A Focus on Glucose-Mediated Drug Delivery to the Central Nervous System

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Abstract: Drug delivery to the central nervous system (CNS) is a timely and challenging issue: 95% of the pharmacological drugs cannot be delivered to the brain. This is mainly due to the blood-brain barrier (BBB), a highly selective boundary that hampers the passage of most compounds into the CNS. To overcome this problem, several approaches exist to deliver a therapeutic drug to the brain that takes into account not only the chemical properties of the drug but also the type of transport used at the BBB. One of those strategies is the glucose-mediated drug delivery which will be the focus of the present review. Glucose-mediated drug delivery requires the attachment of glycosyl moieties to a drug and the use of endogenous glucose transporters as a way to circumvent the blood-brain barrier. Glycosylated drugs display improved cell penetrability, enhanced biodistribution, stability and low toxicity. Examples such as glycosylation of ibuprofen and different opioids result in an enhanced central effect and will be discussed.

Keywords: Blood-brain barrier, central nervous system, drug delivery, glucose derivatives, glucose transporter, glycosylation.

INTRODUCTION

Along the years, biological chemists in both academia and industry have made an effort to find ways to selectively overcome the blood-brain barrier hurdle. It is estimated that only 5% of the drugs can cross the BBB and reach the central nervous system [1]. This is one of the major obstacles in fighting neurodegenerative diseases and pain, for instance, and the reason why only few novel molecular entities to fight pain have entered the clinic in the past 50 years [2]. Glucose-mediated drug delivery to the central nervous system is one of the promising research areas for medicine in the near future. This is the first review completely dedicated to this up-to-date theme, where new important developments on glucose-mediated drug delivery are highlighted. First, we will discuss the systems used to evaluate drug penetrability through the BBB, and several approaches to deliver drugs to the CNS. This will be followed by a revision regarding glucose transporters, the drugs' glycosilation process, and finally examples of glucose derivatives to treat several CNS diseases and pain will be presented.

CNS-DELIVERY OF GLYCOSYLATED DRUGS

BBB is permeable only to small molecules which may enter the brain by passive diffusion between cells (paracellular hydrophilic diffusion) or through cells (transcellular lipophilic diffusion). Other molecules can enter the brain crossing two membranes in series, the luminal and abluminal, through a carrier (ex. polar metabolites like glucose, amino acids, small peptides), a receptor (ex. insulin, transferrin) or an adsorptive mediated endocytosis (ex. histone, cationized albumin) [3, 4]. The efflux transport system removes substances, like small lipophilic substances [5], from the brain back to the circulatory system (ex. P-glycoprotein transporter), Fig. (**1**).

For a substance to reach the brain without a specific transport system it has to be generally lipophilic and of small size to diffuse through the BBB. It may seem simple at first glance but most therapeutic drugs do not meet these characteristics. In addition, metabolically unstable substances are rapidly degraded by enzymes before reaching the cerebrospinal fluid (CSF) protecting the brain, and there is no guarantee that once reaching CSF the drugs will not be exported back to the blood stream [6].

At present, there are different types of strategies to deliver drugs with different properties and sizes to the CNS. One of these strategies is the glycosylation of drugs which can benefit of a carrier-mediated transport: the endogenous glucose transporter. The glucose requirements of the brain are approximately 30% of the total body glucose consumption [7] and are held by a high density of hexose transporters. Considering this, significant efforts have been made to modify drugs in order to make them a substrate for these transporters (see section *Glycosylation* and *Glucose derivatives/drug conjugates to treat CNS diseases*). Glycosylated derivatives compared to non-glycosylated drugs have an increased BBB permeability only in cells expressing glucose transporter 1 - GLUT1 (in adults GLUT1 is present in the plasma membrane of erythrocytes and mainly in vascular endothelial cells, particularly BBB and astrocytes). The affinity between glycosylated drugs and GLUT1 transporter avoids non-specific tissue binding leading to an increase of the pharmacologically active portion in the brain [8, 9]. Moreover, the number of successful glycosylated derivatives with same therapeutic efficiency as the parental drug is privileged [10, 11].

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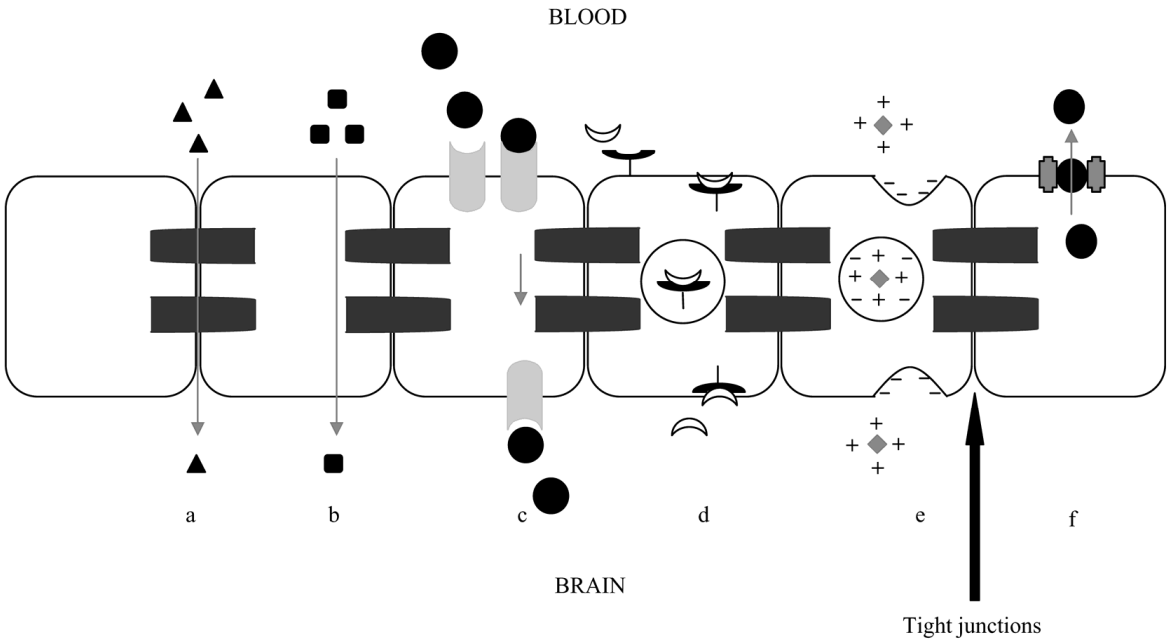

Fig. (1). The six different types of transport between blood and brain. **a**) Paracellular diffusion; **b**) Transcellular diffusion; **c**) Carrier-mediated transport (e.g. glucose transporter 1); **d**) Receptor-mediated transport; **e**) Adsorptive-mediated transport; **f**) Efflux transport.

