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Targeting ischemic penumbra: part I - from pathophysiology to therapeutic strategy

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Abstract

Penumbra is the viable tissue around the irreversibly damaged ischemic core. The purpose of acute stroke treatment is to salvage penumbral tissue and to improve brain function. However, the majority of acute stroke patients who have treatable penumbra are left untreated. Therefore, developing an effective non-recanalizational therapeutics, such as neuroprotective agents, has significant clinical applications. Part I of this serial review on "targeting penumbra" puts special emphases on penumbral pathophysiology and the development of therapeutic strategies. Bioenergetic intervention by massive metabolic suppression and direct energy delivery would be a promising future direction. An effective drug delivery system for this purpose should be able to penetrate BBB and achieve high local tissue drug levels while non-ischemic region being largely unaffected. Selective drug delivery to ischemic stroke penumbra is feasible and deserves intensive research.

Keywords

stroke; cerebral ischemia; neuroprotection; penumbra; treatment; energy state; cerebral energy metabolism

Introduction

Each year, approximately 795 000 people experience a new or recurrent stroke. On average, every 40 seconds, someone in the United States has a stroke. Overall stroke prevalence during 2003 to 2006 is around 2.9%. Of all strokes, 87% are ischemic. (Lloyd-Jones et al.) Due to stroke's high incidence and prevalence rates and the lack of effective treatment, stroke remains one of the major diseases causing most mortality and disability. Stroke is the third leading cause of death, behind diseases of the heart and cancer, and is a leading cause of serious, long-term disability in the United States. Although treatments for ischemic stroke have been rigorously investigated for two decades, up to now there is only one FDA-approved pharmacological treatment for ischemic stroke, the intravenous thrombolytic treatment using recombinant tissue plasminogen activator (r-tPA).(Jahan and Vinuela 2009), which can only be available to a very limited number of patients (Kleindorfer et al. 2004).

Acute stroke causes an irreversibly damaged ischemic core and salvageable surrounding tissue. "Penumbra" is the term used for the reversibly injured brain tissue around ischemic core; which is the pharmacological target for acute ischemic stroke treatment (Astrup et al. 1981a). The goal to treat ischemic stroke is to salvage the penumbra as much and early as possible. It has

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been reported that roughly half of all acute ischemic patients show penumbra on MRI (Rivers et al. 2006) and are potentially treatable. However, only 8% of all ischemic stroke patients eligible for treatment with recombinant tissue plasminogen activator (r-tPA) (Kleindorfer et al. 2004). Effective pharmacological treatment with or without recanalization could be used for the majority of stroke patients, having invaluable clinical significance. The development of neuroprotective treatment for ischemic stroke is obstructed by the blood-brain barrier and reduced blood supply to ischemic brain tissue, facing repeated translational failure in recent 20 years. Drug delivery to brain tissue, especially the ischemic brain tissue has long been the technical bottleneck limiting acute stroke treatments. A breakthrough in this area will possibly bring in numerous related applications. The technology to be developed in this field may also be extended to other fields, such as traumatic brain injury, brain tumor, and CNS inflammatory diseases. This review summarizes advances for ischemic stroke penumbra, and puts special emphases on strategy development from a metabolic point of view for effective drug delivery to ischemic penumbra.

Penumbra and infarct expansion: the “time is brain” concept

In animal studies, the dynamic changes of penumbra area and infarct expansion can be better illustrated based on the data obtained from experimental strokes, in which the timing of occlusion and reperfusion was precisely controlled. After middle cerebral artery (MCA) occlusion, the infarct evolves rapidly in the first few hours, supporting the interventional concept that “time is brain” (Saver 2006). For an example, in a 300 g rat, 2-h MCA occlusion (MCAO) produces a big infarct of 400-450 mm^3 that is close to the infarct caused by 24-h permanent MCAO (Greco et al. 2007; Masada et al. 2001). Ninety minute transient MCAO results in a smaller infarct about 250–380 mm^3 (Eschenfelder et al. 2008; Liu et al. 2006) whilst 60-min MCAO only produces approximately 170 mm^3 infarct (Han et al. 2008). Therefore, in a 300g rat, at 1-h post-MCA occlusion approximately 170 mm^3 brain tissue has already been irreversibly injured. At this moment the occlusion has caused approximately 230 mm^3 tissue in danger. Roughly 140 mm^3 of this 230 mm^3 in-danger brain tissue will die in 30 min, and the left 90 mm^3 will die in 60 min. If we assume the specific gravity of rat brain is 1.0 mg/mm^3, the average speed of infarct expansion for a 300g rat is approximately 3.3 mg/min after MCA occlusion.

