DRUG DISPOSITION

Clin Pharmacokinet 2001; 40 (2): 105-123
0312-5963/01/0002-0105/$22.00/0

The Development of the Bradykinin Agonist Labradimil as a Means to Increase the Permeability of the Blood-Brain Barrier

From Concept to Clinical Evaluation

Dwaine F. Emerich,[1] *Reginald L. Dean,*[1] *Chester Osborn*[2] and *Raymond T. Bartus*[1,3]

1 Alkermes, Inc., Cambridge, Massachusetts, USA
2 Department of Pharmacy and Experimental Therapeutics, Tufts University Medical School, Boston, Massachusetts, USA
3 Alkermes Europe, Ltd., Cambridge, England

Contents

Abstract

Labradimil (Cereport®; also formerly referred to as RMP-7) is a 9-amino-acid peptide designed for selectivity for the bradykinin B_2 receptor and a longer plasma half-life than bradykinin. It has been developed to increase the permeability of the blood-brain barrier (BBB) and is the first compound with selective bradykinin B_2 receptor agonist properties to progress from concept design through to tests of efficacy in patients.

In vitro studies demonstrate that labradimil has a longer half-life than bradykinin and selectively binds to bradykinin B_2 receptors, initiating typical bradykinin-like second messenger systems, including increases in intracellular calcium and phosphatidylinositol turnover. Initial proof of principle studies using electron micros-

copy demonstrated that intravenous labradimil increases the permeability of the BBB by disengaging the tight junctions of the endothelial cells that comprise the BBB. Autoradiographic studies in rat models further demonstrated that labradimil increases the permeability of the BBB in gliomas. Intravenous or intra-arterial labradimil increases the uptake of many different radiolabelled tracers and chemotherapeutic agents into the tumour in a dose-related fashion. These effects are selective for the tumour and for the brain surrounding the tumour, and are particularly robust in tumour areas that are normally relatively impermeable. The increased chemotherapeutic concentrations are maintained for at least 90 minutes, well beyond the transient effects on the BBB.

The increase in permeability with labradimil occurs rapidly but is transient, in that restoration of the BBB occurs very rapidly (2 to 5 minutes) following cessation of infusion. Even with continuous infusion of labradimil, spontaneous restoration of the barrier begins to occur within 10 to 20 minutes. Collectively, these data demonstrate that the B_2 receptor system that modulates permeability of the BBB is highly sensitive and autoregulated and that careful attention to the timing of labradimil and the chemotherapeutic agent is important to achieve maximal effects.

Survival studies in rodent models of both gliomas and metastatic tumours in the brain demonstrate that the enhanced uptake observed with the combination of labradimil and water-soluble chemotherapeutics enhances survival to a greater extent than achieved with chemotherapy alone. Finally, preliminary clinical trials in patients with gliomas provide confirmatory evidence that labradimil permeabilises the blood-brain tumour barrier and might, therefore, be used to increase delivery of agents such as carboplatin to tumours without the toxicity typically associated with dose escalation.

1. The Blood-Brain Barrier as an Impediment to Drug Delivery to the Central Nervous System

The blood-brain barrier (BBB) prevents most hydrophilic substances greater than 400D from gaining access to the central nervous system (CNS) from the cerebral vasculature. Anatomically, the BBB is composed of endothelial cells bound together to form junctions. Compared with the endothelial cells forming capillaries in peripheral (non-CNS) organs, brain endothelial cells have numerous astrocytic processes, mitochondria, tight junctional complexes between opposing cells, and a paucity of fenestrae and pinocytotic vesicles. All of these characteristics contribute to the ability of the BBB to restrict diffusion of blood-borne substances into the brain.[1-3]

By combining low passive permeability for most hydrophilic molecules together with highly selective transport for certain essential molecules (e.g. glucose, amino acids), the BBB provides an exquisite system to regulate the internal chemical environment of the CNS. While this system may protect the brain by maintaining a controlled environment, it also creates a barrier for both researchers and clinicians who wish to introduce drugs to the brain parenchyma.[4]

Because only a fraction of all bioactive drugs possess the attributes required to penetrate the BBB, the treatment of CNS diseases could be improved if a means were available to safely and reversibly modulate the permeability of the BBB to allow greater drug distribution to the CNS. One novel approach explored over the past 2 decades involves the administration of endogenous ligands or their analogues to increase permeability of the BBB by activ-

ation of receptors on the endothelial cells comprising the BBB. Brain endothelial cells possess numerous neurotransmitter and peptide receptors on both their luminal and abluminal membrane surfaces.[5,6] These receptors are coupled to traditional second messenger systems involving calcium fluxes, G proteins and various kinases and phospholipases. Several of these endogenous ligands (e.g. adenosine, arachidonic acid, leukotrienes, histamine and bradykinin) have been shown to increase the permeability of the BBB when administered systemically.[7]

Although the available data with endogenous ligands provide preliminary proof of principle data, they do not convincingly demonstrate that a receptor-mediated approach to BBB modulation may prove medically practical. The use of endogenous ligands is generally compromised by both the high concentrations of ligand required to increase BBB permeability as well as adverse physiological effects that can be severe enough to damage brain capillaries.[8] One method developed to minimise these problems involves infusion of the endogenous nonapeptide, bradykinin, directly into the internal carotid artery.[9,10] Although this approach has enjoyed some success in animal studies, the very short half-life of bradykinin, its potent vasoactive metabolites[11-13] and its narrow therapeutic index[8,9] limit its safety and usefulness, require that it be infused into the carotid artery, and, generally, make it a difficult and potentially dangerous candidate for widespread clinical use. For this reason, the selective B_2 bradykinin agonist labradimil (Cereport®, RMP-7) was developed (for earlier reviews see Bartus,[4] Grous et al.[14] and Boddy and Thomas[15]).

This review discusses some of the attributes thought to be important in developing a bradykinin agonist and the data collected during its early testing. Additionally, the preclinical data supporting the effectiveness of labradimil as a bradykinin agonist is reviewed, as are the currently available clinical results.

2. Developing a Bradykinin Agonist to Increase Permeability of the Blood-Brain Barrier

2.1 Selectivity for the B_2 Receptor

Bradykinin is distributed ubiquitously in the body where it serves both paracrine and autocrine functions. For these reasons, any attempt to modulate a specific physiological response with a systematically administered bradykinin agonist (such as increasing the permeability of the BBB) must do so in the face of competing endogenous bradykinin, both at the intended site (e.g. endothelial cells of brain capillaries), as well as at numerous additional sites throughout the body. Moreover, at least 2 distinct types of bradykinin receptors have been characterised[16] and cloned.[17,18]

The bradykinin B_2 receptor is constitutively expressed on the endothelial cells of brain capillaries[19,20] where its activation of capillary receptors induces a potent vasogenic response.[21-23] In contrast, the B_1 receptor is induced under conditions associated with disease or biological stress.[24,25] Thus, the B_2 receptor is a logical target for a drug intended to permeabilise the BBB. Selective B_2 properties might avoid some of the inflammatory and nociceptive responses associated with injury and disease, where induction of the B_1 receptor is most prevalent.[26]

With these considerations in mind, labradimil was designed as a nonapeptide bradykinin analogue. A number of rationally defined substitutions and modifications were made to confer greater selectivity for the B_2 receptor (fig. 1).[27] Because the cleavage of Arg–9 in bradykinin converts the endogenous peptide from primarily a B_2 agonist to primarily a B_1 agonist,[28] labradimil was designed with a reduced peptide bond inserted between the 8 and 9 positions of the amino acid sequence, thus protecting Arg–9 from proteolytic removal.[27] Additionally, the substitution of a hydroxyproline in position 3 was used to enhance affinity for the B_2 receptor.[29,30] A series of *in vitro* tests confirmed that this goal was achieved (see below).

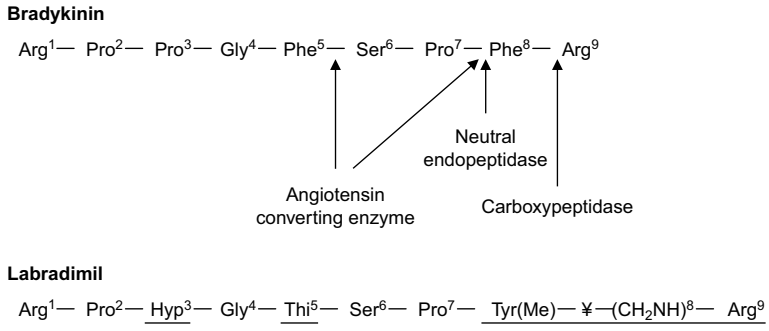

Fig. 1. Chemical structures of bradykinin and labradimil, a synthetic B_2 bradykinin agonist. Bradykinin is rapidly degraded by several enzymes at the sites illustrated. A reduced peptide bond was inserted between the 8 and 9 position of the amino acid sequence to prevent the cleavage of Arg–9, thereby reducing the conversion of the peptide from primarily a B_2 agonist to a B_1 agonist. An additional substitution of a hydroxyproline (Hyp) residue at position 3 enhanced the affinity of the peptide for the B_2 receptor. Finally, the half-life of the peptide was increased by substituting the unnatural (2-thienyl)-Ala (Thi) for Phe in position 5 and substituting methyl-Tyr for Phe in position 8. These changes (underlined) give labradimil increased B_2 receptor selectivity and a longer half-life.

Receptor binding assays demonstrated that labradimil retains affinity for bradykinin receptors on cultured rat brain microvascular endothelial cells,[20] guinea-pig ileum[31] and rat uterus.[31] As summarised in table I, although both labradimil and bradykinin bind to these receptors in a concentration-dependent manner, labradimil does so with a somewhat lower affinity than bradykinin. *In vitro* receptor binding tests further revealed that labradimil did not displace radiolabelled peptide ligands involved with receptor systems commonly associated with vasoactive effects, including angiotensin-II (types I and II), histamine (H_1 and H_2), neurokinin, neurotensin and vasopressin, even at concentrations as high as 100 μmol/L.[31] Thus, labradimil binds selectively to bradykinin B_2 receptors.

