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Principles of strategic drug delivery to the brain (SDDB): Development of anorectic and orexigenic analogs of leptin

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Abstract

The blood–brain barrier (BBB) presents a tremendous challenge for the delivery of drugs to the central nervous system (CNS). This includes drugs that target brain receptors for the treatment of obesity and anorexia. Strategic drug delivery to brain (SDDB) is an approach that considers in depth the relations among the BBB, the candidate therapeutic, the CNS target, and the disease state to be treated. Here, we illustrate principles of SDDB with two different approaches to developing drugs based on leptin. In normal body weight humans and in non-obese rodents, leptin is readily transported across the BBB and into the CNS where it inhibits feeding and enhances thermogenesis. However, in obesity, the transport of leptin across the BBB is impaired, resulting in a resistance to leptin. As a result, it is difficult to treat obesity with leptin or its analogs that depend on the leptin transporter for access to the CNS. To treat obesity, we developed a leptin agonist modified by the addition of pluronic block copolymers (P85-leptin). P85-leptin retains biological activity and is capable of crossing the BBB by a mechanism that is not dependent on the leptin transporter. As such, P85-leptin is able to cross the BBB of obese mice at a rate similar to that of native leptin in lean mice. To treat anorexia, we developed a leptin antagonist modified by pegylation (PEG-MLA) that acts primarily by blocking the BBB transporter for endogenous, circulating leptin. This prevents blood-borne, endogenous leptin from entering the CNS, essentially mimicking the leptin resistance seen in obesity, and resulting in a significant increase in adiposity. These examples illustrate two strategies in which an understanding of the interactions among the BBB, CNS targets, and candidate therapeutics under physiologic and diseased conditions can be used to develop drugs effective for the treatment of brain disease.

1. Introduction

Drug delivery to the central nervous system (CNS) is highly problematic because the blood–brain barrier (BBB) acts through a variety of mechanisms to prevent the unregulated entry of blood-borne substances into the brain [1]. This has been clearly exemplified by drugs targeting the control of feeding and body weight [2,3]. Obesity has become epidemic in

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affluent countries. This epidemic has paralleled an unprecedented explosion in our knowledge of the hormonal and neuronal components that affect body weight, caloric intake, and thermogenesis. Many of the newly discovered hormones, as exemplified by leptin, are peptides or regulatory proteins that must access receptors within the CNS to exert their effects [4,5]. Most of the CNS, about 99.5% of it, is protected by the BBB. As such, many of these peptides and regulatory hormones depend on an ability to cross the BBB to access those deep CNS receptors [6,7]. In many cases, again as exemplified by leptin (Fig. 1), these peptides and regulatory proteins depend on transporters located at the BBB to allow them access to the CNS [8]. When those transporters fail by allowing either too much or too little hormone into the CNS, disease can arise.

The dramatic increase in the percent of the population with obesity has to some extent obscured the equally clinically relevant problem of weight loss. Anorexia nervosa, anorexia from cancers, and anorexia of aging are three conditions in which a decreased caloric intake can result in dramatic decreases in body weight. Anorexia of aging is particularly mysterious, in which elderly individuals insidiously loose 30–40% of their body weight over a decade or more [9,10]. Individuals are often unaware of or relatively unconcerned about this dramatic weight loss. Anorexia of aging in combination with aging itself is associated with loss of muscle mass so that these patients not only have depleted their caloric reserves, but also have greatly decreased strength and stamina. Such patients are at an increased risk for morbidity and mortality. Thus, effective therapeutic agents are needed to treat both obesity and anorexia.

In this review, we will first briefly examine the major problems in developing drugs that need to be delivered across the BBB and then consider the major problems regarding the delivery of leptin to the CNS. We will then illustrate how these problems with delivery actually present unique opportunities and outline two strategies that take advantage of those opportunities.

2. General problems in drug delivery to the brain

Drugs are needed for the treatment of a host of diseases that are believed to have their main pathological expression within the CNS, including depression, dementia, delirium, alcoholism, obesity, anorexia, and Parkinson’s disease. Tentative targets are identified within the CNS for most of these conditions and diseases, but how to get effective amounts of drugs from blood to brain to those targets is problematic [11]. Most CNS-active drugs currently in clinical use are small, lipid soluble molecules. Such molecules diffuse cross the lipid membranes of the cells that comprise the BBB and so can access the CNS. Unfortunately, it is often difficult to use this strategy to develop new CNS drugs because of a series of problems. Chief among these are brain-to-blood (efflux) transporters such as p-glycoprotein that prevent many lipid soluble drugs from accumulating within the CNS [12].