An approach where the drug is transformed to be able to cross BBB, and is afterwards converted to its active parent compound *in vivo* by either a chemical or an enzymatic reaction is named prodrug approach [12]. The objective is that the synthesized prodrug is capable of entering the CNS by virtue of its chemical properties exerting the therapeutic effect of the parent compound. Since many CNS active drugs are unable to enter CNS, several prodrugs are designed to exploit BBB transporters responsible for the uptake of nutrients. One of the best examples are glycosylated drugs [1].

A brain-targeting prodrug that uses an endogenous transport system faces three challenging steps in its delivery: 1) compete with endogenous substrates and, possibly, metabolites; 2) low capacity transporters are only able to efficiently transport drugs that are at extremely low plasma concentrations; 3) the attachment of a drug molecule to a carrier moiety which may itself be an optimal substrate for a transporter does not ensure that the conjugate will remain a good substrate, particularly if the drug is a large molecule [12]. In addition, a slow bioconversion of the prodrug to drug may be responsible for the lack of an *in vivo* activity. This problem may be surpassed through the synthesis of prodrugs with modified linkages that may undergo faster enzyme catalyzed hydrolysis. The glucose transporter is a high capacity transporter used efficiently by substrates, so problems with prodrugs using these transporters as mediators are usually related to possible endogenous competitors and the stereochemical requirements for transport by these carriers [12, 13].

GLUCOSE: THE BRAIN FUEL

The glucose is the obligate energetic fuel of the mammalian brain and the only substrate able to completely sustain neural activity [14]. The maintenance of a relatively constant concentration of glucose in the bloodstream to sustain cerebral metabolism and the delivery of glucose to peripheral tissues for storage and utilization involves key metabolic processes and, in many situations, glucose transport across cell membranes plays a key role [15].

Tight junctions of the brain capillary endothelial cells block blood-borne glucose from crossing between cells into brain extracellular space. Glucose, as a polar hydrophilic compound, does not sufficiently diffuse into or out of the intact monolayer of the brain capillary endothelial cells (BCEC). To maintain the brain's high rate of aerobic metabolism and neuronal homeostasis, the highly regulated transport of glucose must occur through a selective, facilitated transport [16]. The transport of glucose and other hexoses into most mammalian cells is carried by the solute carrier family 2 (SLC2), formed by 13 transport proteins: the GLUT family 1 to 12 and the myo-inositol transporter (HMIT) family. All of the glucose transporters, except for HMIT which is a proton-driven transporter, are facilitative transporters, i.e. they mediate "energy-independent" transport leading to glucose equilibrium by cells. By doing this, they promote a bi-directional transport influencing the presence of intracellular and extracellular glucose [15].

Regarding glucose metabolism, the enzyme hexokinase, abundant in neurons and astrocytes, is required in glycolysis to catalyze the conversion of glucose to glucose-6-phosphate (G6P). Under normal conditions, the rate of brain glycolysis is limited by the phosphosphorylation of glucose to G6P rather than by transport, since hexokinase has a low K_m for glucose (approximately 40 μM) whereas the glucose levels in healthy human brain are between 1 to 2 mM [17]. According to the results from an *in vitro* model, the location and concentration of hexokinase can alter the ratio of

abluminal:luminal GLUT1 transporter [18]. Increasing amounts of G6P inhibits allosterically hexokinase resulting in an increase of the intracellular glucose concentrations. In this study, hexokinase was located closer to the abluminal than to the luminal membrane, which in part determined the concentration gradient of glucose from the luminal to the abluminal side. The concentration of brain hexokinase is largely correlated to local glucose utilization (2 to 3% of glucose is metabolized in the BBB endothelium in normal conditions) and is a crucial component of the glucose transport [17, 18].

Conditions that affect glucose metabolism, blood flow, plasma glucose concentrations, or the integrity of membrane tight junctions have an effect on GLUT1 expression. Under normal conditions (~5 mM plasma glucose levels [19]), there is a relatively stable brain:blood glucose concentration ratio. In unusual conditions such as neurodegenerative diseases, these parameters may be altered. In those cases, the BBB glucose transport can be the rate-limiting step in brain metabolism, leaving neurons and astrocytes vulnerable to glucose deprivation and disease.

There are also genetically inherited diseases related to glucose impairment. In children, the brain glucose requirement is three to four times higher than in adults, equivalent to up to 80% of total body glucose use [20]. Moreover, the ability of the brain to utilize alternative energy is limited; under fasting conditions it can metabolize ketone bodies but not fat. Consequently, impaired glucose supply in children will significantly affect the development and function of the brain, as in the case of GLUT1 deficiency syndrome (GLUT1DS, OMIM 606777). Defective glucose transport across the BBB was firstly described in 1991, by De Vivo and co-workers, in children with infantile seizures, developmental delay, acquired microcephaly, an unexplained low glucose concentration in the CSF termed hypoglycorrhachia, and a reduced CSF/blood glucose ratio [21]. Fortunately, this disease is treatable with an effective form of a high-fat, ketogenic diet [22, 23].

GLUCOSE TRANSPORTER FAMILY: GLUT1 AND GLUT3

The predominant transporters in the brain involved in cerebral glucose use are GLUT1 and GLUT3 which are ubiquitously expressed in the brain and whose characteristics are well established.

GLUT1 is the first GLUT protein identified and one of the most characterized membrane transporters as it is very abundant in the plasma membrane of erythrocytes. It is widely distributed in fetal tissues, but has a more restricted expression in the adult where, apart from the erythrocyte, it is found mainly in vascular endothelial cells, particularly BBB, and astrocytes. The GLUT1 gene has been mapped to chromosome 1 (1p35.31.3) [24]. In brain, GLUT1 has two different molecular weight isoforms encoded by the same gene. They have a different extent of glycosylation but seem to share the same structure and kinetics [25]. The 55 kDa form is present in the luminal and abluminal membranes in the BCEC of the BBB. The 45 kDa form is found in all glial cells, it is present in small amounts in the basolateral and apical membranes of the choroid plexus and ependyma, and also in neurons [14].

