

Thrombolytic Compound Comparison

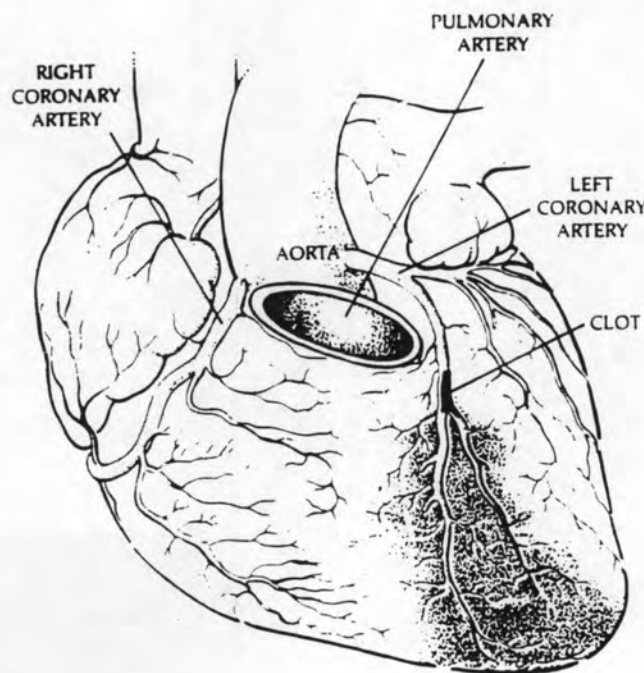
Robert S. Kidd

Introduction

The topic of heart attacks and their treatment may not seem like a very interesting topic, but it is the number one killer in the adult population of the United States. Carey writes that "coronary heart disease 'remains America's number one killer, responsible for about 768,00 deaths each year--or about a third of all deaths,' he [Dr. Windom, assistant secretary for health] said. 'At some point in their lives,' Dr. Windom added, 'one out of every four or five people will feel the pain and constriction of a heart attack--pressure or pain in their chest or pain in their left arm.' When it happens, he advised, 'Don't waste time hoping against hope that the pain will go away... get help quickly (1, p. 23)." Thrombolytic compounds were first used for myocardial infarction in 1959. The problem today is that not enough people receive thrombolytic therapy for various reasons. Victims usually do not get to the hospital soon enough, or do not meet certain criteria that is required to receive thrombolytic therapy. Through public awareness programs and physician screening more people may receive this therapy in the future.

Biological Background

The need for thrombolytic drugs arise from an occlusion in the coronary arteries. Occlusions cause localized anemia by preventing the flow of blood enriched with oxygen to the muscles of the heart. If the heart is not supplied with blood, it cannot pump blood enriched with oxygen to the rest of the body. Ischemia leads to chest pains, pressure in the chest, and onset of myocardial infarction. Death is also possible if the occlusion is not reperused or if the other vessels cannot sufficiently increase the delivery of oxygen to the anemic tissue.

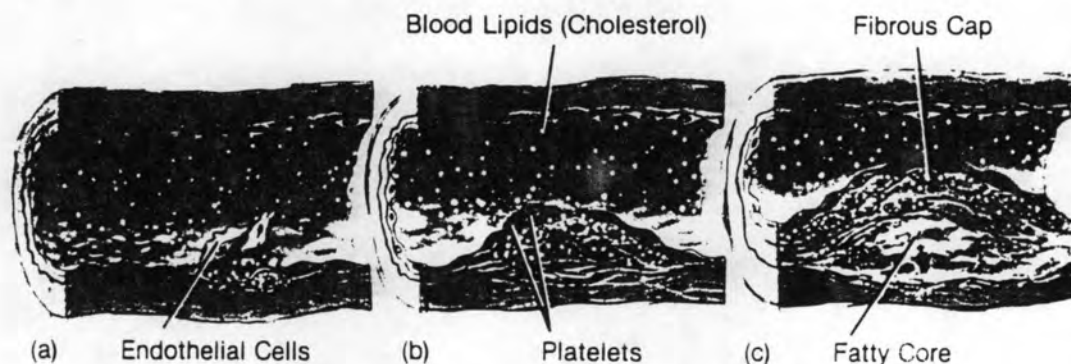


A clot can block the flow of blood to an entire section of the heart. (2, p. 717)

An occlusion in a coronary artery can be a clot formed locally, a thrombus, or it can be a foreign clot that travelled through the circulatory

system and lodged in the coronary artery, an embolus. Artherosclerosis, the reduction of the blood flow due to deposits on the walls of the arteries, can lead to a thrombus, the most common occlusion.