Many strategies for the delivery of drugs to the brain have been investigated or are under investigation. Many of these have “black-boxed” the BBB, whereas others have attempted to use some characteristic of the BBB to deliver drugs to the brain. One oft-repeated strategy is that of disruption of the BBB. It has been shown that under carefully controlled conditions, BBB disruption is relatively safe and can be used to deliver chemotherapeutics to brain tumors [13]. However, disruption under other circumstances allows circulating substances into the CNS that have toxic effects on the brain. Many strategies for CNS drug delivery [14] are “universal” ones; that is, they would be useful to deliver a wide variety of drugs to the CNS for a number of diseases. A major example of a universal carrier is that of the “Trojan horse” delivery system. There are a number of variations on the Trojan horse approach, but a general theme is that a binding site is targeted on the brain endothelium that

will then transcytose (i.e., transport in vesicles from blood side to brain side of the endothelial cell) both the binder/drug cargo combination. Although this is a logical strategy, it is more complicated that it might seem. Infectious agents such as viruses and bacteria have placed an evolutionary pressure on the BBB to deal with their attempts to cross by Trojan horse like mechanisms. Thus, many binders that induce endocytosis are routed to lysosomes for destruction rather than to the abluminal side of the brain endothelial cell. Another concern for universal carriers is that the approach assumes that the BBB of the targeted disease condition is not substantially altered with regards to the delivery strategy. This approach, therefore, does not take advantage of the numerous “special situations” of the BBB. In other words, an approach that would not yield meaningful delivery of a specific drug for a specific disease state might be an ideal approach for a different drug for another disease state.

This review highlights an approach based on strategic drug delivery to the brain (SDDB) in which the complex interactions among the characteristic of the BBB, disease state, CNS target, and potential therapeutic are considered in devising a delivery strategy.

3. General difficulties in delivery of leptin to the brain

Rodents and humans who are unable to express leptin develop extreme obesity [15,16]. These humans and rodents are also extremely sensitive to leptin. However, most rodents not genetically pre-selected for abnormalities in body weightand nearly all obese humans are resistant to leptin [17–19]. In these cases, serum leptin levels are high. A resistance to leptin develops both at the receptor level in the arcuate nucleus and at the level of the BBB leptin transporter [20,21]. Resistance at both the receptor level and the BBB becomes increasingly profound with advancing obesity. In some rodent models, however, BBB resistance predominates in the earlier stages of obesity [19].

Resistance at the BBB is multifactorial. One cause of resistance is based on the fact that access of serum leptin is dependent on transport into the arcuate nucleus by way of a saturable transporter. As serum leptin levels increase, the leptin transporter is increasingly saturated [17]. Leptin transport capacity is already more than 50% saturated at serum leptin levels of 10 ng/ml, a level associated with ideal body weight rather than obesity [22]. The transporter is near capacity at about 40 ng/ml, which means that CNS levels of leptin are unlikely to increase despite further increases in serum leptin. Other factors, including circulating factors, also influence leptin transport. Epinephrine, glucose, ethanol, and insulin tend to increase leptin transport, whereas triglycerides inhibit transport [23–26]. Triglycerides in particular are likely an important cause of leptin resistance at the level of the BBB given the association of hypertriglyceridemia as the dyslipidemia of the metabolic syndrome.

The above relations among leptin, the BBB, and the CNS show two points clearly: 1) the multifactorial resistance at the level of the BBB will make it difficult to treat obesity with leptin or leptin analogs that depend on the leptin BBB transporter for access to the CNS; 2) induction of leptin deficiency within the CNS results in profound weight gain. We illustrate below how we used these principals to develop two analogs for leptin: 1) the first for the treatment of obesity is an agonist capable of crossing the BBB independently of the leptin BBB transporter that results in decreased feeding and weight loss; 2) the second for the treatment of anorexia is an antagonist capable of blocking the transport of endogenous, blood-borne leptin across the BBB that results in increased feeding and weight gain.

