

## Beijing Saisheng Pharmaceutical Co.,Ltd. Introduction

Beijing Saisheng Pharmaceutical Co.,Ltd. is a research-based, growth-oriented enterprise with core competency in health care.

**Location:** It locates in Beijing Economic-technological development Area, It is the only one national development area in Beijing which has excellent environment and policy. There are about 800 companies around us, and about 500 of them are world famous. That is to say, the area is a well organized and open place.

**Workforce:** It employed about 200 employees, 4 to 5 of them are workers the rest are technicians.

**Organization:** The board of directors consists of 4 members who hold special responsibility for manufacture, research and supervised the quality of products and services. There are 3 sub-branches and 8 departments under the lead of the board of directors.

**Financial data:** The general revenue of 2006 is about USD 1.3 million.

**Products:** Beijing Saisheng Pharmaceutical Co.,Ltd. has many products, such as Fibrinogenase for injection, Defibrase for injection, Urokinase for injection, Alprostadil for injection, Muscular amino acids and nucleosides injection, Thymopeptide for injection, and Bozhi glocopeptide injection, and so on.

**Corporate goals:** Our corporate goal is to **Help to satisfy peoples' basic needs and contribute to the continual improvement of the quality of life all over the world.**

*Though Beijing Saisheng Pharmaceutical Co.,Ltd. is young, through the hard work of all her employees we are now undergoing a rapid progress and facing to hopeful and prospective future.*

**We are seeking to retain society`s confidence through: high quality, flexibility and open communication.** Cooperation with internal and external groups is reciprocity. Nowadays we are in an open world, it is impossible to develop by one`s own effort, our coporate goal is to benefit people all over the world. So we are willing to provide our products and service by bilateral even multilateral cooperation, and make all the human being share our achievement.

**Quality:** Quality affects all segments of the company. It means providing our customers, with products and services to meet their needs. Customer satisfaction is therefore a key element in our definition of quality.

For our company, **innovation** is a major factor in product development and optimization. It lays the foundation for competitiveness and growth, and ultimately for the profitability we need to fuel the Group's expansion.

**Research and development** play a particularly important role in the life sciences.

Our company is concentrating its R&D on areas where there is an urgent need for new medicines: infectious diseases, cardiovascular risk management, urology and oncology. The average R&D budget is about RMB Yuan 3 million each year. We also have paid much attention on the intellectual property and now we already had 13 intellectual properties of innovations.

### **FIBRINOGENASE**

Saibai (trade name) Fibrinogenase ,a single chain glycopeptide,  $M_r = 30,000D$  per

SDS-PAGE, IEP 4.55, is a highly purified extract of the snake venom from *Agkistrodon halys brevicaudus Steineger*,

The fibrinolytic enzyme was characterized as zinc metalloproteinase having a methionine turn below and carboxy-terminal to a helical segment containing two of the three histidine residues involved in the zinc-binding site. Most metalloproteinases are fibrinogenases and they release peptides from the C-terminal of fibrinogen. They are classified into  $\alpha$ - $\beta$ -fibrinogenases on the basis of their specificity for the A $\alpha$  or B $\beta$  chain of fibrinogen. Saibai Fibrinogenase is belong to the first group and degraded the A $\alpha$ -chain of fibrinogen preferentially. The proteolytic activity was inhibited by EDTA, L-cysteine, and DTT., indicating Saibai Fibrinogenase was a metalloproteinase requiring disulfide bonds for its activity

Studies of this factor in different animal model have shown that venom fibrinolytic enzyme (VFE) is regarded as one of promising approaches for the treatment of thrombotic disorders such as myocardial infarction and stroke. In a study, we determine if venom fibrinolytic enzyme can reduce exogenous and endogenous thrombosis in two different animal (rats and rabbits) models. The thrombus in experimental model were induced according to the methods of Chandler, one group were injected with venom fibrinolytic enzyme, another group (control group) were injected with equal 0.9% NaCl injection. Then we measured the weight and the length of thrombus in two groups. The result show that venom fibrinolytic enzyme could significantly reduce the thrombosis in two different animal models of thrombosis in different species (rat, and rabbit).