To confirm that labradimil acts as a B_2 agonist, increases in intracellular free calcium (Ca^{2+}) were measured using brain endothelial cells exposed to a concentration gradient of labradimil.[20] A biphasic increase in cytosolic free Ca^{2+} was observed, with a concentration gradient similar to that for bradykinin-induced Ca^{2+} signals in other cell systems. These data were replicated and extended using epithelial Schwan (SH-EP) cells, where the calcium fluxes induced by labradimil were blocked by a selective B_2 antagonist but not by a B_1 antagonist.[31] Labradimil also increased phosphatidylinositol turnover (another second messenger associated with bradykinin) and this response was also blocked by a selective B_2 antagonist (table I).[31] Collectively, these results indicate that labradimil acts as a selective bradykinin B_2 receptor agonist.

2.2 Increased Half-Life

Bradykinin degrades very quickly, and its circulating half-life is only several seconds.[32] For this reason, it is not practical for use as a systemic ther-

Table I. Biochemical and pharmacological events: comparison of labradimil with bradykinin

Property	Bradykinin	Labradimil
B_2 receptor binding (IC_{50}) [nmol/L]		
Cultured rat cerebral microvessels	5-10	50-100
Guinea-pig ileum	0.6	80
Rat uterus	2.5	8.5
Signal transduction (EC_{50}) [nmol/L]		
Phosphatidylinositol turnover	EC_{50}	30-50
Ca^{2+} stimulation	EC_{50}	30-50
Blood-brain barrier permeability (K_i) [μg/kg]		
Brain tumour uptake (IA)	150	1.5
Brain tumour uptake (IV)	Unknown	4.5-9

EC_{50} = 50% effective concentration; **IA** = intra-arterial; **IC_{50}** = 50% inhibitory concentration; **IV** = intravenous; **K_i** = unidirectional transfer constant.

apeutic. Bradykinin is degraded by several different proteases (see fig. 1), which are located in highest concentration in the cell membranes near bradykinin receptors. For example, bradykinin is degraded into inactive peptide fragments by proteolytic cleavage of either the peptide bond between Phe–5 and Ser–6, or between Pro–7 and Phe–8. Labradimil was designed to resist this degradation by substituting the unnatural (2-thienyl)-Ala for Phe in the 5 position and substituting a methyltyrosine derivative for Phe in the 7 position.[27,33]

Studies in rat and human plasma indicate that labradimil has a half-life significantly longer than that of bradykinin, although technical limitations currently make definitive quantification impossible.[33] It is likely for this reason that, despite its lower affinity for the B_2 receptor (relative to bradykinin), labradimil has equivalent potency to bradykinin in cell-based second messenger system assays and is even more potent than bradykinin *in vivo* (see table I).

The structural changes in labradimil also substantially alter the primary pathways involved in the proteolytic degradation of labradimil. The major degradation of labradimil occurs from the amino-terminus, beginning with Arg–1 (Alkermes, unpublished observations), rather than either the carboxyl terminus or the internal peptide bonds favoured by kininase II (or ACE) for bradykinin.

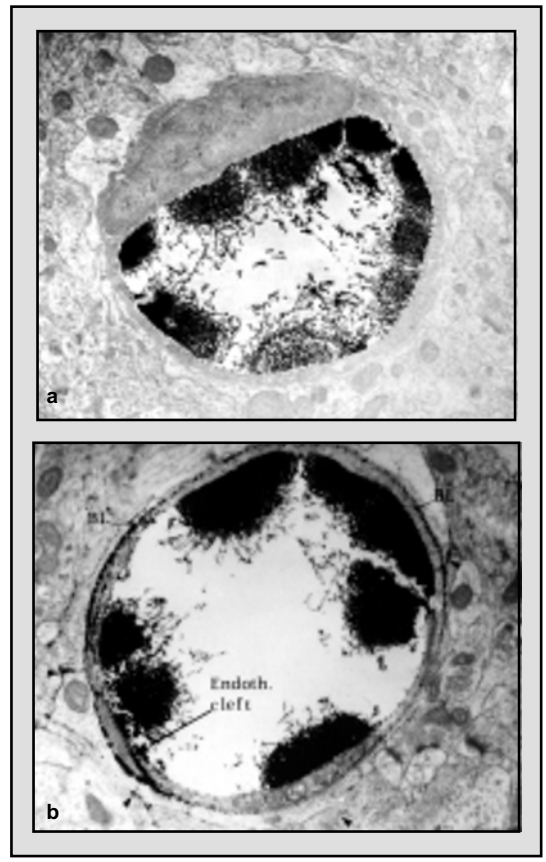

Fig. 2. Electron micrographs of cerebral vessels from nontumour-bearing mice injected intravenously with the electron dense marker lanthanum and either vehicle (**top**) or labradimil 5 μg/kg (**bottom**). In vehicle-treated animals, the lanthanum remained within the vessel lumen and was unable to pass into the surrounding perivascular spaces. In contrast, when labradimil was administered with lanthanum, a clear pathway was opened from the vessel lumen, through the tight junctional complex (denoted as endothelial cleft) to the basilar lamina (BL) and into the perivascular spaces (arrows). Detailed quantitative morphological assessments determined that labradimil increased the permeability of the normal blood-brain barrier by loosening the tight junctional complexes, with no evidence for transcellular passage of lanthanum noted.[34]

2.3 Improved Therapeutic Index

An important issue to consider when developing a bradykinin analogue is the therapeutic index. Bradykinin induces a wide variety of physiological responses, ranging from relaxation of smooth muscle (e.g. vasculature), contraction of smooth muscle (e.g. intestine and uterus), mediation of pain (e.g. stimulation of C fibres in peripheral nervous system) and modulation of inflammation (both through a direct effect on localised tissue involving changes in blood flow, oedema, etc., as well as more indirectly involving the release of potent and longer lasting inflammatory mediators, such as prostaglandins, histamine and substance P). Since most of these diverse responses are induced by activation of the B_2 class of bradykinin receptor, it was critical to determine whether labradimil, as a bradykinin agonist, increases the permeability of the BBB without inducing serious, untoward, adverse effects.

The most conclusive 'proof of principle' evidence was achieved using electron microscopy to observe BBB endothelial tight junctions in mice administered the electron-dense marker lanthanum with or without intravenous labradimil.[34] When given alone, lanthanum permeated the tight junctional complex very poorly and did not pass into the brain parenchymal space. When coadministered with labradimil, however, substantially greater permeation of the tight junctional complex was achieved, with a significant increase in the number of vessels showing lanthanum in the abluminal basilar membrane and adjacent parenchymal spaces (fig. 2). Thus, this study not only demonstrated that labradimil could increase the permeability of the BBB, but demonstrated that it did so by disengaging the tight junctions between the endothelial cells comprising the BBB. In contrast to the clear evidence for paracellular diffusion of lanthanum across the BBB, no evidence was seen for any transcellular route. Most importantly, the increased permeability was not accompanied by any apparent damage to the vasculature of the brain or to the brain itself.[34]

Subsequent studies have demonstrated similarly increased permeability of the BBB within the same intravenous dose range, using quantitative autoradiographic methods,[35] as well as behavioural measurement.[36]

The most prevalent adverse effect with labradimil in animal studies is a decrease in blood pressure.[37] This hypotensive response is observed across species, is dose-related and consistent with a bradykinin mechanism of action.[37,38] Although mild hypotension and increased BBB permeability often coexist, the 2 phenomena have been empirically dissociated, demonstrating that hypotension is neither necessary nor sufficient to increase permeability of the BBB.[37,39] With the exception of an occasional and transient reflexive tachycardia (in response to the decrease in blood pressure) at higher doses of labradimil, no other consistent adverse effects have been observed in animals. Thus, labradimil can increase the permeability of the BBB while maintaining a favourable therapeutic index (see additional support in sections 2.4 and 2.5).

2.4 Utility in a Disease Model

2.4.1 Enhanced Uptake of Chemotherapeutics into Brain Tumour

Labradimil has been initially developed to increase the permeability of the vasculature supplying gliomas, thereby enhancing the delivery of concomitantly administered hydrophilic chemotherapeutics. To provide initial preclinical support for this concept and gain insight into the pharmacodynamic characteristics of enhanced vascular permeability, we developed a rodent model of glioma that shares several important characteristics with tumour vasculature in human glioma.[35,39,40] In this model, rat glioma cells (RG2) are implanted into the striatum of a syngeneic rat strain (Fischer 344). The tumours grow and form a well-defined mass within the striatum (fig. 3a). Immunocytochemical analysis of the vasculature infiltrating these tumours has demonstrated that, like that seen in human glioma,[42,43] the density of the infiltrating vasculature is decreased compared with healthy, nontumour vessels, but the individual vessels are frequently larger and considerably more tortuous as they course through the tumour[41] (figs 3b and 3c). Autoradiographic and scintillation studies[35,39,40,44] have further revealed that the vasculature infiltrating these tumours is 'leaky' relative to the nontumour barrier, again similar to that observed in human glioma (see section 2.4.2 for detailed discussion).