4. Strategies for development of leptin-based anorectic and orexigenic analogs

Overall, modifications of leptin or its analogs with PEG-containing polymers have potentially versatile uses in the development of therapeutic agents targeting the brain. A strategic choice of the polymer should depend on: i) the type of therapeutic desired (i.e., its agonist or antagonist activity), and ii) the desired interactions of the modified drug with the BBB. For example, drug modification with a polymer that efficiently penetrates the BBB is necessary when a leptin agonist is needed. In contrast, a polymer that would allow antagonist attachment to the leptin transporter but prevent actual transport should result in a potent antagonist by blocking the ability of endogenous, blood-borne leptin from reaching its receptors within the CNS. Therefore, in is crucial to identify those structural features that are important for the effects of the polymer on drug interactions with the BBB.

Pluronic block co-polymers are suitable candidates for such modifications. They consist of poly-ethylene glycol (EG) and poly-propylene glycol (PG) blocks arranged in a basic A–B–A structure: EGx–PGy–EGx. Importantly, variations in the number of hydrophilic EG units (x) and lipophilic PG units (y) result in copolymers with different molecular mass and distinct hydrophilic–lipophilic balance (HLB). Co-polymers with a short hydrophilic poly-EG block (PEG) or/and an extended lipophilic poly-PG (PPG) block are highly lipophilic and are characterized by low HLB. In contrast, copolymers with an extended hydrophilic PEG block or a short lipophilic PPG block are hydrophilic and characterized by high HLB. Pluronic P85 has intermediate lipophilicity and HLB values that fall in between the two extremes identified above. We have shown that P85 macromolecules rapidly incorporated into cell membranes and penetrate across the BBB, probably, by a flip-flop mechanism [27,28]. The internalization process occurs presumably in the caveolae-cholesterol rich domain areas of cellular membranes [29–31]. In contrast, molecules of hydrophilic pluronics with extended PEG chains adhere to the surface plasma membrane of the cells and limit the lateral mobility of membrane lipids causing membrane solidification [27]. PEG homopolymers represent an extreme case of such hydrophilic Pluronics.

Based on these findings, two polymers were selected for development of leptin analogs: Pluronic P85 and PEG were used for leptin and and leptin antagonist modifications, respectively. As a result, leptin conjugation with pluronic P85 (leptin-P85) increased its transport across the BBB by a mechanism that is not dependent on the leptin transporter. Furthermore, pegylation of a leptin antagonist resulted in blocking the BBB transporter for endogenous, circulating leptin and increased feeding and body weight gain. These results that the delicate balance between hydrophilic and lipophilic components in the polymer molecule can be manipulated to provide the desired interactions and the most significant therapeutic effect of the designed CNS drug delivery system.

5. Development of a leptin agonist: harnessing forgotten endocytic pathways

Leptin is a protein with a molecular weight of about 16 kDa. The largest molecule to date shown to cross the BBB to any extent by way of lipid solubility is CINC-1, a molecule that is considerably smaller at a molecular weight of 7.9 kDa [32]. Leptin-sized analogs are, therefore, likely too large to be taken up by the brain by way of transmembrane diffusion to a degree sufficient to have an effect on the CNS. Various strategies that could be employed include (but are not limited to): 1) improve leptin pharmacokinetics to take advantage of the extracellular pathways [33], 2) attach leptin to another molecule that can cross the BBB (the Trojan horse approach), 3) modify leptin so as to cross the BBB by vesicular processes that

are independent of the leptin transporter. These and other strategies have their own advantages and challenges. The third strategy as we used it here has the advantages that i) therapeutic amounts of material can be delivered which neither interfere with endogenous transporters nor is subject to their requirements; ii) it is independent of limitations in regional brain distribution imposed by other approaches, which can be a problem with a Trojan horse approach; iii) it can be developed with nontoxic co-polymers; iv) it is compatible with other strategies, such as those that improve pharmacokinetics.

It has been shown that the pluronic modifications can result in several mechanisms that enhance BBB transport and retention by the CNS. These include 1) improved pharmacokinetics primarily caused by decreased clearance and enzymatic degradation; 2) transport across BBB endothelium by way of caveolae-dependent transcytosis; 3) blockade of brain-to-blood efflux by p-glycoprotein [30,34–37]. Additionally, pluronic block copolymers are associated with decreased immunogenicity. Specifically, we covalently conjugated leptin by way of a biodegradable disulfide bridge with pluronic 85 (P85), a triblock copolymer of (ABA) of poly(ethylene oxide) (A) and poly (propylene oxide) (B). This block polymer has previously been shown to increase brain uptake of horseradish peroxidase, a protein that is ordinarily unable to cross the BBB [36].