Imaging penumbra

For identifying the salvageable brain tissue in acute stroke, the direct method is to image penumbra. In acute ischemic stroke, the viability and size of penumbra change dynamically (Kuge et al. 2001; Shimosegawa et al. 2005) in response to regional cerebral blood flow, pathophysiological environment and treatment. Penumbra can be imaged using different technologies, such as MRI, CT (Kumar et al.), PET, and SPECT (Meerwaldt et al. 2009). For targeting penumbra in stroke patients, imaging penumbra is necessary for monitoring treatment response as well as for patient screening. The “mismatch” of perfusion-weighted and diffusion-weighted images (PWI-DWI mismatch) is the most commonly used method for imaging penumbra and may serve for this purpose (Ebinger et al. 2009; Rivers et al. 2006). The diffusion-weighted image may represent reversibly injured tissue in the early hours after stroke (Muller et al. 1995; Sakoh et al. 2001) whereas the perfusion-weighted image may include area of benign oligemia (Sobesky et al. 2005). The mismatched tissue represents “tissue-at-risk”, not “tissue-doomed-to die”; therefore it does not identify lesion growth by itself (Rivers et al. 2006). (For infarct expansion see the following paragraph.) Penumbra may resolve spontaneously (Koga et al. 2005), either by merging with the ischemic core, or becoming normal tissue. When recanalizational therapy started early enough, the mismatched tissue, the penumbra, may be salvaged, which has been observed using both CT (Murphy et al. 2006) and MRI (Olivot et al. 2008) methods.

Penumbra in stroke patients: the majority of potentially treatable patients are not treated

The use of imaging modalities detecting the existence of penumbra in stroke patients brought in new lights in patient management. Theoretically, all patients having penumbra zone should be treated. However, the number of patients treated by recanalizational intervention is only a small portion of all acute stroke patients who have a salvageable penumbra. When further looking into the subtypes according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification (Adams et al. 1993), the existence of penumbral tissue significantly correlates to stroke subtypes. The majority (about 94%)of intracranial large artery atherosclerotic (IC-LAA) stroke patients had perfusion-diffusion mismatch, whereas in cargioembolic strokes the penumbra existed in 35.7% patients (Boomer et al. 2009). Although the initial penumbral volume is similar among large-vessel stroke, cardioembolic stroke and cryptogenic embolic stroke, the mean perfusion defect in IC-LAA stroke was less severe than in other groups. This finding may indicate that the penumbral tissue in intracranial large artery atherosclerotic stroke may be more responsive to acute treatment. When an infarct involves white matter, it is associated with a relatively greater penumbral zone than in gray matter because white matter is more resistant to cerebral ischemia (Arakawa et al. 2006; Bristow et al. 2005; Koga et al. 2005) possibly due to the difference in constituent cell population and NMDA receptor dexpression.. Lacunar infarction is caused by occlusion of perforating artery, which is end-artery without collateral circulation; and its occlusion is thought not to result in a penumbral zone. Because of the small volume of lacunar infarcts, the finding of a perfusion-diffusion mismatch in lacunar stroke is affected by MRI technical issue. Studies using a 1.5-T scanner (Gerraty et al. 2002; Ohashi et al. 2005), or CT perfusion imaging and CT angiography (Vergoni et al. 2000), found no PWI abnormality in patients with a final diagnosis of lacunar infarct. In a most recent study of lacunar infarcts using a 3-T scanner that provide a higher spatial resolution, only 68.2% patients was found having abnormal PWI at the site of the diffusion-weighted imaging lesion (Poppe et al. 2009).