It appears that intracellular transporters are recruited to the luminal or abluminal membrane based on metabolic demand. Approximately 40% of GLUT1 located in the normal human BBB is sequestered within the cytoplasm at any given instance. The luminal:abluminal ratio of GLUT1 transporters ranges from 1:3–4 [26, 27], to 0.85:1 (*in vitro*) [18], to 2:1 [28], and sometimes is symmetrical [29]. The broad range of ratios is hypothetically a mechanism for the change in glucose transport rate at the BBB, thus an increased luminal:abluminal transporter ratio should favor an increment in the glucose uptake to the brain [17]. The amino acid sequence of the human GLUT1 was determined and suggested the presence of 12 transmembrane domains, a feature shared by most members of the major facilitator superfamily [30]. Current experimental evidence suggests that the transmembrane domains 1, 2, 4, 5, 7, 8, 10, and 11 form an inner helical bundle that comprises a water-accessible cavity within the membrane [31]. This inner bundle forms substrate–binding sites near the centre of the bilayer. Helices 3, 6, 9 and 12 are outer helices predicted to encircle and stabilize the inner helical bundle [32], being helices 6 and 12 the most hydrophobic of all transmembrane helices. Mueckler and Makepeace [33] produced point mutations in which each residue along helix 6 was changed to cysteine and the mutants were expressed in the oocytes of *Xenopus laevis* frogs. Unlike previous data on helix 12, in which none of the cysteine substitutions exhibited reduced transport activity [31], cysteine substitutions in 6 residues of helix 6 inhibited or abolished the transport activity. The results suggested that helix 6 plays an active role in the transport mechanism [33]. Most of these suggestions derived from observations using frogs as animal models. Consequently, there is little detailed information about the kinetics of glucose uptake in different mammalian cell types. However, there is a good correlation between the general properties of the GLUT transporters derived from expression studies in frogs with the ones from mammalian cell types.

According to the GLUT1 model proposed by Mueckler & Makepeace [33], the inner pocket of transmembrane helices possesses many hydrogen bond-forming amino acid side chains capable of forming the sugar-binding sites of GLUT1 through the interaction with glucose hydroxyl groups [30]. Furthermore, it has been previously shown that dopamine glucose derivatives that were conjugated at position 6 had the best affinity for GLUT1 compared to conjugates linked to carbon 1 or 3 of glucose [34]. Therefore, the hydroxyl group at the carbon 6 of glucose is likely the most potential functional group to which the drug molecule can be attached in order to maintain the affinity of the glucose conjugate for the GLUT1 transporter.

GLUT1 is transcriptionally regulated by the hypoxia-inducible factor 1 (HIF1). Upstream regulators of the HIF1α subunit include basic fibroblast growth factor and tumor necrosis factor [35]. Other growth factors and proteins such as tight junction proteins (occludin, zonula occludens-1 and claudins) are related to changes that occur in hypoxia or ischemia and affect GLUT1 expression, whereas the vascular endothelial growth factor can regulate GLUT1,

independently of HIF1 [36, 37]. Other proteins that may affect the expression of GLUT1 are the astrocyte transporters [38] and the neuronal transporters [39]. Polyunsaturated fatty acids [40] and sodium dependent glucose co-transporter (SGLT) [41] also have this effect. While GLUT1 is recognized as the main glucose transporter at the BBB, other transporters from a different family also contribute to the transport of energy across the BBB, such as the SGLT located on the luminal side of the brain endothelial cells [41], and the monocarboxylate transporter 1 (MCT1) predominantly located in endothelial cells but also in astrocytes and neurons. MCT1 transports alternate brain energy sources such as acetoacetate, pyruvate, and lactate [42].

GLUT3 has been detected almost exclusively in neurons [43, 44]. In addition, all cell types that exhibit high rates of glucose metabolism (placenta, platelets, and sperm) express this glucose transporter [14]. The neuronal maturation is coincident with an increased expression of GLUT3 which precedes the expression of the GLUT1 45 kDa form [14, 45]. In the human brain the concentration of the GLUT1 45 kDa form is similar to the one of GLUT3, 6.9-7.7 pmol/mg protein and 8-11 pmol/mg protein, respectively, while the amount of the GLUT1 55 kDa form is lower ranging from 2.3 to 2.5 pmol/mg protein [46].

The level of the expression of the main glucose transporters in the brain is regulated in concert with metabolic demand, oxygen tension, mitochondrial function, adenosine triphosphate (ATP) levels and regional rates of cerebral glucose use [47]. That is why the distribution and expression of these transporters may be altered in conditions of energy deprivation. As an example it has been shown that in conditions of hypoglycemia, neurons and astrocytes in some parts of the brain can express different types of glucose transporters (e.g., SGLT) that they do not otherwise [17]. Under pathophysiological conditions, the expression of these transporters may also be altered. In Alzheimer's disease (AD), for instance, the concentrations of GLUT1 (mainly the 55 kDa form) and GLUT3 are significantly reduced presumably secondarily to the underlying pathogenic processes [48, 49]. Moreover, in epilepsy, the decrease of the glucose transport results from a lower expression of GLUT1 at the BCEC and a decreased glucose uptake. Chronic hypoglycemia and cerebral hypoxia or ischemia also leads to an increase (the former one) or to a decrease (the latter ones) in glucose transport [13, 14]. However, Duelli [50] reported an increase in rat GLUT3 but not in GLUT1 under hypoglycemic conditions. Diabetes type I may lead to an increase or decrease of GLUT1. Overall, the effect on GLUT1 expression associated with some neurological disorders is still in debate [17].

GLYCOSYLATION

The attachment of glycosyl moieties to peptides influences the biodistribution, increases BBB permeability [10], enhances metabolic stability [51], and reduces clearance [52]. These properties contribute to the improved analgesic effects shown by glycosylated opioids [53]. Moreover, glycosylated drugs may be highly useful in conditions such as chronic pain or depression where prolonged administration of drugs is required [10] given that glycosylated derivatives exhibit an increased serum half-life Fig. (**2**) [54].