Finally, recent evidence indicates that the endothelial cells of blood vessels within human colorectal tumours express a high preponderance of genes commonly found in healthy endothelial vascular cells and those undergoing angiogenesis.[45] These data offer further evidence for the qualitative similarities between the vasculature across a range of tumours, raising the likelihood that the genetic similarity holds true across most, if not all, blood vessels, including those supplying gliomas in both humans and in rodent models. If true, the genetic, structural and permeability characteristics of the RG2 glioma would seem to provide a valid model

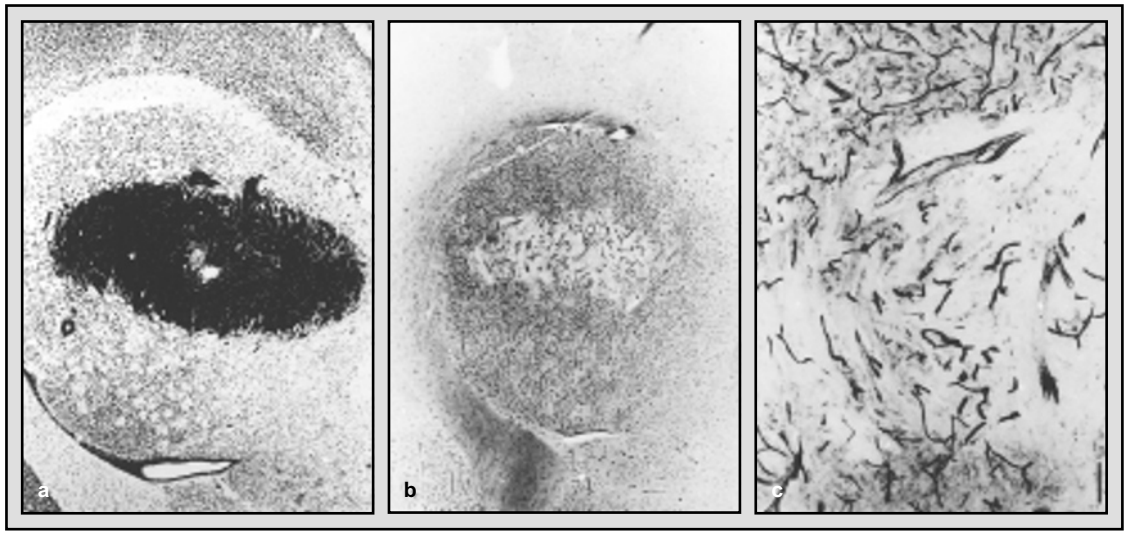

Fig. 3. Photomicrograph illustrating the formation of rat glioma (RG2) tumours in rats and the appearance of host blood vessels infiltrating those tumours. (**a**) shows a low-power photomicrograph of a Nissl-stained section through the striatum of a rat 8 days following implantation of 25 000 RG2 cells. Note the well-formed spherical appearance of the growing tumour mass; (**b**) shows an adjacent section stained for the endothelial cell marker CD31 illustrating the robust expression of CD31 immunoreactive blood vessels in the normal brain and within the tumour itself. (**c**) is a high-power micrograph illustrating the appearance of CD31 immunoreactive vessels around and within the tumour. These photomicrographs (**b** and **c**) highlight the similarities between the vasculature in this model and in human glioma. These similarities include the observation that relative to vessels within the normal brain, the vessels infiltrating the tumour are fewer in number, but are also frequently larger and are considerably more tortuous. The bar in **c** represents 500 μm in **a** and **b**, 100 μm in **c** (from Bartus et al.[41]).

for predicting the effects of labradimil, or any compound intended to modify the vasculature of tumours, in humans.

An extensive series of autoradiographic studies using the RG2 rat model of glioma demonstrated that both intravenous[31,35] and intracarotid[37,39,40] infusions of labradimil produce a dose-related increase in the permeability of the blood brain tumour barrier (BBTB) [fig. 4]. Although comparable uptake effects are achieved following both intravenous and intracarotid labradimil, as expected the doses required are approximately 2 to 5 times greater with intravenous administration (fig. 4).

Recent studies also suggest that the importance of the relative timing of the administration of the chemotherapeutic and labradimil may differ between intravenous and intracarotid infusion. For instance, [^{14}C]carboplatin uptake in tumours is maximally enhanced following intravenous labradimil when the timing of labradimil and [^{14}C]carboplatin infusions are adjusted to produce higher plasma concentrations of carboplatin prior to initiating the labradimil infusion.[44] In contrast, because intracarotid infusions elevate plasma concentrations of [^{14}C]carboplatin more rapidly at the target site (tumour vasculature), the need to precisely control the timing of the carboplatin and labradimil infusion is less important. Accordingly, intracarotid labradimil infusions do not require elevated concentrations of [^{14}C]carboplatin prior to initiating the labradimil infusion to be effective, and simultaneous infusions prove quite effective.[46] These discrepancies in optimal dose administration schedule between the 2 routes, and the shift in dose-response function, seem to easily account for the occasional failure to achieve significant effects with intravenous labradimil.[47]

Despite these dose administration differences, the effects of labradimil following intra-arterial and intravenous administration are quite similar,

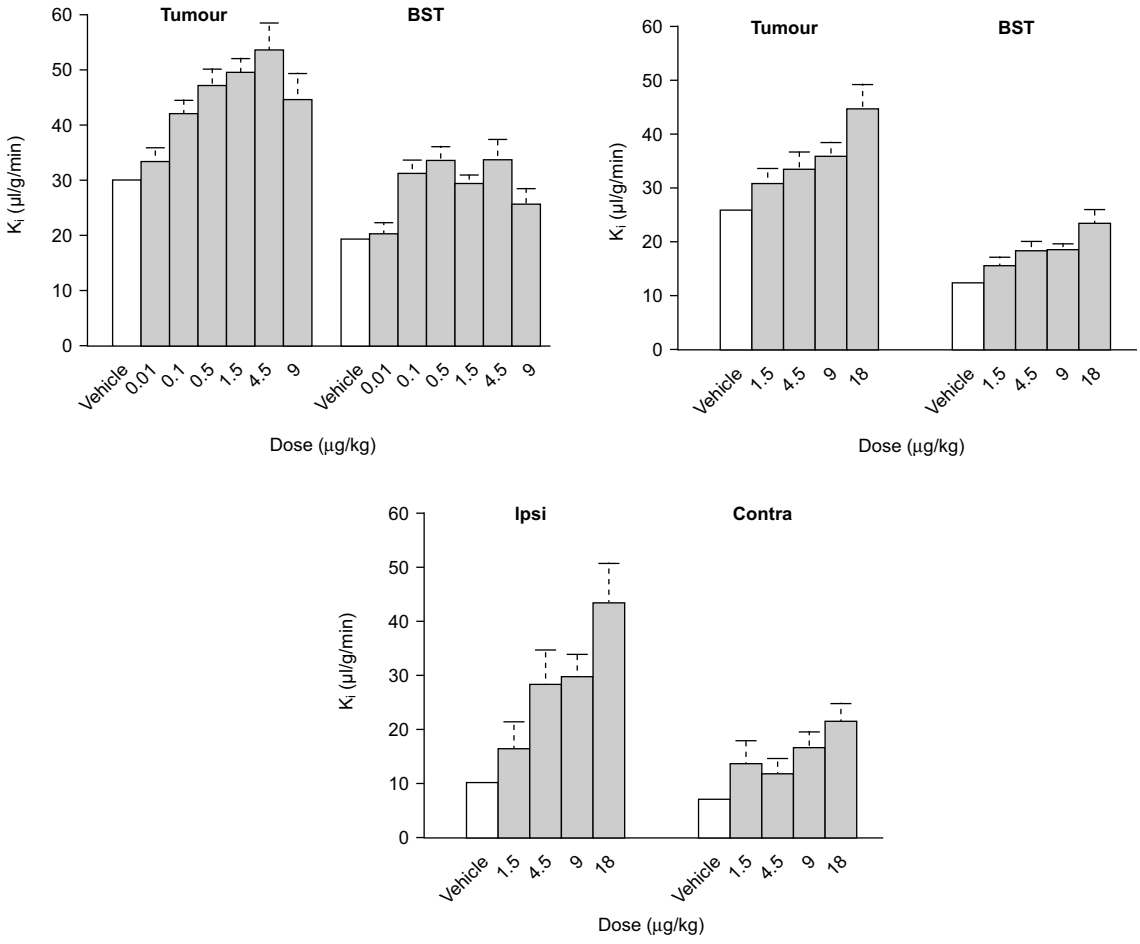

Fig. 4. Intracarotid (top left) and intravenous (top right) dose-response functions for labradimil, measuring uptake of [^{14}C]carboplatin [defined by the unidirectional transfer constant (K_i)] following a 15-minute labradimil infusion. The bottom panel illustrates the uptake of [^{14}C]carboplatin following intravenous labradimil into nontumour brain regions including the cortex ipsilateral and contralateral to the tumour. Note that a dose-related increase in [^{14}C]carboplatin uptake occurs in all regions following labradimil, but that the absolute magnitude of the effects of labradimil are markedly more robust within the tumour and the brain surrounding tumour (BST) than in the nontumour brain regions. Group sizes for top panel were 11 to 48, and 10 to 15 for the middle and bottom panels. Data are mean ± SEM (adapted from Elliott et al.,[37,40] with permission).

with both producing more robust and consistent effects in tumour and 'brain surrounding tumour' (BST, the area of brain immediately surrounding the tumour, in this case, a 1mm band extending around the tumour boundary), relative to nontumour brain. The enhanced uptake in BST is an important observation, since this is the area that tumour cells infiltrate, often escaping detection, surgery and therapeutic levels of chemotherapeutic.[48-50] Being able to increase the delivery of chemotherapeutics to the BST may therefore inhibit the recurrence of gliomas that typically occurs within weeks to months following surgical resection.[51]

In contrast to enhanced permeability in tumour and BST, the effects observed in healthy brain distal to tumour are relatively small and much less consistent. For example, over half of the experiments have failed to achieve statistically significant effects

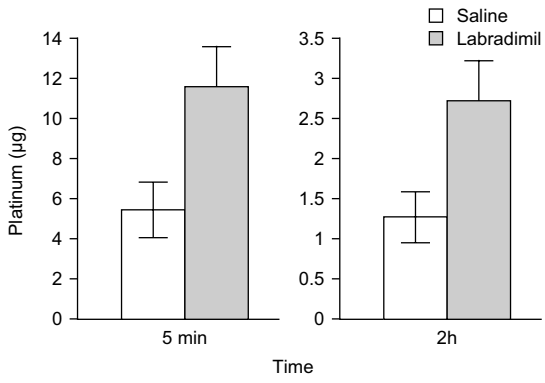

Fig. 5. Comparison of amount of platinum in tumour, as measured by atomic absorption spectrophotometry. Animals received a 15-minute intravenous infusion of carboplatin 10 µg/kg together with a 10-minute infusion of labradimil 9 µg/kg beginning 10 minutes after the initiation of the carboplatin infusion. The left bar of each panel compares the amounts of platinum achieved with labradimil versus the saline vehicle, measured immediately after the labradimil infusion was terminated (and, therefore, 5 minutes following the termination of the carboplatin infusion). The right bar of each panel compares the effects of labradimil versus vehicle either 5 minutes or 2 hours after the carboplatin infusion ended. Note that despite the drop in absolute carboplatin amounts in the tumour over time, the 2-fold increase in carboplatin achieved with labradimil persisted. Longer time-points were not evaluated (n = 12 per group). Data are mean ± SEM (adapted from Bartus et al.,[55] with permission).

in nontumour brain tissue,[4,39] and when an effect was observed, the change in unidirectional transfer constant (K_i) was typically only 3 to 4 µl/g/min (e.g. see fig. 4), an effect unlikely to have significant biological consequences in most cancer situations.