The fate of penumbra: role of energy state

While cerebral blood flow determines both the metabolic process (Hata et al. 2000; Hossmann 1994) and the fate of ischemic tissue (Bardutzky et al. 2007; Murphy et al. 2006; Ohashi et al. 2005), energy state of an ischemic cell determines the pathway (Eguchi et al. 1997; Nicotera and Leist 1997; Nicotera et al. 1998) (Leist et al. 1997; Lieberthal et al. 1998) and the destination (Galeffi et al. 2000) (Wang et al. 2000) of a cell to die or to survive. For detailed discussion please refer to our previous publication (Liu and Levine 2008) and figure 1 and figure 2. Cerebral ischemia causes a disturbance of energy metabolism. In global ischemia, brain ATP levels decrease to approximately 60% of baseline in one minute (Winn et al. 1979). In focal cerebral ischemia, the ischemic core is depleted with ATP whilst the penumbra has decreased ATP level, see figure 3. Theoretically, intervention that maintains cell energy state may provide robust neuroprotection. Such examples can be found in some classic neuroprotectants (Warner et al. 1996). Bioenergetic intervention could be equally important and effective as recanalizational intervention for acute stroke treatment.

Potential of neuroprotection: view from metabolic suppression

Neuroprotection can be achieved through metabolic suppression that decreases energy demand, therefore, maintains energy state. The human brain is metabolically highly active, and the majority of its metabolism is for functional purposes and can be suppressed. The human brain constitutes only about 2% of the body weight, yet the energy-consuming processes that ensure proper brain function account for approximately 25% of total body glucose utilization. The average ATP concentration of normal rat brain tissue is between 2.38 to 2.75 nmole/mg wet

weight (Hsu et al. 1991; Plaschke et al. 1998; Winn et al. 1979). The main energy-consuming process of the brain is the maintenance of ionic gradients across the plasma membrane and function-related activities (Ames 2000). About 87% of total energy consumed reflects function-related activities (Magistretti 2002), which could be suppressed to decrease energy consumption. Metabolic suppression happens naturally in hibernating animals without causing tissue injury. Hibernation and torpid state can reduce basal metabolic rate to 1-5% of resting normothermic metabolic rate below ischemic threshold for causing irreversible injury (Geiser 2004). Decreasing energy demand by metabolic suppression is the classic method for achieving neuroprotection. Metabolic rate could be drastically reduced by hypothermia (Astrup et al. 1981b; Berger et al. 1998; Mori et al. 1998), anesthetics and sedatives (Astrup et al. 1981b; Warner et al. 1996); but hypothermia-related (Jian et al. 2003; Schwab et al. 2001) and drug-related systemic complications (Coupey 1997) have limited their use in acute strokes. Recent advances in CNS drug delivery system may provide a solution for these problems.

Direct energy delivery

ATP molecules are negatively charged and cannot freely pass membrane barriers entering intracellular space (Gordon 1986). Because extracellular ATP are rapidly degraded by ectonucleotidases (Winn et al. 1979), investigators have tried using nanoliposome-entrapped ATP to deliver energy to ischemic tissue. Nanoliposome-encapsulated ATP(Arakawa et al. 1998) has shown protective effects in intestinal injury from hemorrhagic shock (Zakaria el et al. 2005), forebrain ischemia (Laham et al. 1988; Puisieux et al. 1994), myocardial ischemia. (Verma et al. 2005a; Verma et al. 2006; Verma et al. 2005b), and skin wound healing (Chiang et al. 2007). ATP blood levels can be increased drastically after the administration of ATP-loaded nanoliposomes; a similar administration of carboxyfluorescein-loaded nanoliposomes showed that nanoliposomes can reach the ischemic cerebral parenchyma in rats (Chapat et al. 1991).

