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Production and market comparison of urokinase and streptokinase as effective and cheap fibrinolytic agents for treatment of cardiovascular diseases

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ABSTRACT. Failure of hemostasis and the formation of blood clots in the arteries are the main reasons that provoke the onset of cardiovascular diseases (CVDs) such as myocardial infarction and ischemic stroke. Cardiovascular diseases have become the primary cause of deaths and disabilities across the globe. Therefore, this problem needs to be addressed with urgency. The disintegration of blood clots requires fibrinolytic agents, which are involved in thrombolysis. Streptokinase and urokinase are fibrinolytic enzymes; the former is primarily produced from microbial sources and the latter is isolated from urine, respectively. Streptokinase and urokinase have been in use for a long time to treat cardiovascular diseases. This review explains in detail the comparison of employing streptokinase and urokinase for the said purpose in a cost-effectivemanner. The recombinant production of both the agents has been discussed in detail. Furthermore, the efficacy of both the agents has been compared based upon their side effects and retention time in the body. A thorough study has been made to compare the influence of using both the agents on the health of cardiovascular patients in the last decade.

Keywords: Streptokinase; uroquinase; thrombolytic agentes; fibrinolysis; plasminogen activator.

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Introduction

Cardiovascular diseases (CVDs) account for almost 17.9 million deaths every year and have been reported to be the leading cause of death by the World Health Organization. Medical complications such as myocardial infarction, ischemic stroke, peripheral arterial thrombosis, pulmonary embolism, and deep vein thrombosis (Ruscica, Corsini, Ferri, Banach, & Sirtori, 2020), which are majorly caused by atherosclerosis, are all included in the set of CVDs. Clot (blood) dissolving agents or fibrinolytic agents have been proved as efficient means to treat CVDs (Ghosh, Pulicherla, Rekha, Venkat Rao, & Sambasiva Rao, 2012). Various fibrinolytic agents are commercially available amongst which Streptokinase and urokinase are mostly preferred. Generally, fibrinolytic agents activate the fibrinolytic system by converting the proenzyme plasminogen (inactive form) into its active form plasmin. Specific activators (tissue type activators and the vascular type activators) present in various tissues and body fluids are required for the activation of the fibrinolytic system (Arnetz et al., 2020). Thrombolytic treatment is mainly directed towards the activation of plasminogen to plasmin at the location of thrombus, on the surface of fibrin (Stephani et al., 2017). Plasmin is a serine protease that acts on both the fibrin and the fibrinogen, degrading them and thus dissolving the blood clots (Bell, 2002).

Streptokinase is an extracellular enzyme that is produced by several strains of β- hemolytic *Streptococci*, flourishing mainly in the upper respiratory tract (Bhardwaj & Angayarkanni, 2015a), whereas urokinases are produced by the human kidney cells and are later isolatedfrom urine (Mahmood, Mihalcioiu, & Rabbani, 2018). Where streptokinases are exogenous enzymes and may provoke allergic reactions, urokinases are indigenous to human origin and hence can be repeatedly administered without causing any unwanted antigenic response. However, removing the antigenic sites in *Streprococcus* through modern recombinant engineering techniques deprives the streptokinase of its antigenicity (Akbar, Zia, Ahmad, Arooj, & Nusrat, 2020), which can then be produced as a reliable choice for the treatment of CVDs. Streptokinases are neither proteases nor true plasminogen activators like urokinases (Avgerinos, 2017). They bind to one of the circulating plasminogen molecules to form a streptokinase-plasminogen complex (Figure 1), the resulting conformational changes (Figure 2) favor the conversion of surrounding plasminogen to active plasmin (Aghaeepoor et al., 2019; Sahoo & Sahoo, 2020).

Urokinase, unlike streptokinase, is a true plasminogen activator and having been isolated from urine, it is often called UPA: urinary-type plasminogen activator. Mc Farlane and Pilling (Mahmood et al., 2018) first isolated urokinase in 1947; after which in 1972, UPA was first licensed for use in France (Kunamneni, Ogaugwu, & Goli, 2018). It is a serine protease which also functions as a thrombolytic agent by hydrolyzing the peptide link in plasminogen, transforming it into plasmin that can, in turn, hydrolyze the fibrin mesh in the blood clots (Figure 3) (Tan et al., 2017). Urokinase activates both, the fibrin specific plasminogen and the fibrin non-specific plasminogen (Kadir & Bayraktutan, 2020). Activation of fibrin non-specific plasminogen results in excessive bleeding making the process undesirable. However, the activation of fibrin-specific plasminogen is favorable, resulting in the breakdown of blood clots into soluble peptides (Urano, Castellino, & Suzuki, 2018).

