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RESEARCH MODELS

Infusion vs. Injection: Considerations for Administering a Drug Compound Across Preclinical Research Areas

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Overview

When testing a novel compound *in vivo*, rapid elimination can result in mistaken assessment of activity. Rats and mice generally eliminate test compounds more rapidly than humans. After a single injection, plasma concentration rises to a peak and then declines rapidly until the compound is eliminated from plasma and tissues. Often the duration of serum activity following a single injection is limited to several hours, hence biological effects either fail to develop or develop poorly.

If no effect is observed following injection, it is difficult to determine whether the compound is inactive or if it simply was not present in adequate concentration and for a sufficient duration to elicit an effect. Depending on the rate of elimination and the frequency of dosing, injections can result in periods during which the drug is absent from plasma and tissues. Such extreme variability in compound exposure over time can influence the expression of drug action. Thus, the data from such experiments can be misleading as to the nature of compound effects and the dose required to elicit them.

Continuous drug administration using implantable infusion pumps allows researchers to understand and optimise key determinants of drug action, such as the level and duration of drug exposure, and the spatial drug distribution relative to the target tissue. The pumps ensure that test compounds are present in plasma and tissues for a sufficient duration to allow their biological effects to develop fully, with better reproducibility. Therefore, drug effects can be optimised early in preclinical development, allowing clinical studies to be conducted at a lower cost and with better results.

Therapeutic Index of a Drug

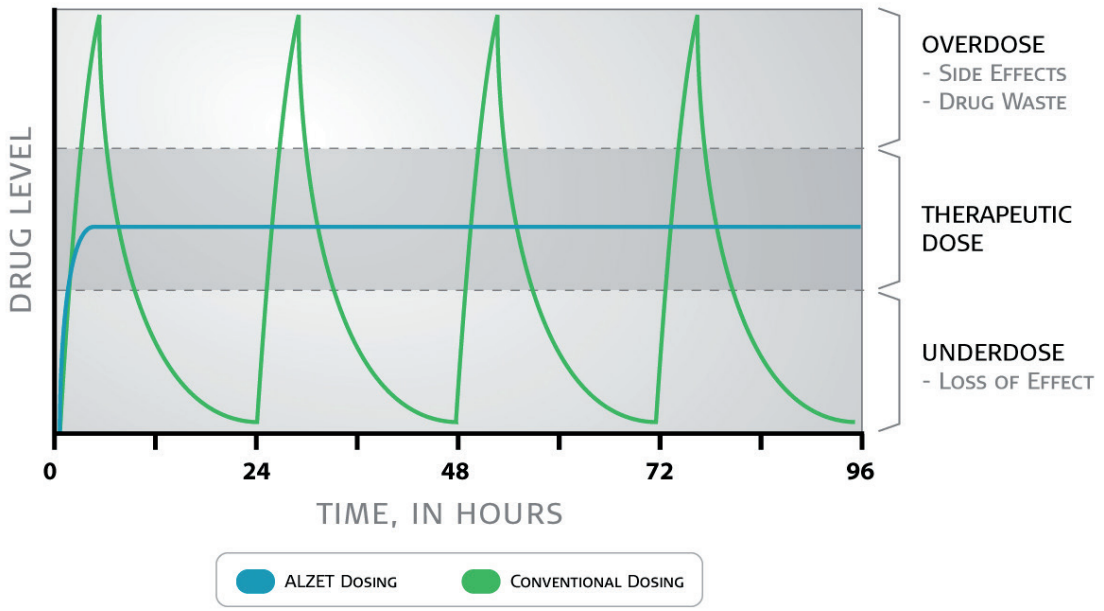
A drug's therapeutic index is a ratio reflecting the quotient of its therapeutic effects and its adverse effects. Varying the schedule of administration can have a major influence on the therapeutic index of some drugs. Relative to bolus dosing, constant infusion can increase efficacy, reduce side effects, or both. All of these changes can increase a drug's therapeutic index, improving its value as a pharmaceutical.