The increased permeability within tumour and BST occurs with many different radiolabelled tracers, traditional chemotherapeutic agents, boronated compounds and more novel biotechnology oncolytic agents. This provides a wide variety of agents for which labradimil might enhance delivery to tumours, ranging from small molecules[39,40,52] through cytokine macromolecules[53] to viral vectors for gene therapy.[54] Finally, studies using atomic absorption spectrophotometry confirmed that the increased uptake of radiolabelled carboplatin into brain tumours reflects an elevation in platinum concentrations, the active biological moiety (fig. 5). The increases in platinum concentrations achieved with labradimil persist for at least 2 hours, offering additional support for the potential therapeutic advantage of labradimil as a chemotherapeutic delivery agent for brain tumours.[55] Thus, labradimil exhibits selectivity for permeabilising the tumour and BST, offering support for its use as a means to increase delivery of chemotherapeutics and other therapeutic agents to brain tumours while exerting relatively little effect on healthy brain. More recently, similar increases in uptake of radiolabelled carboplatin have been seen in a model of brain metastases.[56]

Both autoradiography and scintillation studies demonstrate that labradimil produces a 2-fold or greater increase in [^{14}C]carboplatin uptake into brain tumours and BST. It seems apparent that the enhanced uptake achieved with labradimil in many ways is even greater when the effects are examined within specific microregions of the tumour and BST (fig. 6).[55] To enable this type of analysis, a method was developed that quantified levels of radioactivity within extremely small subregions of tumours and BST. This was accomplished by examining individual pixels (measuring 4.68µm^2) within individual autoradiographic images. By calculating the amount of radioactivity within each pixel and assigning that value to bins of progressively greater levels of radioactivity (e.g. 0 to 10, 11 to 20, 21 to 30 nCi/g, etc.), a profile of both the extent of permeability in the tumour and its point-to-point variability was constructed. Using this detailed, high spatial resolution analysis of the uptake within tumour and BST, it was revealed that the BBTB in this glioma model is highly heterogeneous, with some areas being relatively leaky and others being relatively impermeable (similar to that reported for human gliomas).

Interestingly, labradimil did not simply shift the distribution of [^{14}C]carboplatin uptake uniformly to produce the 2-fold increase in uptake, but rather significantly modified the topographic uptake profile within tumour and BST. For example, under labradimil, almost no portion of the tumour and BST remained impermeable to [^{14}C]carboplatin

and a greater proportion of highly permeable areas was also generated. Thus, the effect of labradimil on [^{14}C]carboplatin uptake is not manifested as merely an indiscriminate doubling of [^{14}C]carboplatin concentrations in the tumour and BST, for the shape of the uptake profile was clearly modified (fig. 7). In fact, in certain subareas of the tumour, the increased uptake achieved with labradimil can be estimated to be several-fold greater than that achieved with vehicle.

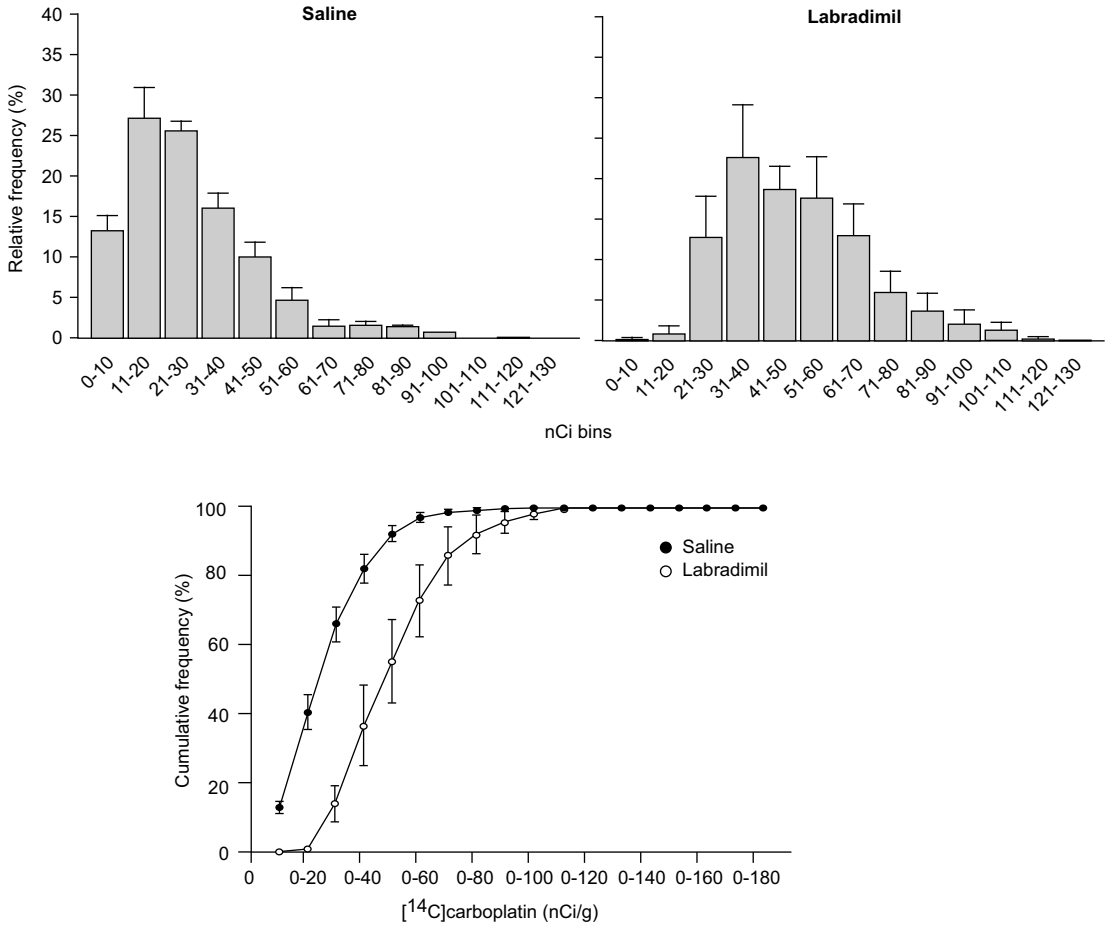

Fig. 6. High spatial resolution profile of [^{14}xC]carboplatin uptake within rat gliomas (RG2). Rats received overlapping infusions of [^{14}C]carboplatin and labradimil as described in figure 5. The horizontal axis defines pixel bins with progressively greater concentrations of [^{14}C]carboplatin. The vertical axis quantifies the proportion of pixels within the tumour that displayed the varying [^{14}C]carboplatin concentrations. Thus, the top graphs depict the proportion of tumour displaying varying degrees of [^{14}C]carboplatin under saline vehicle (**left**) versus labradimil (**right**). Each vertical bar along the axis represents progressively greater degrees of permeability (i.e. progressively greater amounts of [^{14}C]carboplatin). Note the heterogeneity in permeability displayed under both saline vehicle and labradimil conditions. Note also that labradimil both significantly reduced the proportion of tumour that was relatively impermeable (e.g. few pixels exist in the 0 to 20 nCi/g range with labradimil) and increased the proportion of tumour where [^{14}C]carboplatin concentrations were relatively high (e.g. considerably more pixels exist at 50 nCi/g and greater). The bottom graph presents the same data in the form of cumulative frequency (i.e. percentage of pixels within tumour containing increasingly greater amounts of [^{14}C]carboplatin), plotted as function of concentration of [^{14}C]carboplatin. Note the significant drop in the frequency of low-level pixels under labradimil, together with a substantially higher proportion of higher level pixels. Group sizes were vehicle = 7, labradimil = 9. Data are mean ± SEM (adapted from Bartus et al.,[55] with permission).

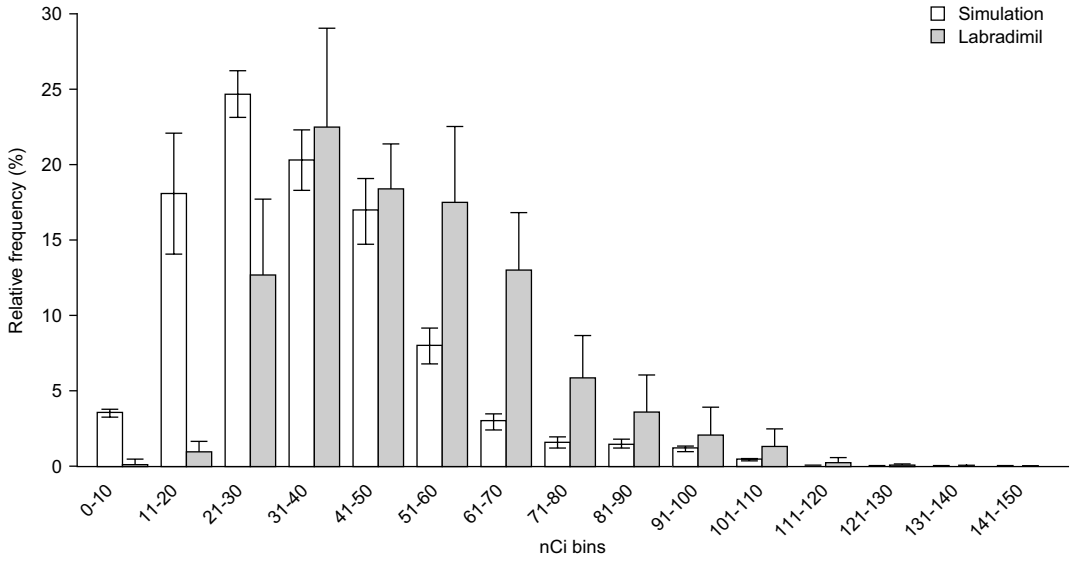

Fig. 7. Direct comparison of the high spatial resolution analysis of [^{14}C]carboplatin uptake into tumours achieved with intravenous labradimil versus a simulated uniform 130% increase in vehicle scores. The labradimil data is the same data presented in figure 6. The 130% increase was chosen because it reflects the mean overall difference in uptake between labradimil- and saline-treated animals. Note that the pattern of uptake is markedly different between the labradimil group and the simulated 130% increase in vehicle scores. These data demonstrate that labradimil does not indiscriminately double uptake throughout the entire tumour. Rather, the 2-fold increase in uptake achieved with labradimil is manifested as relatively selective increases in uptake in the more impermeable areas within the tumour (i.e. those with <20 nCi/g [^{14}C]carboplatin) as well as creating more highly permeable areas (i.e. >60 nCi/g). Data are mean ± SEM (adapted from Bartus et al.,[55] with permission).