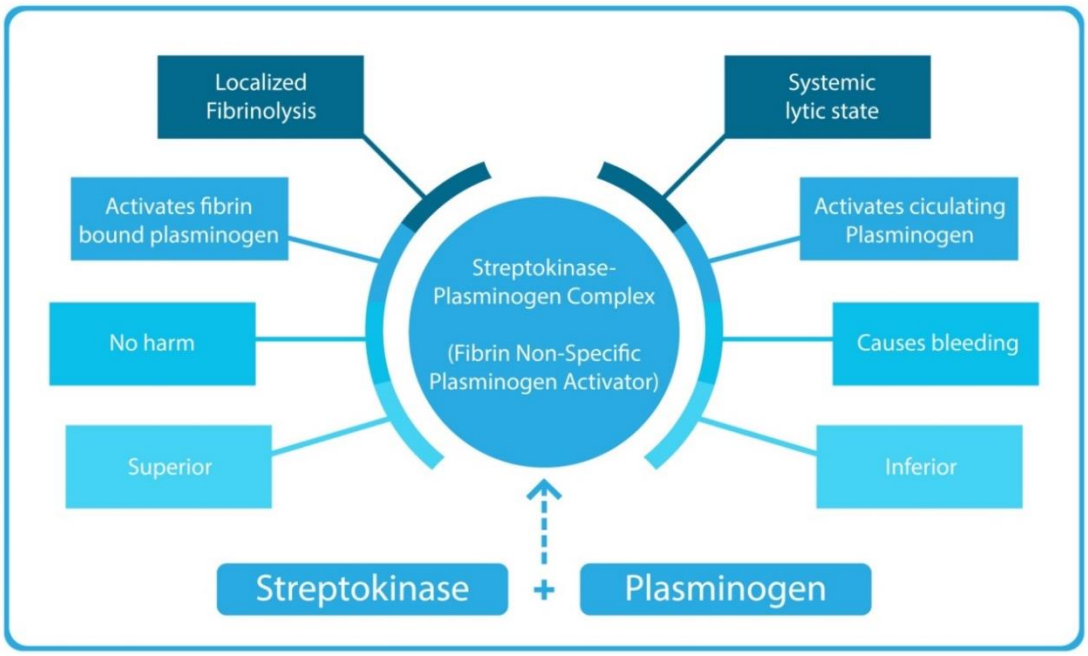

Figure 1. Role of streptokinase in localized fibrinolysis and systemic lytic state.

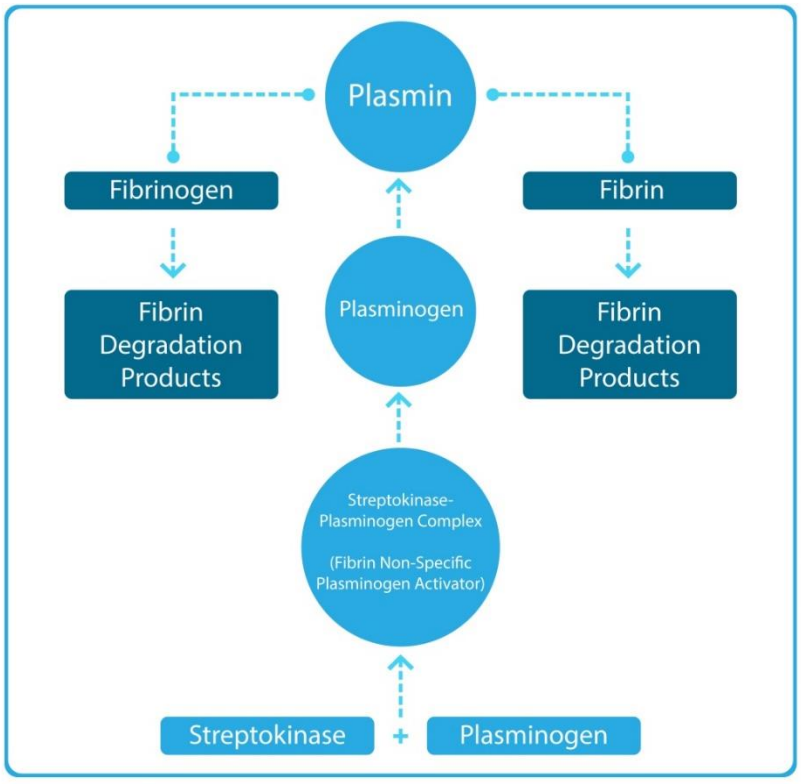

Figure 2. Streptokinase mode of action in blood clot lysis.