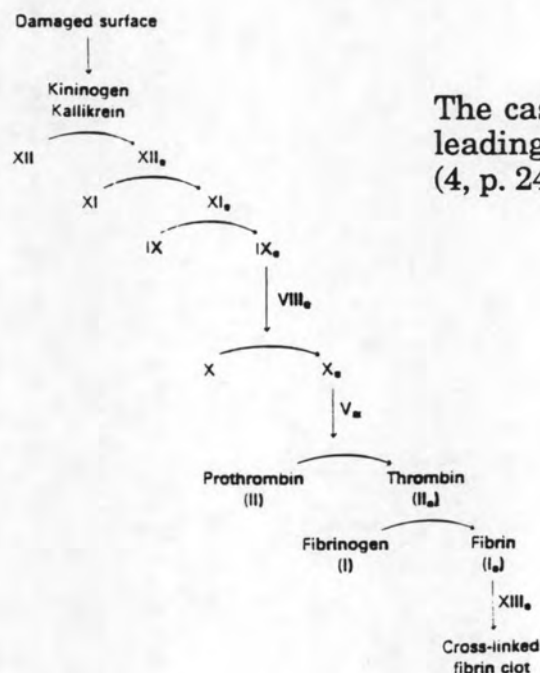
Artherosclerosis begins by the damaging of the epithelial lining of a coronary artery. The damage causes the accumulation of lipids, smooth muscle cells, calcium and other debris around the damaged area. This accumulation is called a plaque deposit. The deposit is soon capped by blood platelets. Wessels and Hopson state that "This problem is very serious because plaque accumulates preferentially in the arteries of the heart and the neck (3, p. 746)."



An example of how a vessel can narrow.
(3, p. 746)

The net effect is the narrowing and hardening of the entire artery, and the roughening of the interior surface of the artery. The rough area can be

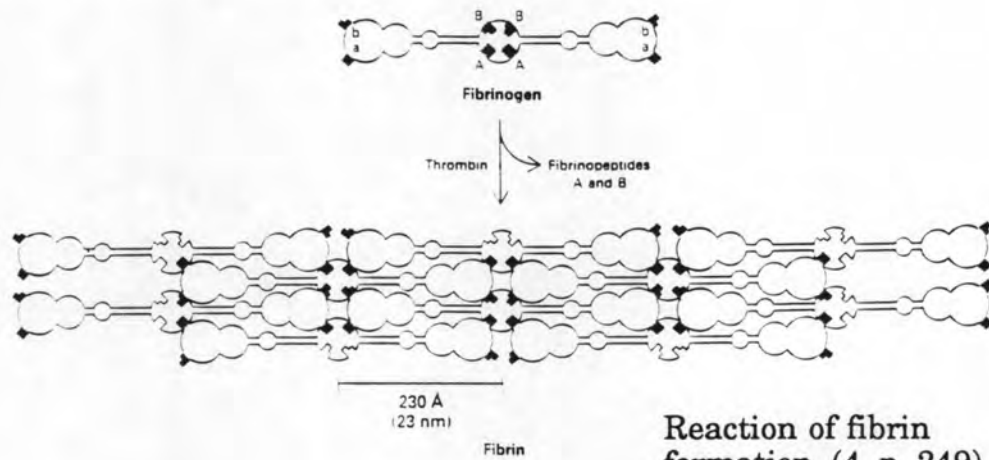
sensed by the clotting factors in the blood which begins a cascade of enzymatic activations that eventually form an array of fibrin molecules.