Direct energy delivery for brain ischemia

ATP molecules are highly recycled in living cells. It is not practical and neither necessary to provide the total consumption amount of exogenous ATP because injured cells still have, although limited, ability to regenerate ATP. Because ATP is released into, and degraded in, extracellular space, theoretically, it could also be beneficial for ischemic cells if such loss of intracellular ATP can be replenished through exogenous resources by targeted intracellular ATP delivery. Administration of liposomal ATP has been shown to be promising in a forebrain ischemia model.(Puisieux et al. 1994)

The liposomal ATP solution for *in vivo* experiments could reach a high concentration about 12 mg/ml (21.8 μmole/ml). (Verma et al. 2005a) With a bolus injection of serum stable pH-sensitive liposomes, 50%, 24%, and 15% of injected dose could remain in the blood at 1-h, 10-h, and 24-h post-injection, respectively (Slepushkin et al. 1997). Considering the regional cerebral blood flow (rCBF) in the inner penumbra being approximately 15 ml/100g/min (0.00015 ml/mg/min), (Murphy et al. 2006; Ohashi et al. 2005) therefore, an injection of 1 ml such ATP-loaded liposomes (12 mg/ml) into a 300g rat could deliver ATP to the inner boundary of penumbra with a speed of **0.079** nmole/mg/min (21.8*0.5/21*0.00015*1000) at 1-h post-injection, assuming the total blood volume being 21 ml. At this delivery speed, it will only need about **30-min** (2.38/0.079) to replenish the total ATP base pool (2.38 nmole/mg wet weight) in the inner penumbra through the residue blood flow.

In a forebrain ischemia model, it has been observed that when being entrapped into nanoliposomes and administered intracarotidally, ATP greatly increased the number of ischemic episodes that can be tolerated before brain electrical silence and death appeared (Laham et al. 1988; Puisieux et al. 1994) because of improvement in energy metabolism. Direct

energy delivery remains an attractive treatment for ischemic stroke, yet it still needs extensive research before its successful translation to clinic settings. Efforts need to be put on aspects such as giving synergistic adjunctive treatments, improving the bioavailability of ATP-loaded nanoliposomes, and minimizing the interaction of exogenous ATP purinergic receptors (Boucsein et al. 2003; Chen et al. 2007; Siow et al. 2005). A non-selective P2 receptor antagonist, such as suramin (Kharlamov et al. 2002; Millart et al. 2009), can be used for minimizing these compounding effects. Suramin can be encapsulated into liposomes (Chang and Flanagan 1994; Chang and Flanagan 1995).

Delivery of a metabolic suppressor

Nanoliposomes have been used as a carrier for CNS drug delivery and can be tissue selective. Selective delivery of a metabolic suppressor to a specific brain region makes it possible to reach a desired regional drug concentration with minimized drug-related systemic adverse effects (CNS depression, hypotension, etc.), therefore, having its application in acute stroke treatments. Some local anesthetics and sedatives have been reported of their liposomal formulation for topical application and controlled release, such as lidocaine (Fransson et al. 2002), benzocaine (Avila and Martinez 2003), diazepam (Fatouros and Antimisiaris 2002; Sznitowska et al. 2000). Because the amphiphilic drug diazepam, which binds to the same $GABA_A$ receptor as pentobarbital does, can be used in liposomal formulation, the more water-soluble pentobarbital will theoretically be better encapsulated in nanoliposomes and be bioactive.

Penumbral drug delivery strategy

Conventional drug delivery methods cause unwanted drug exposure to other tissue or brain regions, leading to severe side effects and toxicity, especially when high dose is being used for reaching therapeutic drug levels in ischemic tissue. For examples, the classic metabolic suppressor pentobarbital could reduce metabolic rate by 56%, (Warner et al. 1996) having a proven neuroprotective effect; but it cannot be used with a sufficient dose to achieve the desired maximal metabolic suppression because of its drug-related respiratory suppression. Neuroprotection by providing exogenous energy has also been facing problems of adverse effects and low bioavailability. With the advancement of CNS drug delivery, those problems can be tackled through innovative approaches (see following paragraph).