The field of glycobiology has grown exponentially, and the interest in the development of new methods to synthesize glucose derivatives is rapidly expanding. Glycopeptides can be produced once the required amino acid glycoside is available. Currently, there are different methods to produce *N*-linked and *O*-linked glycosylated amino acids [55, 56]. Mostly, N-linked and O-linked glycopeptides are synthesized in solution or in solid-phase. Most of the early glycopeptide synthesis was performed in solution-phase

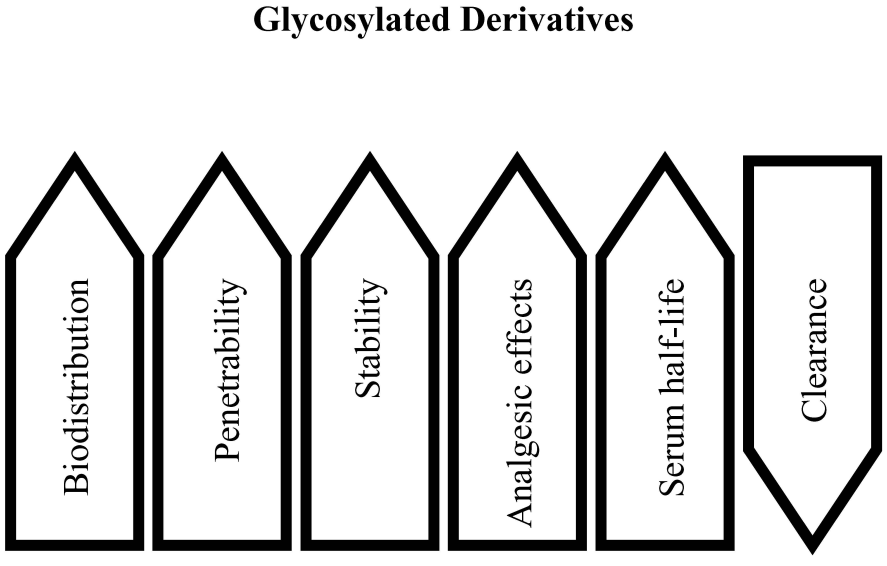

Fig. (2). Glycosylation strategy yields drugs derivatives with improved properties in comparison to non-glycosylated drugs. Glycosylated drugs show an enhanced biodistribution in tissues and organs, improved cell permeability, namely in endothelial cells from BBB, higher stability, superior analgesic effects, an increased serum half-life, and reduced clearance.

synthesis, because it was believed that coupling reactions with large amounts of the glycosyl aminoacids would be low-yielding. This type of synthesis is efficient, economic and leads to the production of small to medium sized oligomers. However, the repetitive isolation of the intermediates renders this method rather slow [55, 56].

Solid-phase synthesis use large excess of the building blocks which may drive peptide couplings to completion and leads to higher yields of glycopeptides. In contrast to solution-phase synthesis, the repetitive process may be automated offering the possibility to implement parallel or combinatorial synthesis and to produce various glycopeptides in a high-speed [55, 56].

Glycosylation may regulate both the structure of a protein (ex. the attachment of a multiple glycan residues may force the peptide backbone to an extended conformation), but does not modify the native function of aminoacids.

Both *O*- and *N*-glycosidic linkages are naturally occurring glycosidic linkages, where the carbohydrate is attached to the protein in a co-translational or post-translational modification [55, 56]. Enhancing the carbohydrate content of proteins can increase *in vivo* potency through increasing serum half-life [57]. Thus, in theory, both linkages provide glycopeptides that are stable with plasma proteins, and are both candidates for brain delivery. Another relevant question concerns the probability of generating neutralizing antibodies by altering the peptide sequence through glycosylation. This can be minimized by the properties of the carbohydrate itself such as antibody repulsion, an increased solubility and reduced aggregation [57].

Despite the advances mentioned above, glycopeptide synthesis is still far less developed than peptide synthesis, largely because glycopeptides are more difficult to synthesize than simple peptides [58]. Glycosylation has been used to produce compounds which penetrate the BBB and some glucose derivatives have been synthesized with success. Neoglycopeptides (non-physiological glycopeptides, such as glycosylated enkephalin analogs) have been extensively explored because their synthesis is easier than natural *N*-linked and *O*-linked glycopeptides [59]. Many neoglycopeptides show greater analgesic potency than morphine, a pharmaceutical agent which penetrates BBB by passive diffusion, and reduced liability when injected in mice [53, 60].

Table 1. Diseases Affecting CNS and the Respective Glycosylated Compounds Already Tested

Diseases affecting CNS	Glycosylated compounds
Alzheimer	Tetrahydrosalen compounds (ion chelating antioxidant molecules) Ibuprofen
Parkinson	Dopamine
Epilepsy	7-Chlorokynurenic acid
HIV	Indinavir, saquinavir, and nelfinavir
Pain/Analgesia	Different opioids

GLUCOSE DERIVATIVES/CONJUGATES TO TREAT CNS DISEASES

This section will focus on some important glucose-mediated drug delivery to the CNS. Most of following studies have been performed recurring to chemical synthesis of modified compounds whose activity was verified by *in vitro* cell culture and *in vivo* experimentation (Table **1**).

The chemical structures of the most commonly used sugars for glucose-mediated drug delivery are displayed in Fig. (**3**).

1. Alzheimer's Disease

The progressive loss of neuronal population, protein aggregation and extensive oxidative stress characteristics of AD are probably caused by a dysfunctional interaction of metal ions with the amyloid–β peptide (Aβ) [61-63]. It was demonstrated *in vitro* that Aβ interacting with metal ions at pH 7.4 leads to aggregation, with Zn^{2+} accelerating more rapidly the deposition process, compared to Cu^{2+} and Fe^{3+} [64]. This interaction leads to the production of H_2O_2 in the presence of O_2 [65], and probably explains the high Cu^{2+} and Zn^{2+} ions concentration in amyloid deposits, recognized as "metallic sinks" [66]. Thus, the disruption of these interactions between metal and peptide through a chelation therapy is an interesting therapeutic strategy. Pursuing this strategic idea, five multifunctional metal ion chelating antioxidant molecules (tetrahydrosalen compounds), namely *N*,*N*'-bis(2-hydroxybenzyl)ethane-1,2-diamine, *N*,*N*'-bis(2-hydroxybenzyl)-(1*R*,2*R*)-cyclohexane-1,2-diamine, *N*,*N*'-bis (2-hydroxybenzyl)-*N*,*N*'-dimethylethane-1,2-diamine, *N*,*N*'-dibenzyl-*N*,*N*'-bis(2-hydroxybenzyl)ethane-1,2-diamine, and *N*,*N*'-bis(4-*tert*-butyl-2-hydroxybenzyl)ethane-1,2-diamine were glycosylated, Fig. (**3**). Glycosylated drugs were chosen for the chelation therapy because glycosylation should prevent systemic metal binding, improve solubility, and enhance BBB crossing [67]. These prodrugs had their primary functions (metal binding and antioxidant activity) masked but once the glycosidic group was removed, the free drugs were able to perform the desired functions in solution [67]. Overall, the authors observed that all these prodrugs have the potential to act as multifunctional therapeutics for Alzheimer's disease in particular the third prodrug which was non toxic *in vitro*.