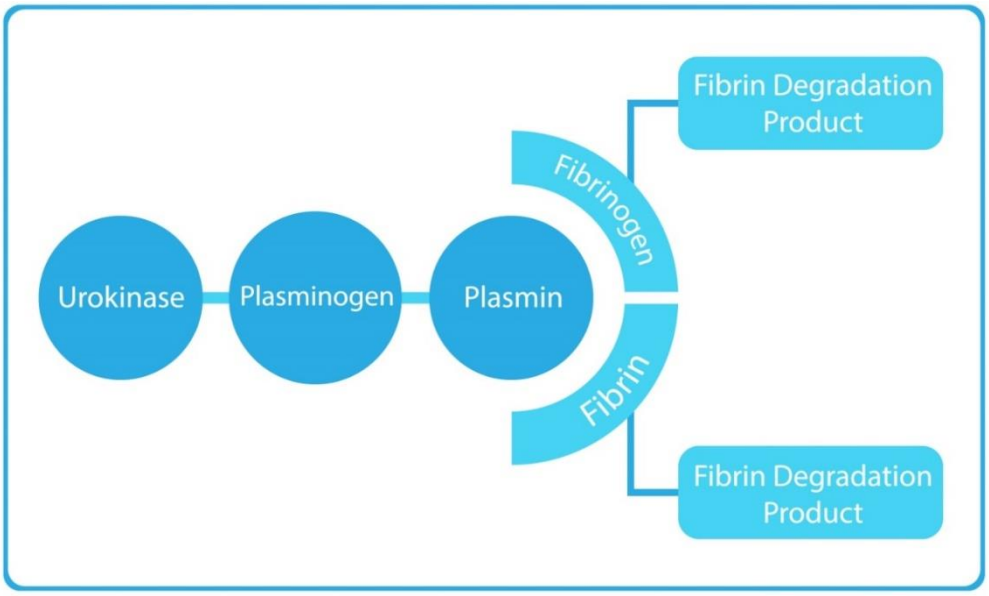

Figure 3. Urokinase mode of action in blood clot lysis.

As a fibrinolytic agent, urokinase is favored over streptokinase essentially because it is a human indigenous protein and thus can be administered repeatedly without the fear of causing any allergic response in the body. It has high fibrin specificity and fewer side effects (Nawaz et al., 2020) and works more or less in the same way as streptokinase. Urokinase can also be isolated from plasma, seminal fluid and tissue cultures of kidney cells (Fedan, 2019); However, the production expenses limit the production feasibility to a great extent; for instance, 1500L of urine is only sufficient to harvest a single dose of urokinase. This has led to the uncovering of bioengineered microbes as potential sources of commercial urokinase (Agrawal & Patil, 2020). Comparison between the characteristics of streptokinase and urokinase has been depicted in Table 1. This article has compared the feasibility of industrial production and the market values of these two agents.

Table 1. Comparison between the characteristics of streptokinase and uroquinase.

Characteristics	Streptokinase	Urokinase
Molecular Weight (kD)	48	32/54
Plasminogen Activation	I	D
Fibrin Specificity	Yes	Yes
Fibrinogeno-lysis	Yes	Yes
Half-life (min)	10/90	2
Elimination	Kidney	Kidney
Immunogenicity	Yes	No
Cost	Less	More

Source: (Baruah, Dash, Chaudhari, & Kadam, 2006).

Microbial production of streptokinase and urokinase

Streptokinases and urokinases are the products of bioprocesses which are highly specific to some microbial species, as is also evident from Table 2. The search for cost-effective and medically sound procedures for their commercial production demands an in-depth insight into the potential of novel microbial sources, and their pathways involved in the synthesis of these enzymes.

Table 2. Microbial sources for the production of streptokinase and urokinase along with their enzyme activity.