One cannot assume, however, that infusion regimens are superior to injections for all drugs. The relationship between dose, regimen, and drug effect must be carefully explored for each drug. Dose-response testing, where the effects of one or more schedules of injections are compared with the effects of constant infusion, helps elucidate schedule-dependent drug effects. This type of testing has been termed the injection-infusion comparison (IIC) protocol. This protocol is an established method for optimising the effectiveness of anticancer agents, and it is important in the preclinical testing of proteins, peptides, and other recombinant DNA products.

"The majority of chemicals are eliminated considerably faster in small laboratory animals as compared with humans, and constant-rate infusion offers several advantages over conventional, bolus delivery regimens in compensating for this difference."

Excerpt from Clarke, David O. "Pharmacokinetic studies in developmental toxicology: practical considerations and approaches." Toxicology Methods 3, no. 4 (1993): 223-251.

Figure 1. Effects of Injection vs. Infusion on Serum Levels



Optimising the schedule of drug delivery can, among other advantages, improve the ratio of therapeutic effects to toxic effects. This is particularly critical in cancer therapy as chemotherapeutics are frequently hampered by their low therapeutic index, wherein side effects occur at relatively low doses while maximal antitumour activity demands high doses. For some agents, continuous infusion is more efficacious and has fewer side effects as compared with administration by immediate release methods, such as injection. The ALZET® pump provides a convenient method for this comparison in research animals.

Research Examples

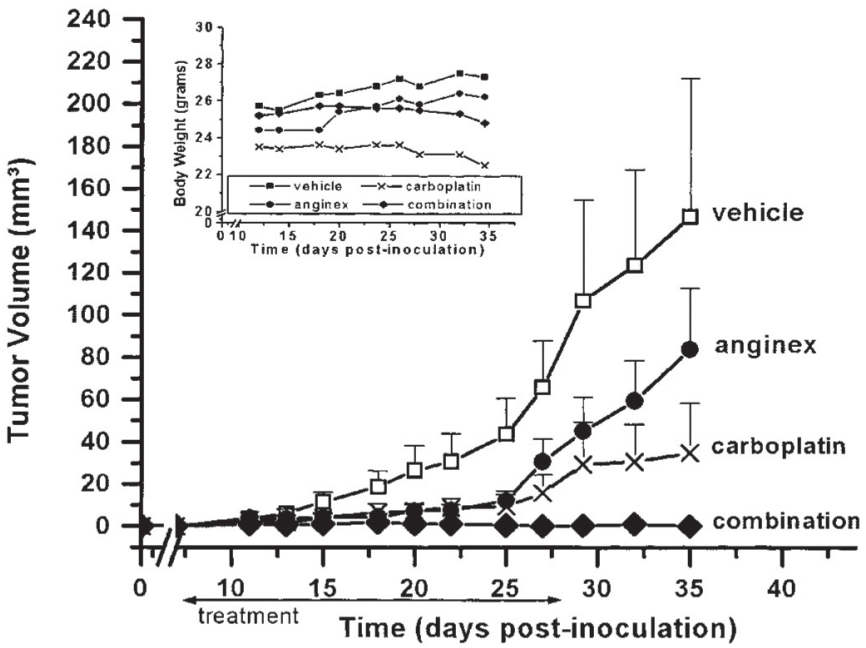
The scientific literature summarised in this white paper provides many examples where continuous administration has facilitated full development of drug effects, including those of proteins, peptides, and other rapidly eliminated compounds.

A. Antiangiogenesis Therapy
B. Continuous Infusion of Somatostatin Analogues
C. Antiobesity and Antidiabetic Effects of FGF21 Infusion
D. Effective Epileptic Treatment Requires Continuous Drug Delivery
E. IL-13-PE and Gemcitabine Combination Therapy for Pancreatic Cancer
F. Successful Implementation of *In Vivo* Imaging Technologies