Considering AD prophylaxis there are a considerable number of studies, published in the last 15 years, oriented to the use of ibuprofen, a non-steroidal anti-inflammatory drug, on the prevention of AD. An epidemiological research revealed that long-term use of ibuprofen is protective against Alzheimer disease, and that protective effect increased with duration of use [68]. Taking this discovery into account, glycosyl derivatives of ibuprofen were synthesized in order to overcome the low permeability of the BBB [69]. Ibuprofen was linked to the C-2, C-3, C-4, and C-6 positions of glucose, Fig. (**3**). The derivatives of ibuprofen crossed the BBB reaching a concentration in the brain three-fold higher than non-derivatives, and its concentration in the brain was

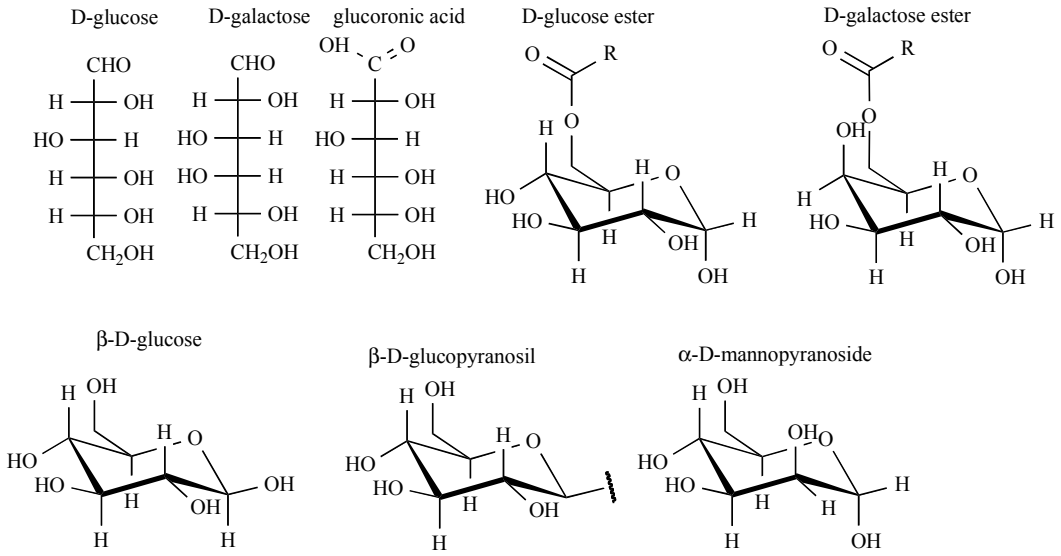

Fig. (3). Most frequently sugars used in glycosylation for drug delivery to the central nervous system: D-glucose, D-galactose, Glucoronic acid, D-glucose-ester, D-galactose-ester, β-D-glucose, β-D-glucopyranosil, α-D-mannopyranoside.

stable for four hours. Thus, these derivatives may potentially be used in the prevention and treatment of AD [69].

2. Parkinson Disease

Parkinson disease is characterized by a reduced concentration of the neurotransmitter dopamine which is unable to cross BBB due to its hydrophilic nature and the absence of active transport mechanism, being rapidly degraded in the endothelial cell through the action of monoamine oxidase. Its precursor 3,4-dihydroxyphenyl-l-alanine (L-Dopa) enters the CNS through active transport and is enzymatically decarboxylated in the brain to give dopamine. However, L-Dopa has low water solubility, is sensitive to chemical and enzymatic oxidation, and suffers peripheral decarboxylation [70].

Several glycosyl derivatives of dopamine, in which the neurotransmitter is linked to the sugar, have been synthesized as antiparkinsonian agents. These compounds presented the amino group of dopamine linked to the C-6 or C-1(β) position of glucose through a succinyl linker, while in others the sugar was attached to the phenolic groups of dopamine through a glycosidic bond. However, none of these compounds exhibited antiparkinsonian properties *in vivo* [34]. Later, Bonina *et al*. [71] evaluated the potential of novel glycosyl derivatives of dopamine and L-Dopa. These two were linked to C-3 position of glucose and to C-6 of galactose, Fig. (**3**), by a succinyl linker. The compounds linked through C-3 glucose position were more active in reversing reserpine-induced hypolocomotion in rats (reserpine model of Parkinson's disease), with minimal vascular effects. These results allowed the authors to highlight the possibility to use them in Parkinson disease therapy [71].

Several studies to deliver dopamine to the CNS were carried using GLUT1. It was shown that only compounds with the dopamine residue linked at position C-6 of glucose, Fig. (**3**), exhibited high affinity for GLUT1 [34, 72]. Recently, it was verified that the C-6 substituted compound, carbamate, did not penetrate erythrocytes cell membranes, whereas other glycosyl derivatives with slight structural improvements in the substituent linked to glucose (ether bond instead of carbamate bond, lack of two phenolic hydroxyl groups, and phenolic groups protected) crossed cell membranes. However, this passage was not mediated by GLUT1 [73].

3. Epilepsy

N-methyl-d-aspartate receptors (NMDA) are members of the glutamate receptor channel superfamily, which mediate most of the fast excitory synaptic transmissions in the CNS. The NMDA receptor is highly permeable to calcium ions and plays a key role in the plasticity of synapses. Abnormal activation has been suggested to lead to neuronal cell death observed in many acute and chronic disorders such as ischemia, stroke, Alzheimer's disease, and Huntingdon's disease [74].

