proteins for the assembly of the cyclic core structure and the general regulatory genes.

In summary, this led to the production of the precursor molecules brasilicardin-aglycon and brasilicardin C (Fig. 1, Fig. 3) in 2gr/ltr scale, which represents a production yield improvement by a factor of 20,000! In a last step, we developed two short but flexible semi-synthesis reactions to finalise the target molecule Brasilicardin A, either starting from the aglycon or from brasilicardin C (Fig. 1, Fig. 3).

Using this combined approach, we are now also able to generate rapidly and economically brasilicardin derivatives with optimised properties, which will be then further evaluated in preclinical trials.

High market potential

Immunosuppressants are pharmaceutical products with a high market value and involve, in the case of organ transplantation, a life-long therapy. In total, the global organ transplant immunosuppressant drug market value is estimated in 2014 to be approximately USD 5.1bn (~€4.2bn) and represents a constantly growing market. In addition, immunosuppressants can be also applied for several autoimmune diseases such as multiple sclerosis, arthritis or psoriasis. This fact even extends the possible indications of a new immunosuppressant drug.

When tacrolimus – one of the current gold standards, and which is also a natural bacterial product – entered the market in 1994, within ten years reached sales of more than 150m per year.

Considering the pressing need for new immunosuppressants that is still given today and the novel promising mode of action of brasilicardin that provides potency but less cytotoxicity, we are convinced that brasilicardin possess a similar economic potential.

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Next Generation Immunosuppressants: Brasilicardin synthesized by *Nocardia* spp.

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