Sr. No.	Microbial Source	Streptokinase Units	Urokinase Units	Reference
1.	*β-hemolytic streptococci*	467.73	-	(Dubey, Kumar, Agrawala, Char, & Pusp, 2011)
2.	*Pseudomonas sp.*	-	785.73	(Dubey et al., 2011)
3.	*Streptococcus equisimilis*	474.56	-	(Banerjee, Chisti, & Banerjee, 2004)
4.	*Streptococcus agalactiae*	147.08	-	(Naeem, Sadia, Awan, & Zia, 2018)
5.	*Enterococcus gallinarum*	-	81.3	(Nawaz et al., 2020)

Streptokinase

There are several microbial strains which can be used for the production of streptokinase. It is obtained mainly from *Streptococcus sp.* isolated from the upper respiratory tract, throat, or sputum samples of infected persons (Hatami & Eghdami, 2018). The other *Streptococcus sp.* reported for streptokinase production include *S. dysgalactiae, S. equinus, S. mutans, S. feacalis, S. uberis, S. equisimilis, S. sanguis, S. lactis,* and *S. pyogenes* (C, V, Babu, Ethiraj, & Naine, 2013; El-Mongy & Taha, 2012; Gupta, Saxena, & Meshram, 2020). The production of streptokinase from microbial sources is an economical process that can be carried out on a mass-scale by maintaining the necessary growth parameters and optimizing media in the industrial fermentations to meet the global demand (Ghosal et al., 2017). This establishes the fact that the optimization of fermentation parameters plays an essential role in enhancing the production rate for commercial consumption (Cheng & Kim, 2015). Despite the ease with which many scientists had been successfully producing commercial streptokinase, various side effects associated to its use declined its industrial value (Chakravarti et al., 2013). The typical undesirable outcome has been the onset of allergic reactions including fever, chills, nausea, itching, skin rashes, and respiratory trouble. This has been linked to the presence of several antigenic epitopes on the streptokinase molecule (Chandran, Singh, Thomas, Basu, & Brahmachari, 2016).

One obvious remedy to overcome the problem caused by the presence of these immunogenic sites was to remove them resulting in a non-immunogenic streptokinase molecule (Akbar, 2020). Another way of producing streptokinase with little or no immunogenicity is through recombinant production or development of a mutant strain. Deitcher and Jaff, for instance, amplified a streptokinase gene from *S. pyogenes* C1, cloned it into pET28a(+) vector and transformed it to a non-pathogenic microorganism like *E. coli* BL21 (DE3) (Deitcher & Jaff, 2002). Next, by inducing the expression vector, high expression of the recombinant protein recSK was produced which held little or no capacity to react as an immunogen (Deepak & Geetha, 2019). Another study carried out by (Arshad, Zia, Asghar, & Joyia, 2019) in an attempt to achieve the said objective, enhanced production of streptokinase was obtained with little immunogenicity by chemical mutagenesis of *S. agalactiae.*

Urokinase

The search for an economical production procedure to synthesize a high fibrin-specific-urokinase still continues. Being immunologically unreactive, unlike streptokinase, urokinases have gained a comparative preference (Fajardo-Espinoza, Romero-Rojas, & Hernández-Sánchez, 2019). There are many sources to obtain urokinases but microorganisms are the best sources because they are easy to grow and the extraction and purification of urokinase is relatively easier. Primitively, urokinase is produced from urine, but the procedure is quite lengthy, complicated and costly (Gupta et al., 2020). These limitations have been countered by utilizing human cell cultures for the production of urokinase. Human embryonic kidney cells, human fibroblasts, human myeloma cells, and human umbilical vein endothelial cells are currently being used for the production of urokinase. Studies show that the quantity of urokinase produced by cultured cells is approximately 50-100 ng mL^{-1}, which is higher as compared to that produced from urine i.e. 10-15 ng mL^{-1} (Roychoudhury, Khaparde, Mattiasson, & Kumar, 2006).

Fermentation technology happens to be the leading application for employing different microorganisms including bacteria (E. Coli, Pseudomonas sp., B. subtilis, Halobacillus sp., Enterococcos sp.), fungi (Asperigillus sp., Penicillium sp., Trichothecium sp., S. cerevisiae), algae, and actinomycetes in the process of urokinase-production (Agrawal & Patil, 2020; Nawaz et al., 2020). The evident attainment of high yields of urokinase from microbial sources validate their acceptance over pre-existing sources of UPA (Sahoo & Sahoo, 2020). In addition, the availability of factors including microbial source, physical parameters, and the cultural conditions make the process sensitive to multi-faceted optimization strategies. One of the significant concerns in the fermentation process is to look for a cost-effective media (Ghaffar, Ahmed, Munir, Faisal, & Mahmood, 2015).