7-Chlorokynurenic acid (7ClKynA) and its precursor kynurenic acid (KynA) are antagonists at the glycine site of the NMDA receptor, being the former more potent and selective as an antagonist. Due to their acidic properties and their inefficient crossing across the BBB, their use as neuroprotective agents is limited. Battaglia *et al*., [75] synthesized D-glucose or D-galactose esters, Fig. (**3**), of 7ClKynA as prodrugs for experimental therapy of epilepsy and neurodegenerative disorders. The approach of sterifying water-soluble molecules with glucose proved to be more advantageous than with D-galactose. The authors showed that D-glucose conjugates of 7ClKynA are protective against NMDA- induced seizures, and the conjugate linked to the 6-hydroxyl group of D-glucose revealed an enhanced BBB crossing and generated 7ClKynA and KynA [75]. Moreover, the transport rate depends on the glucose level. In conditions of hypoglycemia, for instance, GLUT1 will be nearly free to transport the prodrug which may protect neurons against the excitotoxic degeneration associated with hypoglycemic coma

[76]. The therapy of acute and chronic neurodegenerative disorders may potentially beneficiate with these types of prodrugs based on sugar conjugates that are amino acid receptor antagonists [75].

Animal studies have long suggested that BBB glucose transporter activity may be up-regulated some hours after seizures caused by epilepsy. Gronlund *et al*. [77] showed significant increases in GLUT3 8–72 hour after treatment to induce seizures, while GLUT1 was not significantly increased until 72 hour post-treatment. However, Cornford *et al*. [78] found that BBB glucose transporter activity is acutely up-regulated by 30–40% within a span of only 3 minutes. This rapid response is an indicator of how urgently the BBB GLUT1 transporter recognizes the sudden requirement for additional glucose during the seizures caused by epilepsy [78], and results in useful information for the synthesis of the anti-epilepsy drugs described previously.

4. Human Immunodeficiency Virus (HIV)

A common complication of HIV infection is the neurocognitive impairment, ranging from minor cognitive motor disorder to HIV dementia [79]. Unfortunately, the development of appropriate interventional therapies to prevent or treat neurocognitive impairment has been hampered by the relatively poor understanding on how the antiretroviral agents modulate the early neuropathogenic events that lead to neurocognitive impairment [80]. It is accepted that a perivascular inflammation and subsequent BBB breakdown may, by accelerating the rate of viral entry into CNS and enabling the entry of potentially toxic serum proteins, represent a key step in the pathogenesis of neurocognitive impairment [81]. Presently, antiviral drugs do not penetrate the CNS at an efficient inhibitory level or do not penetrate at all. Examples include the protease inhibitors indinavir, saquinavir and nelfinavir.

Confronted with this failure, Rouquayrol *et al*. [82] designed and performed the synthesis of various prodrugs derived from these three protease inhibitors. The drugs were conjugated to amino acids, D-glucose, and to the hydrophilic polymer polyethylene glycol (PEG) with the aim to select the most promising prodrugs that could improve intestinal and brain absorption. The screening was done with Caco-2 monolayers that express efflux carrier systems responsible for the low brain penetration and oral bioavailability of the protease inhibitors. While conjugation with L-tyrosine decreased transport and in some cases increased hydrolysis, conjugation of the protease inhibitors with L-valine, L-leucine and L-phenylalanine (through their carboxyl) improved the translocation across cells and reduced the recognition by efflux carriers. Insertion of the hydrophobic PEG decreased the solubility and passive diffusion across cells. D-glucose conjugates were linked to the 3-hydroxyl group which preserves the recognition and transport capability by the GLUT1. However, the results demonstrated that D-glucose, if recognized by the transporter, was not efficiently transported and/or was substrate of the efflux carrier systems [82]. Probably, the conjugation of D-glucose increased the recognition of the protease inhibitors by efflux carriers. In conclusion, only L-valine, L-leucine and L-phenilalanine amino acid conjugation proved to work as enhancer of transepithelial delivery of protease inhibitors [82].

Horvat and co-workers followed a different strategy. They synthesized Leu5- and Met5-enkephalin glycoconjugates attached by an ether, ester, amide, and N-alkyl bond to a carbohydrate moiety. These glycoconjugates exhibited slight antiviral activity against HIV-1 and owned their viral activity possibly to the glycopeptides' stability and high cell permeability [83, 84]. More experiments are needed to determine the viability of these drug candidates.

5. Nociceptive Pain

Endogenous Opioids

The endogenous opioid peptides have been subject of intensive studies since their discovery in the decade of 1970. They have important roles from cognitive to sensorial functions. Recently, a review centered on glycosylated analgesic neuropeptides was published by Polt *et al*. [59] where theories on how glycosylated opioids interact with cell membranes and cross membrane barriers are also discussed.

Endogenous opioid peptides, like enkephalins, endorphins and dynorphins bind to opioid receptors. There are three primary opioid receptor types in the CNS that mediate analgesia: μ, κ, and δ. Preferentially, enkephalins interact with the δ receptor, dynorphins interact with the κ receptor, and endorphins bind to both μ and δ receptors with similar affinity. These peptides are involved in antinociception. More recently, two short peptides, endomorphin-1 and endomorphin-2, were also identified. They display a high affinity for μ opioid receptors and produce prolonged analgesia in animals. In addition, the opioid-receptor-like 1 (ORL1) was discovered. Its natural ligand is nociceptin or orphanin FQ which appears to be involved in the central modulation of pain [85].

Enkephalins are pentapeptides involved in the regulating of nociception and they have two sequences, one contains leucine and the other methionine. They do not cross BBB at any significant extent and are rapidly destroyed by peptidases in the bloodstream [86]. Therefore, they have poor bioavailability in tissues and organs, similarly to all endogenous peptides. One of the first studies on opioid glycopeptides was carried by Garcia-Anton group [87]. They synthesized a glycosylpeptide, Fig. (**3**), resulting from the covalent bonding of a sugar residue to the C-terminal carboxyl group of an enkephalin related pentapeptide: Tyr-D-Met-Gly-Phe-Pro [N1,5-beta-D-glucopyranosyl] amide. This neo-glycopeptide exhibited an antinociceptive activity 2,000 and 200 times higher than morphine in rats and mice models, respectively, after intraperitoneal administration. Afterwards, the same group added glucose and galactose, Fig. (**3**), through an *O*-linkage producing the first *O*-linked glycopeptides. The antinociceptive activity of one galactoside enkephalinamide analogue (O1,5-[β-D-galactopyranosyl][D-Met2,Hyp5]enkephalinamide) was 57,000 times more potent than morphine in a rat model [88]. Furthermore, there was a clear difference between the analgesic activity of the galactosyl analogue and glycosyl analogue, suggesting that the introduction of a galactose

molecule produces a compound more effective in the control of nociception [88].