2.4.2 Pharmacodynamic Characteristics of Permeability Changes

Several important observations have been made regarding the pharmacodynamic profile associated with the effects of labradimil on BBB permeability. First, the effects of labradimil occur rapidly, within minutes of initiation of the infusion. In a study using autoradiography, pre-exposing the carotid artery to labradimil for 5 minutes prior to infusing a radiolabel produced no greater uptake than simultaneously infusing the label and labradimil.[46] Accordingly, the increased permeability induced by labradimil must have occurred well within 5 minutes under these conditions; otherwise an advantage of the 5-minute pre-exposure period should have been seen. Similarly, using computerised tomography in irradiated dog revealed increased permeability within the first 5 minutes (the first post-infusion time point evaluated).[57]

The effects of labradimil on BBB permeability are not only initiated very quickly, but the barrier is also restored very quickly upon cessation of labradimil administration. For example, by comparing the uptake of label achieved during a short diffusion opportunity within the labradimil infusion, with that achieved following cessation of labradimil infusion, it was estimated that restoration of the barrier begins almost immediately upon termination of infusion (exclusion of the large 70kD dextran occurred immediately) and is essentially complete within 2 to 5 minutes (at which time even the small molecules, such as carboplatin, were excluded).[57]

Another intriguing aspect of the pharmacodynamic response to labradimil response is that even with continuous administration of labradimil, spontaneous restoration of the barrier (i.e. tachyphylaxis) occurs within several minutes. Early studies[31] with direct intracarotid infusion suggested a de-

crease in permeability beginning within 30 minutes of initiation of a continuous infusion. It was subsequently demonstrated that continuous infusions were not required to induce tachyphylaxis, for 2 infusions of 15 minutes, separated by a 15-minute interval, also produced complete tachyphylaxis.[31] These same studies demonstrated that when a 60-minute interval was inserted between two 15-minute infusions, the increase in permeability returned to approximately half of maximum. This phenomenon suggested that once the system was stimulated, an obligatory tachyphylaxis response was precipitated, requiring only the passage of time to manifest itself, and eventually resolving with the further passage of time. This concept of obligatory tachyphylaxis was further supported using sub-threshold infusions of labradimil. A 15-minute infusion of a low dose of labradimil (0.033 μg/kg/min) produced a very modest effect on permeability.[39] Lower doses produced correspondingly smaller, or no, effects. When the same concentrations were infused for 45 minutes, rather than achieving greater effects (caused by more drug being given for longer durations), no evidence of uptake was seen at any concentration. The most parsimonious explanation for the loss of threshold effects following the longer 45-minute infusion is that tachyphylaxis occurred, even though low, sub-threshold, doses were used.

More recent experiments have directly supported the obligatory tachyphylaxis hypothesis. Using both behavioural end-points with the BBB and scintillation end-points with the BBTB, it was shown that once sufficient labradimil is administered to briefly open the barrier, time and not further receptor stimulation was sufficient to induce tachyphylaxis.[58]

Subsequent studies with intravenous infusions of labradimil found that tachyphylaxis occurs more rapidly than with intracarotid infusions, that is to say, within 15 minutes,[44] presumably because the relatively lower concentrations of labradimil at the site of action (the cerebrovasculature) during intravenous administration were not sufficient to overcome the tachyphylaxis initiated at the beginning of the infusion. To support this supposition, when an initial, effective dose of labradimil was followed, under conditions when tachyphylaxis would normally occur, by an even higher concentration of labradimil, no evidence of tachyphylaxis was seen.[46] Presumably, stimulation of B_2 receptors with subsequently higher concentrations of ligand temporarily overcomes the tachyphylactic process.

These studies collectively demonstrate that the B_2 receptor system that labradimil stimulates to modulate permeability of the BBB is highly sensitive and autoregulated. It responds to low doses of agonist, inducing a rapid increase in permeability of the barrier. Moreover, the integrity of the barrier is quickly restored upon removal of the agonist (i.e. cessation of infusion). Even with continuous infusion of bradykinin or labradimil, spontaneous restoration of the barrier begins to occur within 10 to 20 minutes, with a return to baseline permeability occurring within 30 to 60 minutes. For these reasons, the precise timing of the administration of labradimil with that of the chemotherapeutic agent is very important. A series of controlled studies has confirmed that the optimal intravenous administration regimen involves administering the labradimil dose so that its infusion duration encompasses the timing of the maximum plasma drug concentration (C_{max}) of the chemotherapeutic agent.[44]

2.4.3 Enhanced Survival in Rodent Models of Glioma and Metastatic Brain Tumours

To determine if the enhanced uptake of carboplatin achieved with labradimil is biologically meaningful, we examined its ability to prolong survival when combined with chemotherapeutics in rodent models.[55] An initial preliminary survival study demonstrated that intra-arterial labradimil combined with carboplatin in an allogeneic rat strain significantly increased median survival over that achieved with carboplatin alone.[59]

Subsequent studies[55] more fully characterised the enhanced survival produced by intravenous labradimil, using a range of labradimil doses in syngeneic F344 rats. In these studies, carboplatin alone significantly enhanced survival, modestly increasing both median and maximum survival. A low dose (3.0 μg/kg) of labradimil, in combination

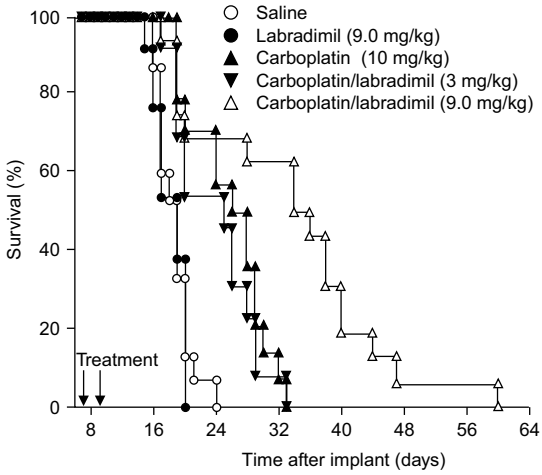

Fig. 8. Kaplan-Meier survival plots for rats implanted with rat gliomas (RG2) and treated 7 and 9 days later with various combinations of carboplatin, labradimil and/or saline vehicle using the dosage paradigm outlined in figure 5. Animals received a 15-minute intravenous infusion of carboplatin 10 mg/kg/treatment together with a 10-minute infusion of labradimil 3 or 9 μg/kg beginning 10 minutes after the initiation of the carboplatin infusion. Note that animals given either vehicle (n = 15) or labradimil (n = 13) show very similar survival functions. Those given carboplatin (n = 14) or carboplatin plus a low dose of labradimil (3 μg/kg; n = 13) survived significantly longer than vehicle, but were not different from each other. However, when carboplatin was combined with a higher dose of labradimil (9 μg/kg; n = 16), a significant increase in median and maximum survival was achieved, relative to the same dose of carboplatin only (adapted from Bartus et al.,[55] with permission).

with carboplatin, failed to further increase survival beyond that achieved with carboplatin alone. However, a higher dose (9.0 μg/kg) of labradimil produced a robust increase in survival, increasing median and maximum survival by 2-fold over that observed with carboplatin alone (fig. 8). Similar effects were seen in a model of brain metastatic disease when labradimil was combined independently with a range of different water-soluble chemotherapeutic agents (fig. 9).

The range of dose-related survival effects reported in these studies[55] provide insight into the plasma concentrations of labradimil required to achieve significant efficacy with carboplatin. Estimates of *in vivo* plasma concentrations of labradimil have been made in lieu of quantitative determinations because of serious limitations in current analytical methods. The enzyme-linked immunoadsorbent methods developed are limited in that the parent (labradimil) and several major metabolic products cannot be distinguished because of cross-reactivity. Additionally, the potency of labradimil (resulting in plasma concentrations in the low to mid nanomolar range) and short half-life (known to be less than 10 minutes and estimated to be less than 3 to 4 minutes) makes detection and quantification extremely difficult. When combined with the cross-reactivity limitation, current assays simply cannot provide quantitative *in vivo* pharmacokinetic data for labradimil. Using standard pharmacokinetic modelling techniques, the plasma concentrations of labradimil following the 10-minute infusion of 3.0 μg/kg were estimated to range from 8 nmol/L (2 minutes from the initiation of the infusion) to 16 nmol/L (at the end of the 10-minute infusion, at which point the concentrations peak).[55] In contrast, the 9.0 μg/kg dose was estimated to produce plasma concentrations that range from 25 to 50 nmol/L (using the same temporal points of reference).

These estimates are reasonably consistent with the K_i values established for labradimil (10 to 50 nmol/L), associated with binding to the B_2 bradykinin receptor and induction of second messenger responses, *in vitro*.[31] Using the same pharmacokinetic methods to model labradimil plasma concentrations in patients, it is estimated that doses in excess of 1500 ng/kg may be required to achieve similar effects of labradimil plus carboplatin in human gliomas.[55] Although these plasma concentrations are substantially higher than those estimated to occur following the 300 ng/kg dose used for most phase II clinical studies, additional dose escalation studies with labradimil have recently been completed in brain tumour patients (see section 3).