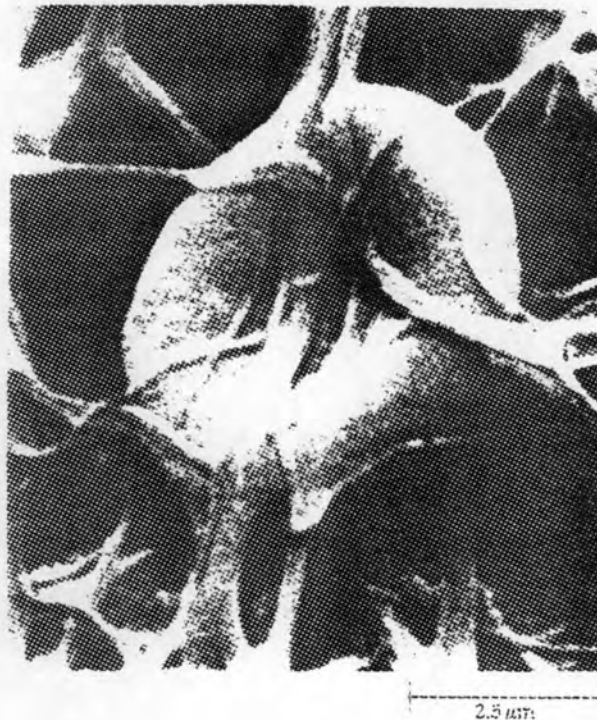
The cascade of reactions leading to clot formation (4, p. 248)

The second to the last step of the cascade is the most important one. The zymogen, fibrinogen, consists of two globular regions connected by triple-stranded alpha-helical rod regions. The globular regions contain two sets of highly negatively charged fibrinopeptides A and B. Thrombin catalyzes the cleaving of four arginine-glycine peptide bonds that link the fibrinopeptides to the center globular region to form a fibrin monomer. The net effect is the increase of the net charge of the center globular region from -8 to +5. Spontaneous ordering into fibrous arrays occurs because the change promotes electrostatic interactions between the center globular

region and the negatively charged terminal units. The newly formed fibrin has a porous character that can be seen in the diagram below and is very important in the action of thrombolytic compounds.



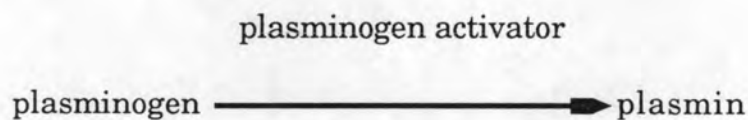
The fibrin molecules attach to one another and begin to catch red blood cells and platelets in their fibrous web.



A red blood cell
caught in a fibrous
web. (2, p. 707)

When platelets are caught and break open, they release their internal agents. Thromboxane A₂, a prostaglandin, is one of these agents that promotes platelet aggregation and vascular constriction. The formation of these insoluble networks constricts the vessel and stops the flow of oxygenated blood through the vessel. The thrombus is the cause of the onset of a cardiac arrest. The sooner the thrombus can be reperfed the better the chances are to save the tissue fed by the blocked artery and to save the victim's life. The problem is that most people do not receive thrombolytic therapy soon enough.

These newly formed clots are open to lysis by plasmin, a serine protease. Plasmin is the active form of the zymogen, plasminogen. Plasminogen activator converts inactive plasminogen to the active enzyme, plasmin:



The porous character of fibrin allows the plasmin molecules to access the interior of the fiber structure. The hydrolyzing of the peptide bonds occurs in the rod regions. If fibrin did not have a porous character, the plasmin molecules would not be able to diffuse into the fibers and dissolve the clot.

The catalytic triad of plasmin carries out this hydrolysis by a mechanism common to all serine proteases. Aspartate, histidine, and serine make up the catalytic triad in plasmin and are helped by an oxyanion hole in transition state stabilization.

Now that the mechanism of clot formation and hydrolysis by plasmin has been explained, the focus can turn to how the activity of plasmin can be enhanced or activated artificially. As noted plasminogen activator activates the clot lysing protease. The three compounds to be discussed are activase, streptokinase, and emminase. All three of these thrombolytic compounds attempt to increase the concentration of plasminogen activator. However, the way the increase is brought about is different. Initially all three compounds have to produce enough active plasmin to overcome inhibitors that naturally circulate in the blood. Alpha-antiplasmin and alpha₂-macroglobulin are depleted rapidly in all cases with therapeutic doses.