Another study we evaluated the Thrombolysis effects of Venom Fibrinolytic Enzyme in rabbit model of pulmonary thromboembolism by determine the changes of thrombus. The rabbits were divided into two groups, one group were injected with venom fibrinolytic enzyme (60ug/kg), control group were injected with equal 0.9% NaCl injection. The weight of thrombus in given venom fibrinolytic enzyme group were less than control group, indicating VFE could accelerate thrombolysis in rabbit model of pulmonary thromboembolism. In addition to show Thrombolysis effects in different animal model, VFE appear to show that it has obviously nerve growth promoting activity and its molecular structure Homology with the nerve growth factor.

In a study to observe the general pharmacology of VFE, we found that VFE could significantly prolong the blood coagulation time and recalcification time. In acute toxicity test, the LD<sub>50</sub> is 19.30 ± 1.77u/10g, the death of the mouse appeared after 24~48 hours. In the subchronic toxic experiment toxicity test all index of the animal was normal suggesting that clinical use of VFE is safe.

Over the years, lots of clinical studies in human have been implemented to help us in the development of VFE for treatment of thrombotic disorder. In 2003, Saibai Fibrinogenase Injection has been approved by State of Food And Drug Administration to market in China for the treatment of acute Cerebral Infarction, myocardial infarction and Femoral Deep Vein Thrombosis.

We evaluate the efficacy and safety of fibrinolytic enzyme injection in patients with acute cerebral infarction. 80 patients with acute cerebral infarction were randomly divided into two groups, the treatment group of fibrinolytic enzyme injection and the positive control group of

Batroxobin. The efficacy of two groups was evaluated before treatment and after treatment at the day 1, 7, 14 respectively. Blood platelet, the time of haemorrhage and meanwhile haemorrhage and adverse events were observed. The results showed there was no significant differences in the two group ( $p < 0.05$ ) and without increasing the bleeding risk and adversity in fibrinolytic enzyme injection group. We conclude that the efficacy of fibrinolytic enzyme injection was the same as Batroxobin in treating acute cerebral infarction patients and fibrinolytic enzyme injection was more safe.

Another well designed study has been done to investigate the efficacy of fibrinolytic enzyme injection in patients with acute myocardial infarction. A standard therapy of myocardial infarction were used. After routine treatment, patients in the observation group received the fibrinolytic enzyme injection and placebo in control group. The efficacy of two groups was evaluated and correlation inspections were observed before treatment and during treatment. The gross efficacy of the observation group was 98.55% and of control group was 80.54% ( $P < 0.01$ ). The mortality rates of two groups was 1.35% (the observation group) and 19.44% (the control group) ( $p < 0.01$ ). During the treatment, adverse event had not shown in fibrinolytic enzyme for observation group. So we conclude fibrinolytic enzyme injection had satisfied clinical curative effect in treatment of acute myocardial infarction patients and no toxin side effect.

Aberrations in normal blood coagulation functions can result in thrombotic disorders or haemorrhage. In thrombosis, largely unknown conditions promote the apparently spontaneous formation of clots large enough to block circulation. Formation of such blocks in the arteries supplying vital organs, such as the heart or brain, can cause myocardial infarction or stroke respectively. Thus a life-saving mechanism of blood coagulation becomes a potentially life-threatening disease mechanism. Several conditions, such as atherosclerosis, contribute significantly to promote the spontaneous initiation of clotting. Anticoagulants are pivotal for the prevention and treatment of thromboembolic disorders, and approx. 0.7% of the Western population receives oral anticoagulant treatment. With the increasingly aging population throughout the world, more people will require antithrombotic therapies in the future. Thus various new anticoagulant and antiplatelet agents are being sought after. Proteins from snake venom affecting blood coagulation and platelet aggregation can provide us with new lead compounds to design novel therapeutic agents, providing new paradigms in the treatment of thromboembolic disorders.

### **A NOVEL SERINE PROTEASE**

A Novel Serine Protease with Kinin-releasing Activity from *Agkistrodon halys* Venom

A high purity novel Serine protease extracted from the venom of *Agkistrodon halys* using monoclonal antibody affinity chromatography. It also has arginine esterase activity. The characters of the protease are as followed:

#### **The purity of the Kininogenase from snake venom:**

The kininogenase was obtained by two chromatography steps including anion exchange on DEAE Sepharose and monoclonal antibody affinity chromatography. The purity of Kininogenase could reach to over 95%.