Modifications of regulatory proteins can alter their potency and efficacy. Assessment of biological activity for feeding hormones is complicated by their tendency to exhibit U-shaped dose-response curves so that feeding behavior may only occur within a narrow therapeutic range. The basis for the U-shaped curve is not always clear but various explanations range from hormesis to nonspecific receptor interactions. Direct biological activities on brain function can be compared by injecting the regulatory proteins into the lateral ventricle of the brain. About 4 times more leptin-P85 was required in comparison to native leptin to inhibit feeding after intraventricular injection into the brain [38]. Interestingly, leptin-P85 had an onset of action between 2 and 4 h after administration, which was faster than for native leptin.

In lean mice, the leptin-P85 crosses the BBB at a rate similar to that of native leptin [38]. However, leptin-P85 crosses the BBB independently of the leptin transporter by a non-saturable mechanism likely involving caveolae (Figs. 2 and 3). As a result, leptin-P85 crosses the BBB of obese and lean mice at a similar rate. Half-time clearance is also increased about 6 fold to 30 min from about 5 min for native leptin. Enzymatic stability is also improved, especially in blood. Maintenance of transport rate coupled with improved stability and prolonged half-life results both in an increase in the percent of dose taken up by brain and a much longer residence time in brain. As a result of these modifications, leptin-P85 acutely inhibits feeding after its intravenous administration.

6. Development of a leptin antagonist: induction of leptin resistance

Lack of leptin action within the CNS either because of loss of the leptin protein or its receptor results in dramatic increased feeding and bodyweight gain [20,39]. Blockade of leptin’s ability to bind either to its BBB transporter or to its CNS receptor would result in a functional deficiency of leptin action within the CNS. We, therefore, developed a leptin analog with antagonist activity [40–42] which we termed MLA-leptin (L39A/D40A/F41A).We found that although MLA did not activate the leptin receptor, it did cross the BBB [43]. This is consistent with the proposal that the protein that transports leptin across the BBB is distinguishable from the leptin receptors [44]. To prolong circulation time in blood, a polyethylene glycol (PEG) group was attached to form PEG-MLA. Pegylation of a protein typically increases half-life in the circulation by decreasing renal clearance and protecting against proteolytic degradation. Several drugs in clinical use are pegylated,

including peginterferon for the treatment of chronic hepatitis C, pegfilgrastin for treatment of neutropenia, and pegvisomant for treatment of acromegaly. Unlike MLA-leptin, PEG-MLA was not transported by the saturable mechanism across the BBB (Fig. 2). However, it did bind to the BBB transporter for leptin, thus blocking the transport of circulating leptin into brain. In essence, PEG-MLA reproduced the defining component of so called "peripheral resistance" by hindering the ability of circulating, endogenous leptin from accessing the CNS. Small amounts of PEG-MLA may have also reached the arcuate nucleus through the extracellular pathways. If so, then PEG-MLA may also have produced a degree of central resistance to leptin's actions.

7. Conclusion

Two examples of the development of drugs to treat CNS conditions, those of obesity and anorexia, are reviewed. These two examples illustrate the use of the SDDB approach for development of such drugs. These principles and drugs can hopefully be used to treated diseases of the CNS.

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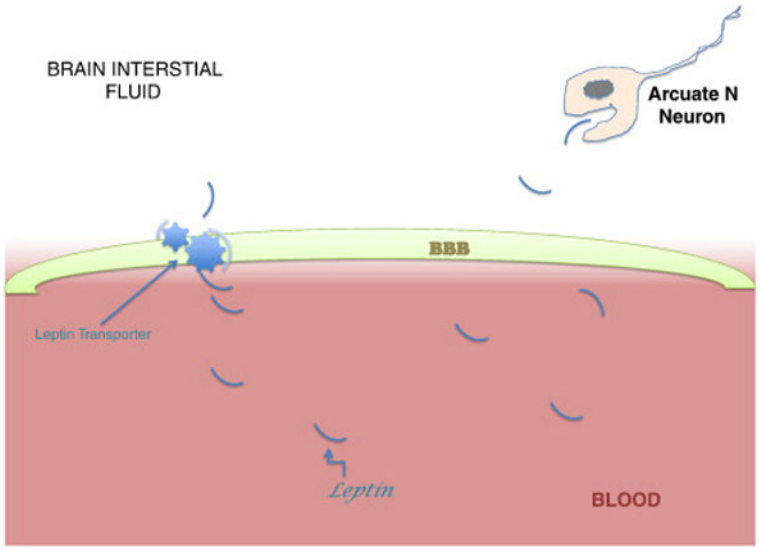

Fig. 1.