Brain ischemia causes a serial of pathological changes that affect drug delivery. In the ischemic local region there is limited blood supply while the blood-brain barrier and the shrunk extracellular space further limit drug access to ischemic brain tissue. However, there are also some pathological changes that may be utilized for facilitating drug delivery to local ischemic tissue. For example, brain ischemia causes a metabolic shift towards anaerobic glycolysis, resulting in a lower intracellular pH value in the ischemic brain tissue. Targeting at this property of ischemic brain tissue, liposomal nanocarrier may be optimized to release their cargos under acidic condition (Collins et al. 1989) similar to the intracellular environment of ischemic brain tissue($pH<6.75$) (Anderson et al. 1999). Another example, ischemia induced molecular structure changes can also be used for selective drug delivery to ischemic brain tissue. A most recently discovered special peptide has showed the homing ability to ischemic brain tissue (Hong et al. 2008). Therefore, the strategy for drug delivery to ischemic brain tissue should be to overcome the disadvantages and to utilize the advantages of ischemia induced pathological changes for achieving maximal bioavailability. And the neuroprotective strategy is to deliver a treatment that has the largest protection potential using the most efficient drug delivery system.

Summary

It is of great clinical significance to develop a neuroprotective treatment that can be made available to most acute stroke patients. Bioenergetic intervention by massive metabolic suppression and direct energy delivery would be a promising future direction. An effective drug delivery system for this purpose should be able to penetrate BBB and achieve high local tissue drug levels while non-ischemic region being largely unaffected. Selective drug delivery to ischemic stroke penumbra is feasible and deserves intensive research. See Figure 1.

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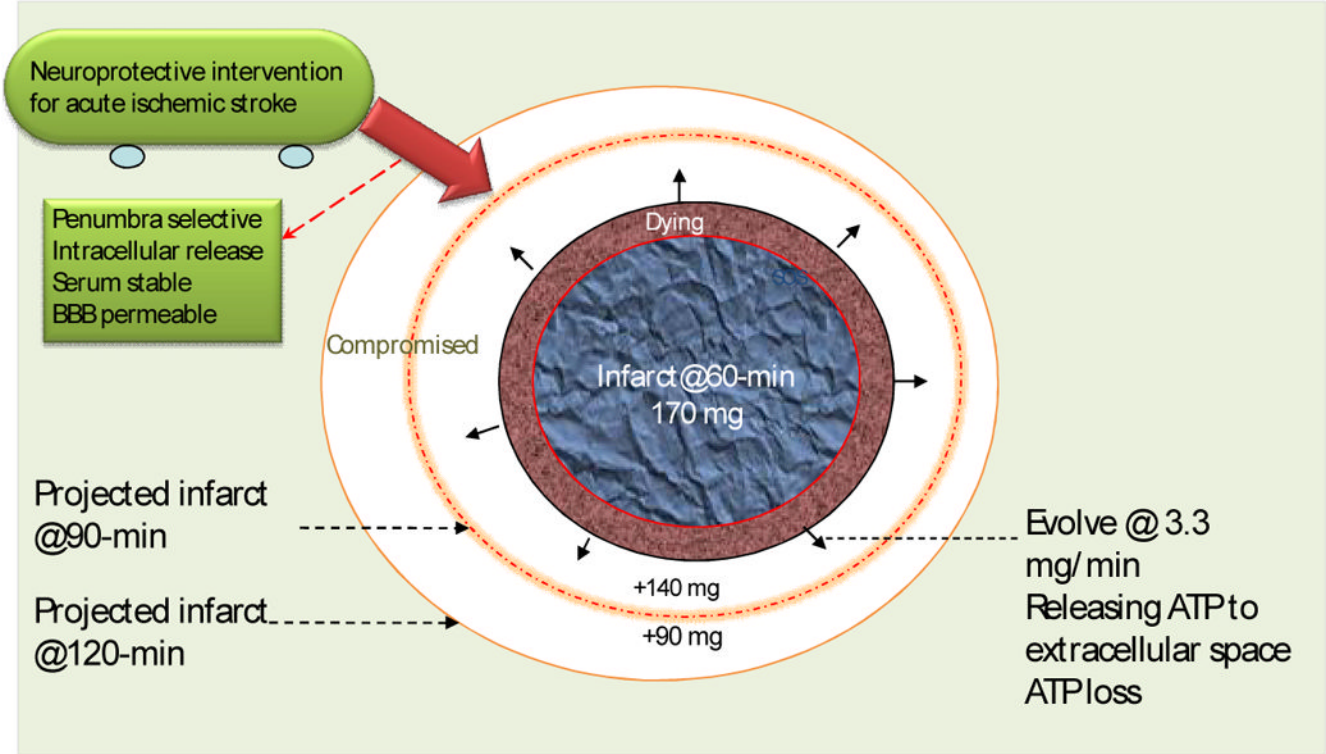