2.5 Toxicological Profile

Another important issue with developing any novel synthetic compound is its inherent toxicity. In the case of labradimil, the use of unnatural amino acids and abnormal, proteolytically protected,

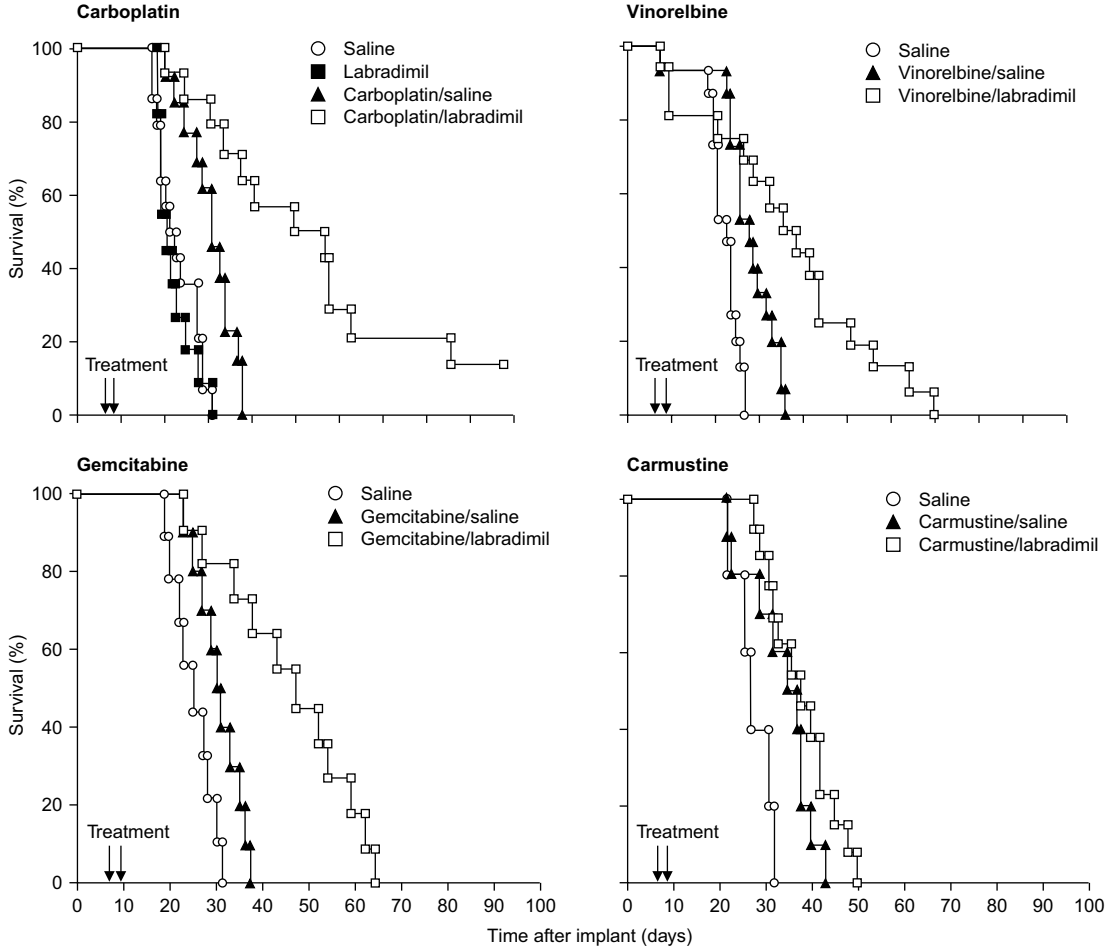

Fig. 9. Kaplan-Meier survival plots for rats implanted with MATB-III metastatic tumours in the brain. Using the same dosage paradigm described in figure 8, animals were treated 7 and 9 days after tumour implantation with various combinations of chemotherapy, labradimil and/or saline vehicle. In addition to testing carboplatin 10 mg/kg in this model, separate sets of animals received intravenous infusions of either the hydrophilic drugs vinorelbine 5 mg/kg or gemcitabine 15 mg/kg, or the lipophilic drug carmustine 2.5 mg/kg alone or in combination with labradimil. Note that labradimil significantly increased survival in the animals treated with carboplatin, vinorelbine or gemcitabine, relative to chemotherapy alone. Note also that carmustine did not benefit from being combined with labradimil. These data are consistent with the action of labradimil on the tight junctions of the blood-brain barrier, which provides a transient, aqueous pathway from the vasculature into the brain for hydrophilic, but not lipophilic, compounds (n = 10 to 16 for all groups) [from Emerich et al.[56]].

dipeptides produces some degree of uncertainty. However, following detailed metabolism studies, no evidence of accumulation of any abnormal peptide or peptide fragment has been observed (Alkermes, unpublished data). Additionally, extensive animal testing of labradimil, involving daily administration over weeks of high doses of labradimil alone, and months of periodic administration of combinations of labradimil plus carboplatin, has revealed no evidence of serious toxicity.[14,38]

An additional problem associated with a compound intended to increase delivery across the BBB is the potential neuropathology that might be induced, specifically by its action on the BBB. A still-

common concept of the BBB is that its primary function is to protect the brain from toxic elements in the blood. While true to some degree, it is becoming clear that the function of the BBB is much more complex than initially thought. Indeed, recent studies indicate that routine changes in its permeability may be the norm rather than the exception, with bradykinin playing a major role in this homeostatic activity (see Bartus et al.[31,39] for more detailed discussion of this hypothesis). At any rate, extensive testing of labradimil alone and in combination with carboplatin has consistently shown no vasogenic oedema, cerebral vascular damage or neurotoxic effects, even with direct infusion of high doses of these drugs directly into the carotid artery.[38] The lack of cerebral vascular neurotoxicity probably rests with the fact that the increased BBB permeability induced by labradimil is both transient and (at least in healthy, nontumour brain tissue) limited to relatively small compounds (<1kD). Essential criteria for induction of brain oedema and secondary brain and vascular damage are permeation of large molecules into healthy (nontumour) brain for prolonged periods of time. To date, no evidence has been seen that either effect occurs when labradimil is administered to increase the permeability of the BBB.

3. Clinical Results

Given that the preclinical data demonstrate that labradimil safely and transiently permeabilises the BBTB in rodent models of glioma, clinical trials evaluating both the safety and potential efficacy of labradimil have been conducted in patients with glioma.[60-64] Initial studies using intracarotid infusions of labradimil 10 to 300 ng/kg demonstrated significantly enhanced transport of ^{68}Ga-EDTA into tumours of patients with recurrent malignant glioma.[60] Overall, the increase in delivery (as determined by calculating the K_i) was 46% in tumour tissue with no detectable alterations in permeability of the BBB in healthy brain tissue. This study and a subsequent study using intracarotid administration of labradimil and carboplatin[61] also provided preliminary evidence of tumour response. In each study, 3 of 6 patients who received 300 ng/kg of labradimil, together with carboplatin, exhibited a reduction in tumour volume.

Because permeabilising the BBTB using conventional intravenous administration of labradimil offers practical advantages and greater safety than with intra-arterial administration, phase I and II clinical trials were conducted to evaluate the potential of intravenous labradimil combined with carboplatin. Phase I dose escalation (up to 300 ng/kg) studies in patients revealed that labradimil was well tolerated, with the primary adverse effects being transient and mild flushing, nausea, headache and subclinical increase in heart rate.[63] Neutropenia and thrombocytopenia were consistent with the known effects of carboplatin alone. One early pharmacokinetic study reported elevated carboplatin concentrations with labradimil (when compared with literature standards),[65] but another found no change in carboplatin concentrations.[66] In neither study was any evidence of enhanced carboplatin toxicity observed.[65,66]

Subsequent phase II studies in patients with recurrent glioma indicated that the combination of labradimil and carboplatin has activity against glioma.[64] A total of 87 patients (45 with no prior chemotherapy and 42 with 1 prior course of chemotherapy) received intravenous infusions of labradimil 300 ng/kg and carboplatin every 28 days. Clinical evaluations revealed that 37% of the chemotherapy-naive patients had a clinically significant improvement and another 24% showed stabilisation of their neurological symptoms. Radiological evaluations further showed that 32% of these patients had either a complete or partial response and an additional 47% showed stabilisation of tumour growth.

Although the effects of combining labradimil with carboplatin were less robust in patients who had received a prior course of chemotherapy, the rate of clinical response in these patients was still 39%, with 24% of these patients showing stable, partial or complete radiological response.[64] The average duration of response was 30.3 weeks in the chemotherapy-naive patients and 19.6 weeks in the

patients who previously received treatment. It is important to note that these studies were phase II evaluations that did not include contemporaneous controls.

Although there is limited information available on the efficacy of carboplatin on recurrence in patients with chemotherapy-naive malignant glioma, the results with labradimil compare favourably with those reported by Yung et al.[67] They evaluated a group of young patients with good performance status, approximately half of whom had a glioblastoma and half an anaplastic astrocytoma. The partial response rate reported in this study was only 14%.[67]

The chemotherapy-naive patients receiving carboplatin plus labradimil also exhibited statistically enhanced survival when compared with historical controls (patients that received carboplatin alone) chosen prospectively from the Medical Research Council database.[68] Once again, no evidence of enhanced neutropenia or other carboplatin-related toxicity was observed.

In summary, these data demonstrate that labradimil can permeabilise the BBTB in patients with glioma and offer suggestive evidence that significant patient benefit might be achieved when labradimil is combined with carboplatin.

A more recent dose-escalation study investigated a range of labradimil doses in patients with brain metastases originating from small cell (SCLC) and non-small–cell (NSCLC) lung cancer (unpublished data). Doses began at 300 ng/kg and were escalated to 1800 ng/kg. Response rates were determined by magnetic resonance imaging (MRI), with patients grouped into 3 general dose levels (300 ng/kg, prior maximum human dose, n = 53; 450 to 1050 ng/kg, intermediate dose escalation, n = 13; 1200 to 1800 ng/kg, target range based on calculations from animal studies, n = 13). Patients were reasonably well matched across a number of important variables, including number of cerebral metastases, prior brain surgery, prior radiation therapy and prior chemotherapy. Blinded analysis of the MRIs was used as a surrogate marker for response rates (i.e. partial and complete response, combined).