Appropriate media formulation can also help produce high yields of urokinase. Minor changes in the media formulation, cultural conditions, and physical parameters can affect enzyme production to a great extent (Zhu, Mollet, Hubert, Kyung, & Zhang, 2017). Submerged and solid-state fermentation procedures are famous with respect to urokinase production. Submerged type is preferred as it provides high yield due to máximum nutrient availability and accessible approach for downstream processing (Sharma, Sharma, & Shivlata, 2015). The most significant concern about fermentation technology is the cost involved. In this regard, recombinant technology proves to be a cost-effective and reliable alternative. For instance, thrombolytic agents are being

produced using various expression platforms like bacteria (Adivitiya & Khasa, 2017). Dubey has reported the production of 12.5U 50 μL^{-1} urokinase by the recombinant method. Low molecular weight fragment of DNA, isolated from *Pseudomonas sp.,* was ligated into pET28a(+) vector (Dubey et al., 2011). It was transformed to *E.coli* BL21- RIL and induced for expression regulated by the T7 promoter. Not only did this method proved to be feasible, but was also found much efficient in contrast to fermentation (Graor, Young, Risius, & Ruschhaupt, 1987).

Industrial production and componential costs

Isolation

The isolation process of a potential microorganism for the production of thrombolytic agents takes up a significant fraction of the total production cost. As streptokinase can quickly be produced from microbes that are isolated from sputum samples or dental plaque (Qiao et al., 2018), it makes the process cost-effective. However, the production process can be made more cost-effective by optimizing the media required for cell growth and enzyme production. Production of urokinase is also possible by the isolation of microorganisms (bacteria) from various sources like soil, seawater, and fermented food samples such as Sardine, pickle, chickpea, soybean, tuna, soya sauce, olive, hot sauce, corn, red kidney beans, sweet corn, and tomato ketchup (Nawaz et al., 2020). Isolation of microorganisms from these sources produces urokinase that is much cost-effective and efficient as compared to its production by recombinant techniques and tissue culture methods (Huish, Thelwell, & Longstaff, 2017).

Production

Streptokinase is preferably produced through continuous culture methods that contain glucose as an energy source (Dubey et al., 2011). Producing streptokinase on a large scale using industrial fermentation can significantly decrease the production cost by optimizing different factors like pH, inoculum size, temperature of the fermentation medium, fermentation time, agitation speed, and aeration rate (Singh, Saxena, & Yadav, 2017). The microbial synthesis of streptokinase has been further reported to increase using mutant microbial species and optimization of fermentation medium. Arshad et al., (2019) reported a 4.14-fold increase in streptokinase production by optimization of fermentation media. Similarly, Wu et al., (2019) developed a cheap fermentation media for the cost-effective synthesis of streptokinase.

Contrarily, separation of urokinase from urine is not cost-effective as it produces very low yields with equally low efficiency as well. Therefore, recombinant methods stand more valid for its production (Adivitiya & Khasa, 2017). High secretion of urokinase by cells is an essential factor in using cell cultures for the production of urokinase. Keeping in view the therapeutic importance and cost efficiency of producing a plasminogen activator from human source, cell lines like human kidney cell lines are used for urokinase production (Roychoudhury et al., 2006). These cell lines are supplied with a complex growth medium including sugars, amino acids, and growth factors which contribute majorly towards high yield in lesser input (Li, Yuan, & Yuan, 2020).

Shelf-life

Shelf life and reusability after the opening of the thrombolytic agent's vials are the noteworthy factors considered when calculating the cost-effectiveness of both, streptokinase and urokinase. Comparing the shelf-life of streptokinase and urokinase, (Hasanpour, Esmaeili, Hosseini, & Amani, 2021) stated that unopened vials could remain stable for 3 years at less than 25°C, and 4 years at below freezing temperatures, respectively. The reconstituted solution should not be stored in a refrigerator for more than 24 hours at +2 to +8°C. However, if urokinase is diluted to 5000 IU mL^{-1} in sterile water or 0.9% sodium chloride, it retains its in vitro fibrinolytic activity for up to 6 months when stored at -20 to -70°C (Hickman, Pawlowski, Sekhon, Marks, & Gupta, 2018). These studies provide yet another striking advantage of urokinase over streptokinase.