Figure 1.

Infarct expansion and treatment strategies. During the first few hours after middle cerebral occlusion of a 300 gram rat, the infarct expands quickly at an average speed of 3.3 mg brain tissue per minute assuming the specific gravity of rat brain is 1.0 mg/mm^3. Neuroprotective treatment should be able to penetrate the blood-brain barrier and reach penumbral zone. Such treatment should be made available to most acute stroke patients who have salvageable penumbral tissue.

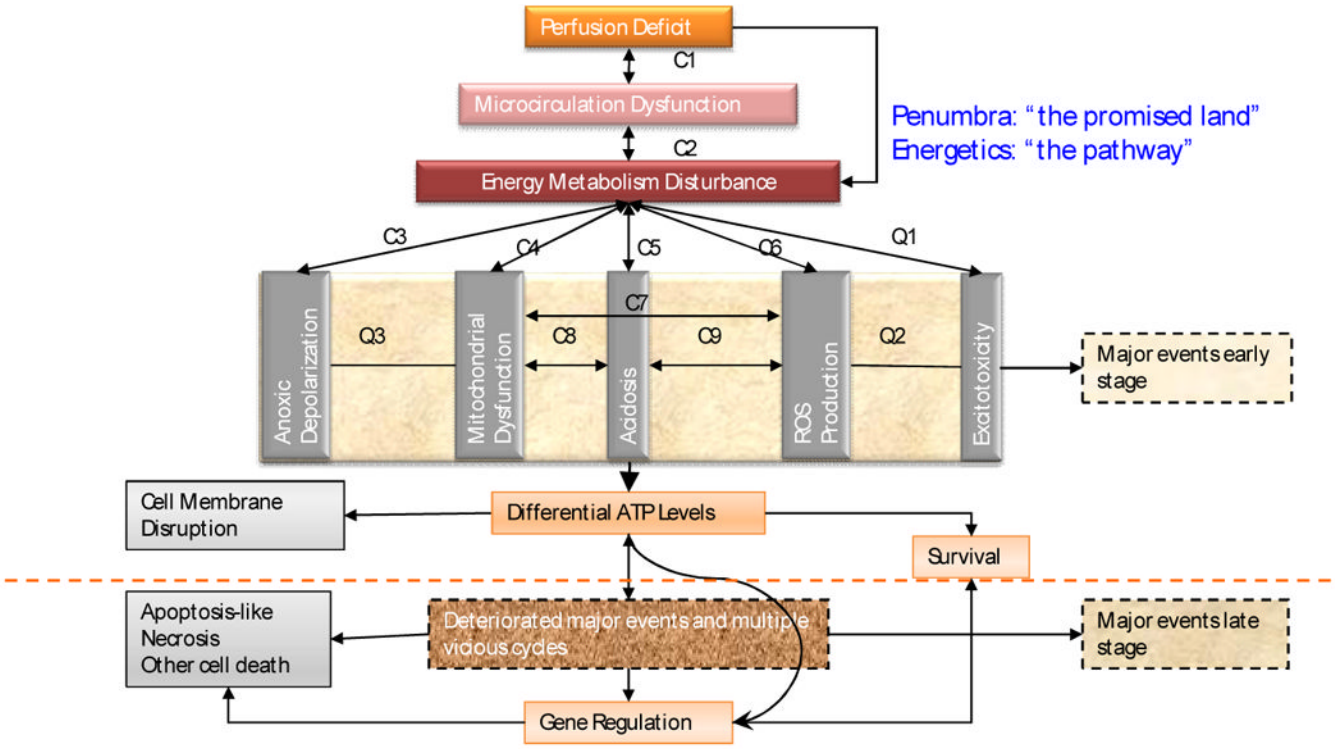

Figure 2.

From pathophysiology to therapeutic strategy. Salvaging penumbra is the goal for acute stroke treatment. Neuroprotection for acute ischemic stroke should target the upper stream event that determines the fate of ischemic penumbra. Bioenergetic intervention could be the