Later, Toth and co-workers added glucoronic acid, Fig. (**3**), to enkephalins at the C-terminal synthesizing C-terminal glycosyl enkephalin amide analogs [89, 90]. The authors wanted to test if the glucoronidated derivatives of enkephalin were more effective. It was confirmed that Leu-enkephalin derivatives attached to glucoronic acids had an even increased activity, improved affinity to δ-opioid receptor, and some showed significant BBB permeability *in vitro* [90, 91].

Polt and co-workers [92] focused on the cyclized met-enkephalin analogue, [d-Cys-2,5] enkephalin. The authors introduced Ser β-D-glucose, Fig. (**3**), at various structural positions of the peptide forming l-serinyl β-D-glucoside analogues of [Met5]enkephalin (glycopeptides) and investigated receptor binding and analgesia. With these results, they demonstrated for the first time that the strategy of linking glucose to a brain targeting drug could be useful not only to treat neurogenenerative disorders but also to induce analgesia without the need to administer the drug intracranially. The addition of the glyco group within the cyclized region of the peptide led to large decrease in binding to opioid receptors, while the addition of the glyco group at position six of the drug led to minor changes in receptor binding and a significant increase in analgesia following peripheral administration. The improved analgesia was due to an increased metabolic stability and delivery to the brain, potentially through a glucose transporter [92]. This study highlighted the importance of the glycosylation position within the molecule on the biological activity of the drug. Placement of the Ser β-D-glucose outside of the active region at position 6, i.e. glycosylation at the Ser6, did not affect the active region of the peptide which is critically important to maintain its efficacy [92, 93]. Since these initial studies on glycosylated enkephalin opioids, a great number of opioid analogues have been tested, the most promising of which were based on linear Leu-enkephalin analogues [53]. Egleton *et al*. [53] compared the bioavailability of the Leu-enkephalin Tyr-D-Thr-Gly-Phe-Leu-Ser-NH_2 and its analogue glycosylated at Ser6 to the brain, and conclude that the β-glycosylated analogue had 2-fold improved BBB permeability and enhanced analgesia. The improved analgesia of glycosylated analogues can be accounted for both improved BBB transport and increased half-life within the serum (metabolically and possibly clearance). The increased bioavailability means that more peptide is available to cross the BBB *via* the improved transport mechanism. These studies have therefore shown that glycosylation may be a useful tool for delivering small peptides to the CNS. However, before a clinically viable candidate is available, more research is required [10].

Non-Endogenous Opioids

Non-endogenous opioids like deltorphin and dermorphin have been subject to structure modifications for an improved analgesia. Both deltorphin and dermorphin are heptapeptides isolated from frog skin and are highly selective ligands to μ or δ opioid receptors, respectively [94]. Tomatis *et al*. [95] prepared opioid deltorphin (H-Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH_2) and dermorphin (H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2) analogues in which a D-glucopyranosyl moiety, Fig. (**3**), was β-*O*-glycosidically linked to a Thr4 or Thr7 side chain. They demonstrated that Thr4-glycosylated peptides displayed low opioid properties, while Thr7-glycosylated peptides retained high μ or δ opioid receptors selectivity, and remarkable *in vivo* activity. Particularly, Tyr7-glycosylated analogues of deltorphin and dermorphin were more potent than the parent unglycosylated peptide counterparts as systemic antinociceptive agents, showing a high influx from blood to brain possibly due to GLUT1 glucose transporter [95].

According to Polt *et al*. [92], GLUT1 transporter for glycosylated enkephalins shows a selective preference for the β-*O*-links and three times more affinity for glucose than for galactose. However, these two premises were not observed by Negri *et al*. [96], who synthesized deltorphin and dermorphin analogues β-*O*-glycosylated and α-*C*-galactosylated on the C terminal amino acid residue (7^{th} residue). They found that the analogues yielded higher analgesic potency than their parent compounds as predicted by the studies of Tomatis *et al*. [95]. The authors also observed that [α-Gal]-deltorphin and [α-Gal]-dermorphin were more analgesic than [β-Glc]-deltorphin and [β-Glc]-dermorphin, respectively, even when they exhibited comparable or even lower μ and δ receptor affinity. Thus, galactosylation of deltorphin and dermorphin through α-*C*-link yielded analogues with more antinociceptive activity than the β-*O*-glycosylated compounds. In a previous study, Mulder [97] had already suggested that *C*-glycosylated peptide analogues have a higher resistance to proteolysis by tissue peptidases than *O*-glycosylated peptide analogues, and consequently this type of modification increases the peptide half-life. The higher analgesia observed for *C*-glycosylated opioids which was not related to a proportional opioid receptor affinity can be explained by the existence of a different carrier mediated transport systems for opioid peptides, particularly for deltorphin analogues which were more potent than dermorphin [96].

In clinical practice the analgesic opioids most commonly used bind selectively to the μ receptor and are called μ agonists. Morphine is considered the prototypical μ agonist. Although there are many similarities between morphine and the other μ agonists, the different drugs can produce varied effects in the individual patient which suggests that there are different μ receptors (at least two) [85]. When administered, morphine is primarily glucuronidated into two compounds excreted by the kidneys: 90% is converted in the inactive morphine-3-*O*-glucuronide (M3G) and only 10-15% into the active morphine-6-*O*-glucuronide (M6G) [97]. M6G has a high analgesic potency and is actively transported through BBB by GLUT1 and by a digoxin sensitive transporter, which could be the organic anion transporting polypeptide 2. The active M6G could be transported from the blood to brain or *vice versa* by these two transporters according to its concentration gradient, but with a weak transport capacity considering its very low brain uptake [98]. Recently, Arsequell *et al*. [99] provided new insights concerning morphine derivatives. The authors synthesized more potent M6G analogues replacing the glucuronide part of the

molecule by a mannose moiety namely, 6-morphinyl-α-d-mannopyranoside (M6Man), Fig. (**3**). When administered intraperitoneally in rats, the antinociceptive action of the compound was 100-fold higher and twice as long lasting as morphine. The M6Man is a μ opioid receptor ligand with twice the affinity of morphine, although the receptor binding selectivity was not fully examined.