Since previous studies of carboplatin for treating patients with SCLC and cerebral metastases demonstrated concordance between the response rate for the primary lesion and the metastases[69] it has been hypothesised that the BBB in SCLC metastases is not a barrier to carboplatin therapy. Rather, SCLC metastases appear to be insensitive to carboplatin treatment. The results from the current trial in patients with SCLC are consistent with this idea. The response rate for the SCLC metastases was equivalent to that reported elsewhere for primary tumours and their cerebral metastases treated with carboplatin and, as expected, no benefit of labradimil plus carboplatin was seen in these patients.[69] A preliminary analysis of the NSCLC patients given less than 1200 ng/kg exhibited response rates of only about 15%. However, patients given the preclinically-defined target dose of over 1200 ng/kg of labradimil exhibited a response rate of 50%. Because it is generally accepted that the response rate of NSCLC to chemotherapy is very low, a response rate of 50%, without severe toxicity, might be considered remarkable by many authorities.[70] Thus, these preliminary data in NSCLC confirm the prior results obtained in animals as well as the extrapolation to humans regarding the dose level required (and projected plasma concentrations necessary) to achieve reliable benefit from labradimil.

Interim reports of adverse events have been also obtained from a preliminary analysis of the first 2 cycles of treatment in the dose-escalation studies (unpublished data). At doses above 300 ng/kg, 2 new adverse events were seen: urinary urgency and a mental sensation similar to a post-alcohol 'hangover'. The events were mild to moderate in intensity, began during the infusion and resolved rapidly upon completion of the infusion. The incidence of both events was 36% at the higher dose. A number of adverse events that had occurred occasionally at 300 ng/kg were seen more frequently in the mid and higher dose groups, including dry mouth, diarrhoea/urge to stool, dyspepsia and abdominal pain. All events began during the infusion and resolved rapidly upon completion of the infusion. In a single

patient in the mid-dose group, the abdominal pain was severe, but did not require discontinuation of the infusion. All these events were dose-related, mild to moderate in intensity, and considered disagreeable rather than hazardous.

4. Conclusions

The information presented in this review collectively describes the first successful attempt to design and develop a bradykinin B_2 agonist to exploit one particular characteristic of bradykinin, increased BBB permeability, without inducing substantial adverse effects. These data argue that it is possible to develop a tolerable and potentially efficacious B_2 agonist as a drug for extensive testing in animal models, and preliminary testing in patients indicates that labradimil enhances the effects of concomitantly administered chemotherapeutic agents by increasing their delivery across the BBTB. Thus, these data suggest this compound could be useful as an adjunctive treatment in combination with chemotherapeutic agents for brain tumours.

The data collected with labradimil satisfy 3 key criteria that have been identified for any drug intended to pharmacologically modify the BBTB.[71] First, labradimil consistently increases the amount of chemotherapeutic delivered to the tumour and BST.[31,35,37,39,40] Secondly, detailed autoradiography studies in animals demonstrate that the distribution of the chemotherapeutic within the tumour and BST is improved, so that with labradimil, no area of the tumour or BST escapes elevated chemotherapeutic concentrations.[55] Thirdly, the duration of elevated chemotherapeutic concentration within the tumour and BST is increased.[55] Importantly, each of these effects occurs selectively in tumour and tumour-associated tissue, with little or no effect in normal brain distal to tumour.

On the basis of the data reviewed here, it seems likely that similar advantages can be obtained from many other hydrophilic chemotherapeutic agents. Taken together, these data support the potential use of labradimil as a unique therapeutic tool for gliomas and other brain tumours and argue that doses higher than those tested in earlier phase II studies will be required to achieve reliable effects in humans.

Although labradimil shows promise for neuro-oncology applications, additional indications for labradimil, involving different brain-related maladies, remain to be explored, including other forms of acute neuropathology (e.g. stroke and CNS infections) and possibly chronic applications (e.g. multiple sclerosis or Alzheimer's disease). In this regard, labradimil has been shown to enhance uptake of fairly large molecules such as 20kD cytokines[53] and 70kD dextran[31] across the BBTB, while only much smaller compounds (e.g. aminoisobutyric acid, loperamide and carboplatin) are able to permeate the normal BBB with labradimil, and even then, at only one-tenth of the rate of the tumour barrier.[39,52]

It is noteworthy that while quantitative autoradiographic studies do not show robust or consistent enhancement of small molecule penetration into nontumour brain (although small effects are often seen), evidence of reliable increases in non-brain tissue have been reported with electron microscopy[34] and behavioural[44] end-points. A detailed series of studies has also recently demonstrated that intravenous labradimil enhances delivery of chemotherapeutics to solid peripheral tumours and that this leads to suppressed tumour growth and improved survival.[72] Interestingly, while initiated by the B_2 receptor, this phenomenon nonetheless occurs through intracellular mechanisms that appear distinct in many ways from those involved with increased BBB permeability.[73] Thus, one challenge for future research will be to define the biological conditions under which labradimil is most likely to work effectively and the chemical constraints of the drugs that it might help deliver to the brain and periphery, and the magnitude of the effects that might be expected.

References

1. Davson H, Segal M. Physiology of the CSF and blood-brain barriers. Boca Raton (FL): CRC Press, 1996
2. Pardridge W. Transport of small molecules through the blood-brain barrier: biology and methodology. Adv Drug Deliv Rev 1995; 15: 5-36

3. Rapoport S. Blood-brain barrier in physiology and medicine. New York: Raven Press, 1976
4. Bartus RT. The blood-brain barrier as a target for pharmacological modulation. Curr Opin Drug Disc Dev 1999; 2 (2): 152-67
5. Betz A. An overview of the multiple functions of the blood-brain barrier. In: Frankenheim J, Brown R, editors. Bioavailability of drugs to the brain and the blood-brain barrier. Washington, DC: US Government Printing Office, 1992: 54-72
6. Zlokovic B, McComb J, Perlmutter L, et al. Neuroactive peptides and amino acid at the blood-brain barrier: possible implications for drug abuse. In: Frankenheim J, Brown R, editors. Bioavailability of drugs to the brain and the blood-brain barrier. Washington, DC: US Government Printing Office, 1992: 26-42
7. Black K. Biochemical opening of the blood-brain barrier. Adv Drug Deliv Rev 1995; 15: 37-52
8. Raymond J, Robertson D, Dinsdale H. Pharmacological modification of bradykinin induced breakdown of the blood-brain barrier. Can J Physiol Pharmacol 1986; 13: 214-20
9. Inamura T, Black KL. Bradykinin selectively opens blood-tumor barrier in experimental brain tumors. J Cereb Blood Flow Metab 1994; 14: 862-70
10. Nomura T, Inamura T, Black K. Intracarotid infusion of bradykinin selectively increases blood-tumor permeability in 9L and C6 brain tumors. Brain Res 1994; 658: 62-6
11. Bhoola K, Figueroa C, Worthy K. Bioregulation of kinins: kallikreins, kininogens and kinases. Pharm Rev 1992; 44: 1-80
12. Erdös E. Some old and some new ideas on kinin metabolism. J Cardiovasc Pharmacol 1990; 15 Suppl. 6: S20-4
13. Kumakura S, Kamo I, Tsurufuji S. Role of bradykinin in the vascular permeability response induced by carrageenin in rats. Br J Pharmacol 1988; 93: 739-46
14. Grous J, Elliott P, Bartus R. Development of RMP-7: a novel bradykinin agoinst that increases delivery of chemotherapeutics to gliomas. In: Salmon S, editor. Adjuvant therapy of cancer. Philadelphia (PA): Lippincott-Raven, 1997: 19-31
15. Boddy A, Thomas H. RMP-7: potential as an adjuvant to the drug treatment of brain tumours. CNS Drugs 1997; 7 (4): 257-63
16. Regoli D, Rhaleb N, Drapeau G, et al. Kinin receptor subtypes. J Cardiovasc Pharmacol 1990; 15 Suppl 6: S30-8
17. Hess J, Borkowski J, Young G, et al. Cloning and pharmacological characterization of a human bradykinin (B2) receptor. Biochem Biophys Res Commun 1992; 184: 260-8
18. Menke J, Borkowski J, Bierlo K, et al. Expression cloning of a human B1 bradykinin receptor. J Biol Chem 1994; 269: 21583-6
19. Hamayoun P, Harik S. Bradykinin receptors of cerebral microvessels stimulate phosphoinositide turnover. J Cereb Blood Flow Metab 1991; 11: 557-66
20. Doctrow SR, Abelleira SM, Curry LA, et al. The bradykinin analog RMP-7 increases intracellular free calcium levels in rat brain microvascular endothelial cells. J Pharmacol Exp Ther 1994; 271: 229-37
21. Unterberg A, Wahl M, Baethmann A. Effects of bradykinin on permeability and diameter of pial vessels in vivo. J Cereb Blood Flow Metab 1984; 4: 574-85
22. Wahl M, Young A, Edvinnson L, et al. Effects of bradykinin on pial arteries and arterioles in vitro and in situ. J Cereb Blood Flow Metab 1983; 3: 231-7
23. Yong T, Gao X, Koizumi S, et al. Role of peptidases in bradykinin-induced increase in vascular permeability in vivo. Circ Res 1992; 70: 952-9
24. Regoli D, Barabe J. Pharmacology of bradykinin and related kinins. Pharmacol Rev 1980; 32: 1-46
25. Regoli D, Marceau F, Lavigne J. Induction of B1 receptors for kinins in the rabbit by a bacterial lipopolysaccharide. Eur J Pharmacol 1981; 71: 105-15
26. Dray A, Perkins M. Bradykinin and inflammatory pain. Trends Neurosci 1993; 16: 99-104
27. Kozarich J, Musso G, Malfroy-Camine B. Increasing blood-brain barrier permeability with permeabilizer peptides. Cambridge (MA): Alkermes, Inc., 1993
28. Marceau F, Gendreau M, Barabe J, et al. The degradation of bradykinin (BK) and of des-Arg9-BK in plasma. Can J Physiol Pharmacol 1981; 39: 131-8
29. Regoli D, Drapeau G, Dion S, et al. New selective agonists for neurokinin receptors: pharmacological tools for receptor characterization. Trends Pharmacol Sci 1988; 9: 290-5
30. Rhaleb N, Telemaque S, Rouissi N, et al. Structure-activity studies of bradykinin and related peptides: B2-receptor antagonists. Hypertension 1991; 17: 107-15
31. Bartus RT, Elliott P, Hayward N, et al. Permeability of the BBB by the bradykinin agonist, RMP-7: evidence for a sensitive, auto-regulated, receptor-mediated system. Immunopharmacology 1996; 33: 270-8
32. McCarthy D, Potter D, Nicolaides E. An in vivo estimation of the potencies and half-lives of synthetic bradykinin and kallidin. J Pharmacol Exp Ther 1965; 148: 117-22
33. Straub JA, Akiyama A, Parmar P. In vitro plasma metabolism of RMP-7. Pharm Res 1994; 11: 1673-6
34. Sanovich E, Bartus RT, Friden PM, et al. Pathway across blood-brain barrier opened by the bradykinin agonist, RMP-7. Brain Res 1995; 705: 125-35
35. Elliott PJ, Hayward NJ, Dean RL, et al. Intravenous RMP-7 selectively increases uptake of carboplatin into rat brain tumors. Cancer Res 1996; 56: 3998-4005
36. Emerich D, Snodgrass P, Pink M, et al. Central analgesic actions of loperamide following transient permeation of the blood brain barrier with Cereport (RMP-7). Brain Res 1998; 801: 259-66
37. Elliott P, Hayward N, Dean R, et al. Dissociation of blood-brain barrier permeability and the hypotensive effects of the bradykinin B2 agonist RMP-7. Immunopharmacology 1996; 33: 205-8
38. Riley M, Kim N, Watson V, et al. Intraarterial administration of the bradykinin agonist, RMP-7 and carboplatin: a toxicologic evaluation in swine. J Neurooncol 1998; 36: 167-78
39. Bartus RT, Elliott PJ, Dean RL, et al. Controlled modulation of BBB permeability using the bradykinin agonist, RMP-7. Exp Neurol 1996; 142: 14-28
40. Elliott PJ, Hayward NJ, Huff MR, et al. Unlocking the blood-brain barrier: a role for RMP-7 in brain tumor therapy. Exp Neurol 1996; 141: 214-24
41. Bartus R, Snodgrass P, Dean R, et al. Evidence that Cereport's ability to increase permeability of rat gliomas in dependent upon extent of tumor growth: implications for treating newly emerging tumor colonies. Exp Neurol 2000; 161: 234-44
42. Del Maestro R. Angiogenesis. In: Berger M, Wilson C, editors. The gliomas. Philadelphia (PA): WB Saunders, 1999: 87-106