Physiological condition: circulating leptin is transported across the blood-brain barrier by a saturable mechanism. Once in the brain, leptin interacts with its receptor at the arcuate nucleus to influence body weight.

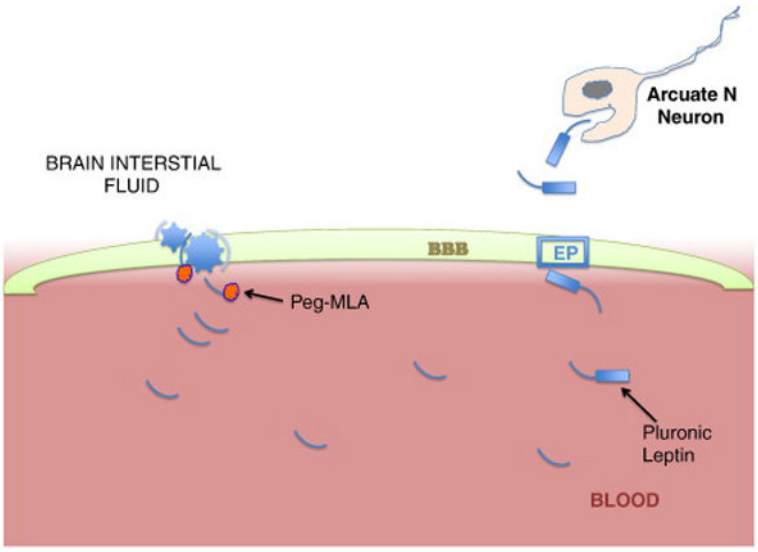

Fig. 2.

Drug development strategies: the leptin antagonist PEG-MLA binds to the leptin transporter at the blood–brain barrier but is not transported across by it. This binding blocks the ability of endogenous leptin in the blood from being transported into brain. Pluronic leptin crosses the BBB by way of an endocytic pathway (EP) to reach CNS receptors.

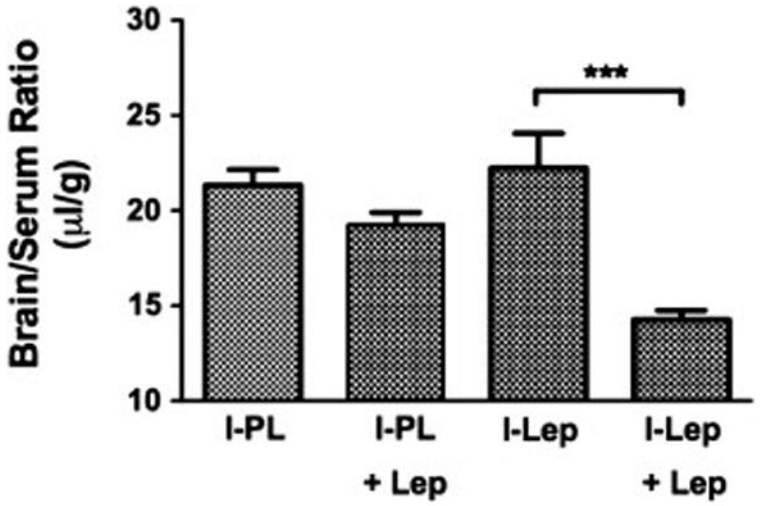

Fig. 3.

Pluronic leptin transport is independent of the leptin BBB transporter: brain/serum ratios greater than 10 microl/g represent extravascular leptin (that is, leptin which has crossed the BBB). Radioactively labeled pluronic leptin (I-PL) and radioactively labeled leptin (I-Lep) are taken up by brain at similar rates. However, unlabeled leptin (Lep) inhibits the uptake of radioactively labeled leptin (I-Lep+Lep) but not of radioactively labeled pluronic leptin (I-PL+Lep). ***$p<0.001$. Adapted from [38].