Several studies have demonstrated that glucose conjugates can bind to GLUT1 but none of these studies proved that these derivatives indeed cross the BBB *via* GLUT1 [33, 34]. In addition, two interesting works revealed some important aspects concerning glucose derivatives of enkephalin, anti-inflammatory drugs and transport across BBB. Palian *et al*., [100] suggested that the incorporation of hydrophilic carbohydrate moieties into enkephalin analogues renders them amphipathic, promoting exchange between lipid and aqueous phases. This would promote the capability of the glycopeptides to reversibly insert into lipid phases allowing the membrane-mediated transport across the BBB through adsorptive endocytosis. Recently, Gynther and co-workers [101] studied two model compounds, ketoprofen and indomethacin to show that GLUT1 can be utilized to carry prodrugs by conjugating a drug molecule to D-glucose with bioreversible linkage. These two hydrophilic drugs, ketoprofen, (2*S*)-2-(3-benzoylphenyl)propionic acid and indomethacin, which are non-steroidal anti-inflammatory drugs with analgesic and antipyretic effects, were attached to the hydroxyl group of D-glucose at the carbon 6 to synthesize ketoprofen-glucose and indomethacin-glucose prodrugs [101]. The authors showed that both prodrugs crossed the BBB in a temperature-dependent manner, suggesting that their brain uptake is GLUT1 mediated. The prodrugs also significantly inhibited the uptake of glucose by GLUT1, indicating the existence of an affinity to the transporter.

The group of Vandelli has been focused on developing polymeric nanoparticles as carriers for brain delivery. They developed a biodegradable polymeric poly(lactic-*co*-glycolic acid) (PLGA) nanoparticle modified with a similopioid peptide (NH2-Gly-L-Phe-D-Thr-Gly-L-Phe-L-Leu-L-Ser(O-β-D-Glucose)-COOH which was able to deliver model drugs, such as Loperamiden, within the brain after intravenous administration in animal models [102-104]

Recently they conjugated PLGA with sialic acid derivative forming nanoparticles with the surface decorated with the derivative. Sialic acid may be recognized by specific receptors in the brain allowing nanoparticles to be retained for a prolonged period of time in the brain parenchyma [105].

Compared to other nanoparticle systems (ex. nano-particles conjugated with polysorbate 80-coated polybutyl-cyanoacrylate [106]), PLGA nanoparticles associated with Loperamiden enhanced the opioid activity of this drug. Its central opioid activity increased for 24h probably due to the interaction between sialic acid residues and brain specific receptors leading to a prolonged activity and long lasting residency in the brain. With this strategy the authors provided a new approach to develop prolonged treatments in brain pathologies [105].

CONCLUSIONS

The currently available brain targeting drugs for the treatment of depression, epilepsy, multiple sclerosis, neurodegenerative disorders, pain, and schizophrenia have molecular weights lower than 500 Da and are lipophilic, as required for BBB permeation. Thus, they do not require any secondary mechanism of permeation to the CNS delivery [107]. With the discovery of biological mechanisms underlying neurological disorders along with the advances in drug discovery, new drugs potentially more active became available. The drawbacks associated with alternative methods to deliver drugs that do not have the requirements for BBB permeation are: 1) not all delivering approaches are suitable for a certain drug, and 2) even when the strategy is adequate, the drug may present a low affinity for the receptor, low brain distribution and low bioavailability. In the case of glucose-mediated drug delivery there are several recognized weaknesses. The first concerns to the limitations of glucose as a pro-moiety. The structure of glucose limits the amount of drugs that can be linked with biodegradable bonds to glucose. In general, drugs that bear a carboxyl acid group are more easily linked with glucose without the need of a linker or a spacer. In addition, the stability of prodrugs in systemic circulation might not be adequate for clinical use for drug brain targeting [101]. Another limitation is that GLUT1 is very stringent in its stereochemical requirements, forming highly stereospecific pores and tolerating only a minimum of molecular substitutions on the parent hexose. Thus, GLUT1 will not transport a pseudo-substrate much different to the endogenous substrate [13]. Possibly, this fact is one of the reasons for the reduced number of available glucose derivative drugs that can cross BBB through GLUT1.

It is also of significant importance continuing to identify changes in the brain glucose transport, particularly when different therapeutics are considered. These changes may be induced by or may be a consequence of serious neuropatho-logies. Their knowledge is a way towards the development of effective glucose-mediated drugs. Therefore, alternative methods to deliver drugs to brain demand further development about the specific disease mechanisms, the transporter requirements, and new strategies for the design of safer and active drugs.

CONFLICT OF INTEREST

Declared none.

ACKNOWLEDGEMENTS

We thank Fundação para a Ciência e a Tecnologia, Portugal, for the grants SFRH/BPD/37998/2007 to Isa Serrano, SFRH/BD/42158/2007 to Marta Ribeiro, and Project PTDC/SAU-FCF/69493/2006. Marie Curie Industry-Academia Partnerships and Pathways (European Commission) is also acknowledged for funding (FP7-PEOPLE-2007-3-1-IAPP, project 230654). The authors declare not to have any potential conflict of interests.

ABBREVIATIONS

CNS = Central nervous system

BBB = blood-brain barrier

CSF = cerebrospinal fluid

GLUT1 = glucose transporter 1

BCEC = brain capillary endothelial cells

SLC2 = solute carrier family 2

HMIT = myo-inositol transporter

G6P = glucose-6-phosphate

HIF1 = hypoxia-inducible factor 1

SGLT = sodium dependent glucose co-transporter

MCT1 = monocarboxylate transporter 1

ATP = adenosine triphosphate

AD = Alzheimer's disease

Aβ = amyloid–β peptide

L-Dopa = 3,4-dihydroxyphenyl-l-alanine

NMDA = N-methyl-d-aspartate receptors

7ClKynA = 7-Chlorokynurenic acid

KynA = kynurenic acid

HIV = human immunodeficiency virus

PEG = polyethylene glycol

ORL1 = opioid-receptor-like 1

M3G = morphine-3-O-glucuronide

M6G = morphine-6-O-glucuronide

M6Man = 6-morphinyl-α-d-mannopyranoside

PLGA = poly(lactic-*co*-glycolic acid)

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Received: April 18, 2011 Revised: October 11, 2011 Accepted: October 15, 2011