Activase

Activase, developed, patented and produced by Genentech, was cleared for marketing by the United States Food and Drug Administration in November of 1987. The Food and Drug Administration also approved Genentech's claim that activase saves lives in February of 1989. Sales of

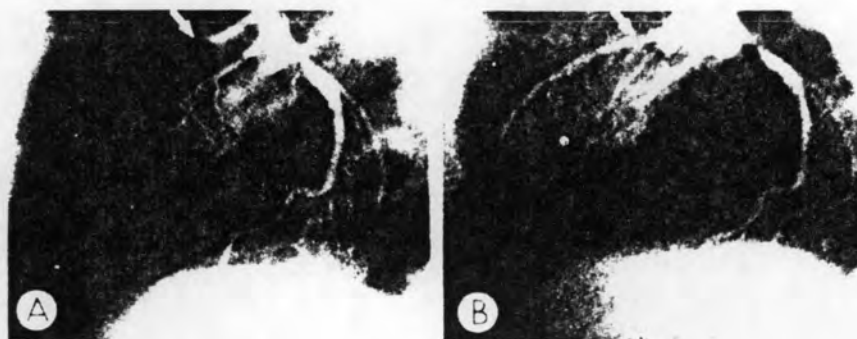
activase totaled \$151.4 million in 1988 and increased thirty percent to \$196.4 million in 1989 (5, p. 947M). Activase, the only biosynthetic thrombolytic compound produced by recombinant DNA technology, costs \$2332 for a single treatment (6, p. 2). Genentech invested \$220 million in researching and developing activase and must reclaim their investment through its high prices. Sales of activase accounted for over *fifty percent* of Genentech's total sales in 1989. This high percentage makes Genentech very protective over the market it has gained for the product. An example of their protectiveness occurred in midsummer of 1989. Genentech, a San Francisco based company, asked two California senators to write the Food and Drug Administration and request that a special advisory committee be formed to review eminase, the most recently developed compound. Two weeks later, SmithKline Beecham, developer of eminase, was notified that it would have to appear before the committee on October 31, 1989. This move by Genentech proved to postpone eminase's approval until November of 1989 and postpone distribution in the United States until January 1, 1990.

The structure of activase is very similar to that of naturally occurring plasminogen activator. The active 527 amino acid sequence is identical, but slight variations occur in the carbohydrate side chains. Intravenous activase is applied over a period of approximately four hours through an intravenous infusion. It works by increasing the levels of plasminogen

activator by approximately one thousand times. This increase in turn activates more plasmin to lyse the clot. One of the heralded traits of activase is its clot specificity. Activase is supposed to preferentially bind to fibrin in clots, and not to affect circulating fibrinogen. Hussar states that "It [activase] binds to fibrin in a thrombus and converts the trapped plasminogen to plasmin, but it has comparative little effect on free circulating plasminogen (7, p. 41)." However, Boyle states that "In practice, some reduction in circulating fibrinogen does occur (8, p. 84)." The small effect of activase on circulating fibrinogen seems to be an advantage of activase, but it could be a disadvantage of activase. McEvoy states that "Reocclusion of the infarct-related coronary artery following therapy with rt-PA [activase] generally has occurred in approximately 10-20% of patients, although higher reocclusion rates (up to 45%) have been reported (9, p. 729)." Activase's relatively small effect on circulating fibrinogen could be the cause of this problem. The other two compounds (especially streptokinase) lack activase's specificity and break down more of the circulating fibrinogen.