therapeutic modality equivalent to recanalizational therapies at metabolic levels because the disturbance of energy metabolism after acute brain ischemia differentiates the ischemic cascades. C1-C9: pathological cycles between major events that are supported by literature; Q1-Q3: suspected pathological cycles between major events that need more literature support.

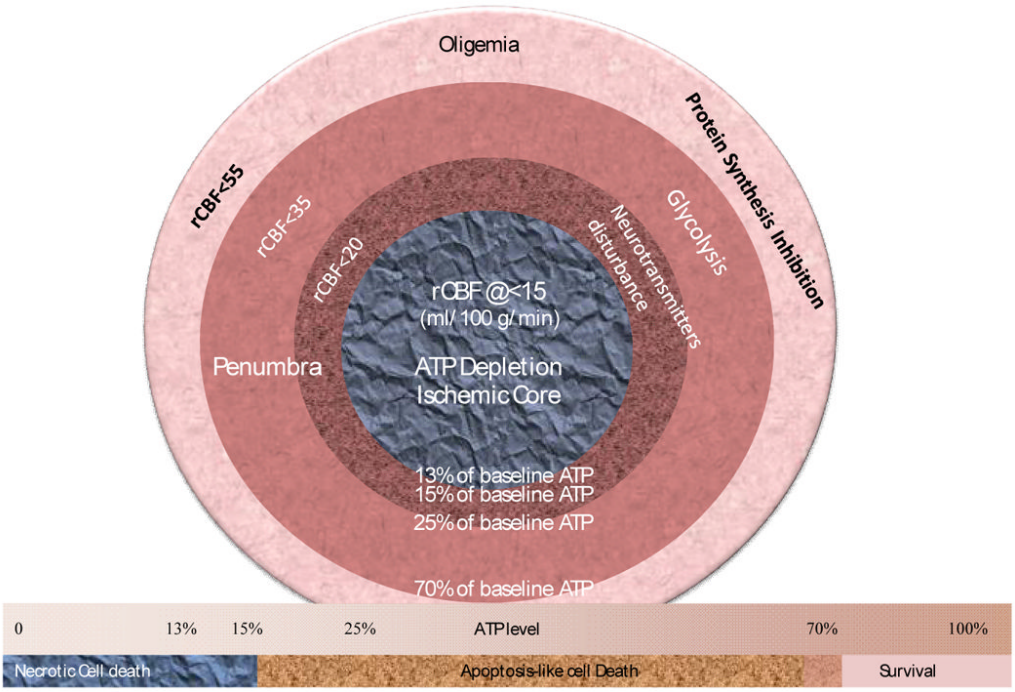

Figure 3.

Correlation between energy thresholds and blood flow thresholds. The form and pathway of cell death closely are closely associated with energy state levels. Blood flow reduction causes specific metabolic disturbances at certain blood flow thresholds. The ischemic core has depleted ATP level whilst the penumbra has gradient reduction of ATP level between normal or oligemic tissue and ischemic core.