Recovery

Various mechanisms have been explained for the recovery of streptokinase, either from fermentation broths or crude samples that are commercially available. According to a study (Dubey et al., 2011), a five to six-fold increase in purity was observed by first performing column chromatography and then column electrophoresis in sucrose density gradients. In a likewise study, Xu et al., (2019) obtained a final specific activity of 100,000 units of streptokinase per mg of protein by coupling ion-exchange and gel-permeation chromatography.

Unlike streptokinase (obtained from a microbial source), urokinase (obtained from a human source) is recovered in shallow concentrations which makes the process costly (Mousa & Broce, 2017). Various methods are being applied for the recovery of urokinase, including ion-exchange chromatography, ammonium sulfate precipitation, affinity chromatography, and gel permeation chromatography (Badhe & Nanda, 2018). Among all of these, affinity chromatography has emerged as the most powerful method for purification of urokinase involving synthetic and biological ligands that bind specifically to urokinase. The recovery methods for urokinase are mostly multi-step processes that produce lower yield but require high operating costs (Lijnen & Collen, 1995). The development of new methods however, does produce enhanced recovery at reasonable cost. Xu et al., (2019) coupled processes involving foam fractionation and silica gel adsorption which increased urokinase activity and purification yield by 25.3% and 79.2%, respectively, and rendered it cost-effective.

Efficacy

Severe allergic reactions which may progress to fatality are very likely with streptokinase dosages. As streptokinases are of bacterial origin, these molecules are coated with several antigens which can initiate an allergic response within the patient's body (Krishnamurthy, Belur, & Subramanya, 2018). If such a response does occur, the administration of streptokinase is immediately ceased and is not recommended to be consumed again by the patient for a minimum duration of the following four years (Bell, 2002). In such cases, the patient is then treated with alternative agents like alteplase or urokinase (Gurewich, 2019). Draxler, Sashindranath, & Medcalf, (2017) showed that patients previously infected with streptococci required high doses for thrombolysis due to the extent of its antibodies within the patient's body.

In addition to the violent allergic response, streptokinase is also associated with a higher risk of developing hemorrhage (Banerjee et al., 2004; Sawhney, Katare, & Sahni, 2016). A recent study subjected a cohort of 80 patients to fibrinolytic therapy; 15 among which showed hemorrhage effect included five patients who were given urokinase and ten who were given streptokinase (Picard et al., 2019). Results by Yazdi et al., (2017) have shown that bleeding was the most significant complication seen in patients injected with streptokinase as it binds with both circulating and non-circulating plasminogen. Bleeding may occur spontaneously or at the puncture site. Intracranial hemorrhage and hemorrhage stroke are of significant concern in this case (Prasad, Singh, Kanabar, & Vijayvergiya, 2020). Different factors including tumor, aneurysm, infarction, bleeding diathesis, advanced age, uncontrolled hypertension, severe heart disease, low body weight, trauma, or surgical intervention in the cerebral system may increase the risk of bleeding (Aslanabadi, Safaie, Talebi, Dousti, & Entezari-Maleki, 2018). Minor bleeding from the venous entry port can be stopped by applying pressure but in case of severe bleeding, discontinuation of the drug as soon as possible remains the best approach (Skottrup, Dahlén, Baelum, & Lopez, 2018).

Thrombolysis with urokinase is a more potent alternative to streptokinase. The advantageous effects of urokinase promoting revascularization and nerve regeneration make it a much efficient fibrinolytic agent (Kadir & Bayraktutan, 2020). In a study, as an example, 27% ± 8% of thrombus was dissolved by streptokinase, while 64% ± 9% of an equivalent thrombus sample was dissolved by urokinase within the same time frame of 60 minutes (Ouriel, 2002). A recent study falls in agreement, stating that streptokinase partially consumes plasminogen (and plasmin) while forming the activator complex, whereas urokinase converts all of the plasminogen to plasmin, making it more efficient (Xu et al., 2019). Furthermore, for the local thrombolytic therapy, improved efficacy of lysis and a decreased percentage of systemic fibrinolytic effect and bleeding problems make urokinase preferable over streptokinase again (Tan et al., 2017). Treatment of cardiovascular diseases CVDs require a balance to be maintained between fibrinolytic and thrombolytic activity that is also efficiently sustained by urokinase, while streptokinase only poorly manages it (Ouriel, 2002). Moreover, considering the antigenic effects, being indigenous to human origin, urokinase makes itself perfectly applicable for repeated administration with little or no complications (Stump, Lijnen, & Collen, 1986). Despite all the merits of urokinase discussed above, studies also suggest that it seemingly takes longer time in comparison for streptokinase to dissolve the same amount of clot (Tanaka, Key, & Levy, 2009).