43. Stewart P, Farrell C, Del Maestro R. The effect of cellular microenvironment on vessels in the brain. Part 1: vessel structure in tumour, peritumour and brain from humans with malignant glioma. Int J Radiat Biol 1991; 60: 125-30
44. Emerich D, Snodgrass P, Dean R, et al. Enhanced uptake of carboplatin into brain tumors with intravenous Cereport (RMP-7): dramatic differences as a function of dosing parameters. Br J Cancer 1999; 80: 964-70
45. St Croix B, Rago C, Velculescu V, et al. Genes expressed in human tumor endothelium. Science 2000; 289: 1197-202
46. Bartus R, Snodgrass P, Dean R, et al. Use of Cereport (RMP-7) to increase delivery of carboplatin to gliomas: insight and parameters for intracarotid infusion via a single-lumen cannula. Drug Deliv 1999; 6: 15-21
47. Barth R, Yang W, Moeschberger M, et al. Enhanced delivery of boronophenylalanine for neutron capture therapy of brain tumors using the bradykinin analogue, Cereport (RMP-7). Neurosurgery 1999; 47: 189-98
48. Hochberg F, Pruit A. Assumptions from the radiotherapy of glioblastoma. Neurology 1980; 30: 907-11
49. Lesser G, Grossman S. The chemotherapy of high grade astrocytomas. Semin Oncol 1994; 21: 220-35
50. Walner K, Galichih J, Krol G, et al. Patterns of failure following treatment of glioblastoma and anaplatic astrocytoma. Int J Radiat Oncol Biol Phys 1989; 16: 1405-9
51. Nelson D, McDonald J, Lapham L, et al. Central nervous system tumors. In: Rubin R, McDonald S, Qazi R, editors. Clinical oncology. Philadelphia (PA): WB Saunders, 1992: 617-44
52. Inamura T, Nomura T, Bartus R, et al. Intracarotid infusion of RMP-7, a bradykinin analog: a method for selective drug delivery to brain tumors. J Neurosurg 1994; 81: 752-8
53. Nakano S, Matsukado K, Black K. Enhanced cytokines delivery and intercellular adhesion molecule 1 (ICAM-1) expression in glioma by intracarotid infusion of bradykinin analog, RMP-7. Neurol Res 1997; 19: 501-8
54. Ikeda K, Ichikawa T, Wakimoto H, et al. Oncolytic virus therapy of multiple tumors in the brain requires suppression of innate and elicited antiviral responses. Nat Med 1999; 5: 881-7
55. Bartus R, Snodgrass P, Marsh J, et al. Intravenous Cereport (RMP-7) modifies topographic uptake profile of carboplatin within rat glioma and brain surrounding tumor, elevates platinum levels and enhances survival. J Pharmacol Exp Ther 2000; 293 (3): 903-11
56. Emerich D, Dean R, Marsh J, et al. Intravenous Cereport (RMP-7) enhances delivery of carboplatin and increases survival in rats with metastatic tumors in the brain. Pharm Res 2000 Oct; 17 (10): 1212-9
57. Fike J, Gobbel G, Mesiwala A, et al. Cerebrovascular effects of the bradykinin analog RMP-7 in normal and irradiated dog brain. J Neurooncol 1998; 37: 199-215
58. Snodgrass P, Emerich D, Bartus R. Obligatory tachyphylaxis of bradykinin B2 receptors following increased permeability of the blood brain barrier by intravenous Cereport [abstract]. Society for Neuroscience; 2000 Nov 4-9; New Orleans
59. Matsukado K, Inamura T, Nakano S, et al. Enhanced tumor uptake of carboplatin and survival in glioma-bearing rats by intracarotid infusion of bradykinin analog, RMP-7. Neurosurgery 1996; 39: 125-34
60. Black K, Cloughesy T, Huang S, et al. Intracarotid infusion of RMP-7, a bradykinin analog, and transport of gallium-68 ethylenediamine tetraacetic acid into human gliomas. J Neurosurg 1997; 86: 603-9
61. Cloughesy T, Black K, Gobin Y, et al. Intra-arterial Cereport (RMP-7) and carboplatin: a dose escalation study for recurrent malignant gliomas. Neurosurgery 1999; 44: 270-8
62. Ford JM, Miles KA, Hayball MP, et al. A simplified technique for measurement of blood-brain barrier permeability using CT: preliminary results of the effect of RMP-7. In: Faulkner K, Carey B, Crellin A, et al., editors. Quantitative imaging in oncology. London: British Institute of Radiology, 1996: 1-3
63. Ford J, Osborn C, Barton T, et al. A Phase I study of intravenous Cereport (RMP-7) with carboplatin patients with progression of malignant glioma. Eur J Cancer 1998; 34 (11): 1807-11
64. Gregor A, Lind M, Newman H, et al. Phase II studies of RMP-7 and carboplatin in the treatment of recurrent high grade glioma. J Neurooncol 1999; 44: 137-45
65. Thomas H, Newell D, Calvert A, et al. Clinical studies with the novel bradykinin analog RMP-7 and carboplatin in patients with glioma [abstract]. Br J Cancer 1996; 73: 15
66. Siddik Z, Grous J, Chang S, et al. Pharmacokinetics of carboplatin and RMP-7 in patients with recurrent malignant glioma [abstract]. American Society of Clinical Oncology Annual Meeting; 1996 May 18-21; Philadelphia
67. Yung W, Mechtler L, Gleason M. Intravenous carboplatin for recurrent malignant glioma: a phase II study. J Clin Oncol 1991; 9: 860-4
68. Osborn C, Stenning S, Downing G. Survival following treatment with RMP-7 and carboplatin in malignant glioma who grade III-IV: comparison with matched controls. European Congress of Clinical Oncologists; 1997 Sep; Hamburg, Germany.
69. Postmus PE, Smit EF, Haaxma-Reiche H, et al., and the European Organization for Research and Treatment of Cancer: Lung Cancer Cooperative Group. Teniposide for brain metastases of small-cell lung cancer: a phase II study. J Clin Oncol 1995; 13: 660-6
70. Boogerd W, Sande JVD, Zandwijk NV. Teniposide sometimes effective in brain metastases from non-small cell lung cancer. J Neurooncol 1999; 41: 285-9
71. Jain R. Critical issues in tumor microcirculation, angiogenesis and metastasis: biological significance and clinical relevance [course notes]. Cambridge (MA): Harvard Medical School, 1996
72. Emerich D, Snodgrass P, Dean R, et al. Bradykinin modulation of tumor vasculature I: activation of B2 receptors increases delivery of chemotherpaeutic agents into solid peripheral tumors, enhancing their efficacy. J Pharmacol Exp Ther 2001; 296: 623-31
73. Emerich D, Snodgrass P, Dean R, et al. Bradykinin modulation of tumor vasculature II: activation of nitric oxide and phospholipase A2/prostaglandin signaling pathways synergistically modify vascular physiology and morphology to enhance delivery of chemotherapeutic agents to tumors. J Pharmacol Exp Ther 2001; 296: 632-41

Correspondence and offprints: Dr *Raymond T. Bartus*, Preclinical R&D, Alkermes, Inc., 64 Sidney Street, Cambridge, MA 02139, USA.
E-mail: rtbartus@alkermes.com