Another problem associated with activase is its short half-life. Its half-life is only five minutes compared to eminas's half-life of approximately ninety minutes. McEvoy states that "More than 50% of t-PA is cleared from plasma within 5 minutes after discontinuance of IV

infusion of altepase [activase], and approximately 80% is cleared within 10 minutes (9, p. 728)." The use of an anticoagulant with activase is required to prevent reocclusion. Heparin is a natural anticoagulant that is available for use with activase. Abram states that "Heparin acts in conjunction with antithrombin III to inactivate several clotting factors (IX, X, XXI and perhaps XII) and thrombin so that thrombus formation is prevented (10, p. 535)." Disadvantages of heparin are similar to those of activase. It has a relatively short half-life, so continuous IV infusion is required and it is obtained at a fairly high cost. Weiss records that "t-PA [activase] must be given simultaneously with the anticoagulant heparin, increasing the risk of bleeding. The bottom line, said Sherry, is that t-PA now proves to have comparable bleeding to streptokinase (11, p. 22)." The following picture shows how a coronary vessel's flow is actual restored after activase is administered.



Picture of reperfusion
with activase. (12, p. 611)

One distinct advantage that activase has over its two competitors is that it is not antigenic. Activase treatment can be given repeatedly without problems of antibody build up against it. After repeated treatments, the body will build a resistance to antigenic streptokinase and eminasase. Subsequent treatment of these two compounds will be less effective and possibly produce allergic reactions.

Streptokinase

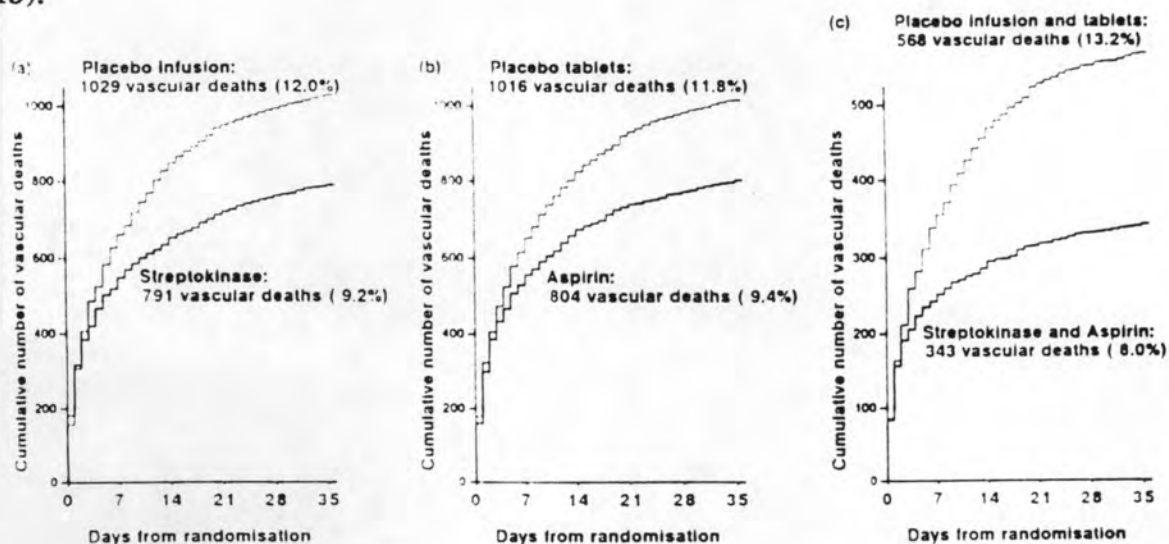
Streptokinase has reportedly been used used to treat myocardial infarction since 1959. Since its patent has long expired, several companies market the compound under different names. Hoechst-Roussel markets it as streptokinase, and other smaller companies market it under the names Kabikinase and Streptase. A single treatment of the nonenzymatic protein, streptokinase, costs only \$186.48. Streptokinase is derived from streptococci bacteria from which it got its name. Intracoronary or intravenous streptokinase therapy is available. Intravenous therapy is more common because of limited facilities for intracoronary therapy and other difficulties that arise from its use. Once treatment is initiated, instead of artificially

increasing plasminogen activator level, McEvoy states that "Streptokinase acts in a complex manner with plasminogen or plasmin to produce an activator complex that converts residual plasminogen into the proteolytic enzyme plasmin (9, p. 733)." In addition to increasing the conversion of plasminogen to plasmin, streptokinase also decreases plasma and blood viscosity, decreases erythrocyte aggregation, decreases blood pressure, decreases vascular resistance and possibly alters platelet function. These additional functions of streptokinase may help to prevent the reocclusion of reperfused arteries.