#### **Activity assay:**

After affinity purification, the hydrolytic BAEE activity can be up to more than 800 units/mg. The hydrolytic rate for TAME is only 5% of that for BAEE.

**The character of the kininogenase:**

The molecular weight assay: SDS-PAGE showed the molecular weight of the kininogenase was 38KD, it was similar to the tissue-kininogenase.

**N-terminal amino acid sequence:**

The N-terminal sequence of the kininogenase was VIGGDECNINEHRFL.

**The optimum pH:**

The kininogenase' optimum pH is 7-8, which was the same as tissue-kininogenase of mammal. BAEE break down above pH 10.

**The stability for the kininogenase under different pH:**

The kininogenase was stable between pH 4 and pH 9. Below pH 4 and above pH 9, the enzymatic activity decreases rapidly.

**The stability for the kininogenase under different temperature:**

Below 30°C the BAEE activity was almost not change, above 40°C the enzymatic activity decreases rapidly, above 60°C the enzyme precipitated because of denaturation.

**EDTA affection :**

EDTA have no affection to the kininogenase activity.

**Metal Ion Affection:**

Ca<sup>2+</sup>, Mg<sup>2+</sup>, Ba<sup>2+</sup> have no affection to the kininogenase, while Zn<sup>2+</sup> (0.1M), Hg<sup>2+</sup> (0.01M), Co<sup>2+</sup> (0.1M), can restrain the kininogenase activity completely. 0.1mol/L EDTA can renew it activity.

**The PI**

The PI of the protease is pH 6.0-8.0.

**Acute toxicity:**

Mice can maximum tolerate Venom kallidrein 2,000 units /20g weight by iv Injection.

**Pharmacology and pharmacodynamics analysis:**

Kininogenase are becoming an ideal drug for mild to moderate primary hypertension patients, since it possess the important physiological functions in vivo which include to release of bradykinin, participate coagulation reaction by activating factor XII, closely relate to complement system, dilate small arteries, lower blood pressure, reduce the heart stress, decrease myocardial oxygen consumption, improve cardiac function, enhance myocardial contractility, improve ventricular function with hypertension. In addition, kininogenase can also expand glomerular vascular, reduce the production of mesangial matrix, lower and reverse urinary protein, decrease the trace protein in diabetic nephropathy patients<sup>[9]</sup>. Thus, the drug was widely used as the treatment in primary hypertension, angina, arteriosclerosis, retinal blood disorder, acromegaly perceived anomalies, occlusive thrombotic vasculitis.

**LUMBROKINASE**

According to the ancient Chinese medical publication Ben Cao Gang Ma (Compendium of Materia Medica), earthworm (Lumbricus rubellus) was said to unblock the body's meridians and channels, and was used to support blood circulation. In 1883, in a book discussing the action of worms, Charles Darwin observed that earthworm digestive fluids can dissolve fibrin. In the

1980s, Japanese researchers extracted a fibrin dissolving enzyme from *Lumbricus rubellus*, and found that it consisted of six proteolytic enzymes, collectively named lumbrokinase (LK). Since 1992, lumbrokinase derived from earthworms has been extensively studied and used in China. Research has shown lumbrokinase to support healthy coagulation of blood within normal levels and enhance fibrinolytic activity. LK capsule technology was awarded a certificate of National Significance Achievement in Science and Technology in China.

Lumbrokinase was described as a group of biologically active components in the extracts of some kinds of earthworms such as *Lumbricus rubellus*, *Lumbricus bimastus*, and *Eisenia fetida*. Its molecular weight, as estimated by SDS-PAGE, is fluctuated from 18KD to 42KD. Its isoelectric point were about 3~5. It is stable to heat. Its activity doesn't decrease until it is heated to 65°C. It is displayed a very broad optimal pH range (pH 3~11). The metal ions  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$  can increase its activity and  $\text{Ca}^+$ ,  $\text{Hg}^{2+}$  show some inhibition.

The lumbrokinase (LK) group of proteolytic enzymes, extracted from the researched species of earthworm, includes plasminogen activator and plasmin. The plasminogen activator (e-PA) in LK is similar to tissue plasminogen activator (t-PA) from other sources, which makes it possible to show the thrombolytic activity only in the presence of fibrin. Therefore, LK has the advantage of not causing excessive bleeding.