Market Potential

The first industry for streptokinase was established in 2001 (Zhong et al., 2020). Being cost-effective, streptokinase production through recombinant techniques has a more significant market potential (Oh, Modiano, Bachanova, & Vallera, 2020). The cost of streptokinase for 250,000 units is approximately $21 as compared to urokinase that costs for $133 for the same amount, which is much expensive. The Chinese market

for urokinase progressed to 50.56 CNY million from 2013 to 2017, representing that market for urokinase is further developing in the coming years (Kapoor, 2016). The strategies of domestic production, import and export, and consumption have helped businessmen to analyze and capitalize on potential opportunities (Tripon et al., 2020). The major industries for urokinase are NDPHARM, Wanhua Biochem, and LIVZON which accounted for 22.35 percent, 10.11 percent, and 21.86 percent of revenue in 2019, respectively. The urokinase market across the globe is estimated to reach USD 56 million by 2026 (Gurewich, 2019).

However, to meet the globally increasing demand of urokinase, various researches are being conducted to increase the bioreactor volumetric productivity by using various methods like media manipulation, novel bioreactor design, and feeding strategies for perfusion culture (Kabir, Moreino, & Siam, 2019). The recent significant developments done by the vendors in the United States Urokinase Market include ImaRX Therapeutics, Jiangsu Aidea Pharmaceutica, Jiangxi Haoran Bio-Pharma, NDPharm, and Wanhua Biochem (Opacic, Paefgen, Lammers, & Kiessling, 2017). Growing cases of thrombolytic disorders across the world is the driving force of the urokinase market – however, the lack of proper personnel monitoring the condition of patients while diagnosing hampers the growth. The challenge for cost-effective production hence remains valid (Vimal & Kumar, 2019). The market potential of thrombolytic therapy using drugs like streptokinase and urokinase has been divided into segments, classified based on drug type, application, distribution channel, and region (Arshad et al., 2019) (Table 3).

Table 3. Worldwide market potential of streptokinase and urokinase in terms of market price.

Sr no.	Trade name	Thrombolytic agent	Quantity	Price (USD)	Company
1	Bharat Serum and Vaccines Ltd	Streptokinase	100 uL	18.13	Bharat Serum
2	TTK Healthcare Ltd	Streptokinase	-	26.60	Wdrugs/streptokinase
3	TTK Healthcare Ltd	Urokinase	-	33.02	Ndrugs
4	MERCK	Urokinase	-	40.25	Sigma Aldrich
5	United States Biological	Streptokinase	100 uL	247	Usbio
6	Medical Isotopes	Streptokinase	5 mg	950	Medical Isotopes
7	Congruent Pharma	Streptokinase	5 mL	25	Congruent Pharma
8	Salvavidas	Streptokinase	4 mL	27	Salvavidas Pharmaceutical
9	Yogeshwari	Streptokinase	5 mL	32	Yogeshwari Medicals
10	Alfa Aesar	Urokinase	1 mg	281	Alfa Aesar
11	Apollo	Urokinase	1 mg	174	Apollo Scientific Ltd.
12	American Custom	Urokinase	1 mg	728	American Custom Corporation

Source: Bhardwaj and Angayarkanni (2015b); Ghosh, Saha, and Sahoo (2021); Mendieta et al. (2019); Ponnada, Pulicherla, and Rao (2012).

Conclusion

Streptokinase and urokinase are widely used across the globe as fibrinolytic agents. Urokinase appears to be costly but more useful than streptokinase. Streptokinase possesses specific side effects due its bacterial origin, but they can be overcome through recombinant techniques. Recombinant production of streptokinase and urokinase has a significant market potential that will further develop in upcoming years. Generally, whatever method implied, reducting or optimizating the process steps involved can lead to an overall decrease in the operational costs and a relative increase in the product yield. The previous trends and recent experiences suggest that fibrinolytic therapy has been progressively incorporated into the routine management of patients with thrombolytic disorders. The analysis of the comparative efficacy of different thrombolytic agents in combination and the inspection of different methods of administration of the thrombolytic agents are the two facets that need be explored further in future researches to identify a considerably efficient production process for these agents.

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