Streptokinase is most effective on recently formed clots, so the sooner the treatment is started the better survival rates. If applied later than recommended the treatment can still be beneficial, but not as beneficial as when applied early. When thrombolytic treatment was initiated within four hours of onset of pain, 6.4% of the victims died from vascular causes within thirty-five days and 9.2% died in the same time period if the treatment was initiated from five to twenty-four hours after onset of pain as shown by ISIS-2 (13, p. 352). This study shows an approximately thirty percent reduction in vascular death if the treatment was initiated early.

In contrast to activase and heparin, streptokinase is usually supplemented with aspirin. Aspirin is an effective antiplatelet which prevents platelet aggregation. It prevents the synthesis of thromboxane A₂

in platelets. Thromboxane A₂ stimulates the aggregation of platelets and promotes vascular constriction. The effects of aspirin act for the entire ten day life span of the platelets. Dosages for antiplatelet effects are 150-325 milligrams daily and adverse effects are uncommon. Aspirin used alone after the onset of cardiac arrest significantly reduces vascular mortality as shown by ISIS-2 (13, p. 349). When the two compounds are used together, there is a marked decrease of vascular mortality over either one used alone. ISIS-2 states that "Their [streptokinase and aspirin] separate effects on vascular deaths appeared to be additive: 343/4292 (8.0%) among patients allocated both active agents vs 568/4300 (13.2%) among those allocated neither (odds reduction: 42% SD 5; 95% confidence limits 34-50%) (13, p. 349)."



Three graphs showing the effects of each compound and a combination vs. placebo. (13, p. 349)

A problem associated with streptokinase is that its antigenic. The body will produce antibodies against the protein if it is used repeatedly. This response should decrease streptokinase's effectiveness and possibly cause allergic reactions.

Eminase

Eminase, the newest competitor in the thrombolytic market, is being distributed by a joint effort of SmithKline Beecham and Upjohn. It is the culmination of fifteen years of research and development at a cost of \$190 million. Eminase is a modified, second generation form of streptokinase. It is also known by the acronym, APSAC, which stands for Anisoylated Plasminogen Streptokinase Activator Complex. As the name implies, it is a complex. This complex is composed of acylated, inactive streptokinase combined with human lys-plasminogen. Human lys-plasminogen, truncated human plasma, is supposed to preferentially bind to fibrin in clots and not to circulating fibrinogen. The acyl group is hydrolyzed slowly to produce a time release of the active compound that converts the plasminogen into plasmin.

Eminase has a few distinct advantages that were obvious even before clinical trials began. Perrin records one distinct advantage eminase has is that "'Eminase is administered over a two- to five-minute period with a simple syringe,' said Anderson. 'Other thrombolytic agents such as streptokinase or tissue plasminogen activator [activase] take a one to three hours or more to administer and require an intravenous line as well as a monitoring pump' (14, p. 20)." If it proves to be as an effective life saver as the other compounds, many experts see this single advantage as one that could win overwhelming support of eminase. Sun writes that "Jeffery Anderson of the University of Utah, who was an investigator in the APSAC study, points out that a heart attack victim has many clinical complications. 'With APSAC, you don't have to worry about an i.v. drip. You just give it in 2 minutes and then you can deal with the other things,' Anderson says (15, p. 1268)."

Another distinct advantage eminase has over the other two compounds (especially activase) is its half-life. Eminase has a half-life of about ninety minutes which is attributed to the acyl group's slow hydrolysis which releases the active compound. Activase has a half-life of only five minutes which causes many problems with reocclusion. Streptokinase's half-life is twenty three minutes. The long half-life of eminase prevents the need for potentially hazardous anticoagulants. One of the primary objections to

eminase's quick administration is that if bleeding occurs the drug infusion can not be stopped. If bleeding occurs with streptokinase or activase the administration of the drug can simply be stopped. However, there is a antidote for streptokinase that should work for eminase since it is only a streptokinase complex. Aminocaproic acid is the antidote for streptokinase and it can be given orally or intravenously.