Lumbrokinase's mechanisms of action include participation in the activation of plasminogen, and direct activity on fibrin itself. LK primarily proteolyzes fibrinogen and fibrin, hardly hydrolyzing other plasma proteins including plasminogen and albumin. There are studies showing that lumbrokinase can significantly decrease ESR, C-RP, and TXB<sub>2</sub>. And it can decrease platelet and lower blood and plasma viscosity and reduce cholesterol and triglycerides.

Lumbrokinase showed protective action against myocardial infarction in rats. The possible mechanisms of anti-ischemia could be attributed to decreasing  $\text{ICa-L}$  and  $[\text{Ca}^{2+}]$  of ventricular myocytes in rats. Chailing Guiqi Decoction (CLGQD) combined with lumbrokinase can reduce proteinuria, regulate lipid metabolism, protect renal function, and delay progressive renal damage in rats. LK might significantly decrease the immunoreactions of P-selectin and E-selectin in ischemic lesion. Lumbrokinase has an in vivo thrombolytic effect in a rabbit cerebral embolism model. Lumbrokinase has potential antithrombotic effects in a small diameter vascular prosthesis.

Pharmacological and toxicity studies have been done. No negative effects of Lumbrokinase on the nervous, cardiovascular, respiratory or blood systems of rats, rabbits and dogs were recorded in acute and sub-acute toxicological experiments. Long-term animal toxicological experiments did not show any damage in hepatic and renal functions. No negative influences on embryonic development were found in embryonic rats, or any teratogenic or mutagenic effects. In clinical experiments, no undesirable effects were observed in hepatic or renal function, blood levels of glucose or lipids, or other laboratory parameters. The LD<sub>50</sub> has been established: LD<sub>50</sub>=144,600U/Kg.

Lumbrokinase has been studied as a treatment for various clinical conditions, including acute, sub-acute, and chronic conditions that are associated with the presence of hypercoagulation and hypoperfusion. LK is generally used in cardiovascular and cerebrovascular diseases. Furthermore, it can be applied in the diseases related to hemorheology such as Miniere's disease, sudden deafness and so on.

In a Chinese study conducted at Beijing Tongren Hospital, patients with hyperfibrinogenemia were orally administered six tablets of lumbrokinase per day for three weeks. Serum fibrinogen levels were examined before and after treatment. In comparison with the control groups given antilipemia or antiplatelet aggregation drugs, serum fibrinogen in the lumbrokinase treated group significantly decreased two weeks after treatment in a time related manner. These results showed lumbrokinase could relieve hyperfibrinogenemia, and that the formation of thrombi would be decreased. Lumbrokinase had few reported side effects.

Additional research investigated the effect of lumbrokinase on anticoagulation and fibrinolysis in treating cerebral infarction. Patients were randomly divided into the lumbrokinase treatment group (n=31) or a control group (n=20). The single blind method was used in this investigation and the Chinese stroke score was used to evaluate the results of treatment before and after administration of lumbrokinase. Kaolin partial thromboplastin time (KPTT), prothrombin time (PT), fibrinogen content, and vWF content were analyzed, while tissue plasminogen activator (t-PA) activity, plasminogen activator inhibitor (PAI) activity, D-dimer level were assayed. In both groups the stroke score decreased after administration, but in the treatment group, it was more obvious. In the treatment group KPTT was prolonged, t-PA activity and D-dimer level increased, while the content of fibrinogen decreased significantly. There were no significant changes of PT and PAI activity in both groups. The researchers concluded that lumbrokinase is beneficial to the treatment of cerebral infarction and the effect of lumbrokinase is related to the inhibition of intrinsic coagulation pathway and the activation of fibrinolysis via an increase of t-PA activity.

The capsules of the extracts of lumbrokinase, which are commercially referred to as Panford or Boluoke (as a dietary supplement), have been used in Southeast-Asian countries like in China, Japan, and Korea, and also in North American countries such as in Canada and United States. Lumbrokinase Enteric-Coated Capsules (extracted from *Eisenia fetida*.) have been used in hospitals in China. Lumbrokinase (extracted from *Eisenia fetida*.) for injection has been completed preclinical studies. The preparation process of the lumbrokinase is complex and the lumbrokinase preparation contains some unwanted components. Now we can purify a single component from the extracts.