Since the market for thrombolytic therapy has been estimated to be up to a billion dollar a year industry in the near future, all the companies are trying to get mass approval of their product. Perrin states that "SmithKline Beecham and Upjohn have begun a program to both inform physicians of the benefits of thrombolytic therapy and help educate the general public about heart attack symptoms and the importance of seeking prompt medical attention (14, p. 20)." The possible victims could take daily doses of aspirin, which is currently recommended by doctors. The education of these people to the early symptoms of heart attack could decrease the time before a thrombolytic compound can be administered. The use of eminase would allow the injection of a thrombolytic compound at home, or in an ambulance on the way to a hospital. As studies have shown, the sooner the thrombolytic compound is administered the sooner the occlusion can be reperfused, and the less chance of permanent damage to the heart tissue.

Analysis

The results of all the studies show that thrombolytic therapy does save lives no matter which compound is used. Sun writes that "Not since the 1960s, when coronary care units were introduced has a single technology improved a heart attack victim's chances of survival so dramatically as thrombolytics, according to Braunwaly and other experts (15, p. 1267)." Thrombolytic therapy does have it drawbacks. McEvoy states that "Plasmin is a relatively nonspecific serine protease that is capable of degrading fibrin, fibrinogen, and other precoagulant proteins such as factors V, VIII, and XII (9, p. 726)." Increased plasmin concentration will then not only lyse clots in coronary arteries, but also prevent clots from being formed until new precoagulation proteins can be made. The consequences include the prevention of immediate surgery on the patient and any other type of therapy that would break the skin. The plasmin will also lyse clots elsewhere in the body that are considered to be beneficial. Side effects are mostly associated with bleeding complications, but vomiting, fever, and hypotension have also been reported. The most serious side effect is a stroke. Marx states that "The incidence of stroke is sufficiently low--from about 0.5% to 1.0% of the patients who receive thrombolytic agents have the problem--compared to the life-saving benefits

of the therapy to justify its use (16, p. 1506)." All the side effects combined were rare and occurred in less than three percent of all patients in all the studies. Another problem with thrombolytic therapy is its high cost especially with activase and eminase. Medicare does not cover the high expense of the compounds separately, it is just included in the limited coverage of the overall treatment for a heart attack. Activase and eminase may decrease the amount of time the victims need to stay in the hospital, but at only \$186.48 streptokinase may prove to reduce the stay equally well (6, p.2). However, if one compound is found to be better than the other compounds can one put a limit on the price of a treatment that can save a human life.

Although thrombolytic therapy does have problems with side effects, the benefits far outweigh the disadvantages. The AIMS trial study done on 1004 patients showed a forty-seven percent mortality reduction in those treated with eminase versus those victims given placebo (17, p. 545). The advisory committee decided to discontinue use of placebo because of the great decrease in mortality with the use of a thrombolytic compound. Thrombolytic compounds are also known as fibrinolytic compounds because of their general ability to lyse fibrin clots. For this reason, thrombolytic technology can be applied to a number of other clot related problems. Any clot that has formed that is not medically desirably can be

lysed in a similar procedure as used to lyse coronary clots. Examples include: subclavian vein occlusion, femoropopliteal artery occlusion, basilar artery occlusion, cerebral infarction, deep vein thrombosis, and pulmonary embolism. Future thrombolytic drugs may be linked to monoclonal antibodies that will truly direct the drug to a specific clot.

The ultimate question is not which compound dissolves clots the best or which one has the least number of side effects, but it is which compound has the best survival rate. All the studies previously done have used different techniques and compounds to supplement the particular thrombolytic that is being tested. For example, some patients receive aspirin, heparin, angioplasty, beta-blockers, etc. A single large scale study with direct comparisons between the different compounds is needed for a definitive winner. Eventually, I believe the answer will be which compound works best when supplemented with other compounds and treatments. For example, a study using eminas and aspirin should show that their effects are also additive.

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