A. Antiangiogenesis Therapy

The formation of new blood vessels, or angiogenesis, is essential to sustain tumour growth. Thus, methods that inhibit angiogenesis provide a unique therapeutic approach for cancer treatment. Many angiostatic agents have been identified; however, the most promising ones are those that directly inhibit endothelial cell proliferation, such as anginex. Dings et al. evaluated the *in vivo* efficacy of anginex in mouse xenograft models of ovarian carcinoma (Dings, van der Schaft et al. 2003). Anginex was administered continuously via ALZET® pumps at increasing doses of 5, 10, and 20 mg/kg/day. Anginex treatment resulted in a dose-dependent inhibition of tumour growth in nude mice. Optimum efficacy was achieved with the 10 mg/kg/day dose, which led to up to 80% tumour growth reduction compared to a maximum of 50% in the lower dose group. The therapeutic effects of various treatment regimens were also evaluated on established MA148 tumours. Anginex was administered either by locoregional injections (once- or twice-daily), slow-release alginate beads, or continuous administration by osmotic pumps for 28 days. Continuous administration by osmotic pumps was most effective, leading to up to 80% reduction in tumour growth, compared to 60% and 30% reduction with alginate beads and daily injections, respectively. Anginex was also shown to have a synergistic effect with angiostatin or carboplatin on the inhibition of established ovarian tumours in nude mice (Dings, Yokoyama et al. 2003). Although continuous, single-agent administration was effective, combination therapy of anginex with angiostatin resulted in enhanced tumour inhibition of up to 80%. When anginex was combined with suboptimal doses of carboplatin, animals experienced tumour remission to undetectable levels (**Figure 2**).

Figure 2.



Mean tumour growth curves in a human ovarian carcinoma model. Mice were treated with (□) vehicle, (●) anginex (10 mg/kg/day), (X) carboplatin, or (♦) a combination of both agents. Carboplatin was administered IP once every 3 days at a suboptimal dose of 32.5 mg/kg. Vehicle and anginex were administered continuously via SC minipump. Treatments were given for 28 days starting 7 days after tumour cell inoculations. The inset shows animal body weight changes during the treatment period as an indirect assessment of toxicity.

B. Continuous Infusion of Somatostatin Analogues

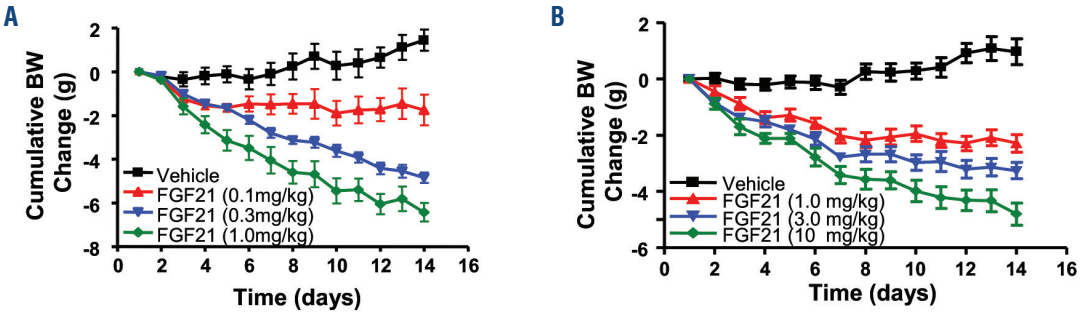
Somatostatin analogues have been found to induce apoptosis and reduce proliferation of cancerous cells in animal studies. Tejeda et al. found that the therapeutic efficacy of TT-232, a novel somatostatin analogue, is significantly dependent upon its mode of administration (Tejeda et al. 2005). Leukemia tumour-bearing mice were treated with various doses of TT-232 either by daily injections for 14 days, or continuous infusion via ALZET® pumps for 14-28 days. While a dose-dependent tumour reduction was evident in both treatment groups, continuous administration was the most efficacious form of administration. In the P-388 tumour cell model, "infusion of TT-232 by ALZET® osmotic minipump resulted in 70-80% tumour growth inhibition and 20% tumour-free survival." Administration by injections resulted in only a modest 26-44% tumour growth inhibition, with no impact on the survival rate. Tejeda et al. also favoured osmotic pumps over injections since "serial injections represent significant stress to the animals and require precautions in terms of drug administration." On the other hand, "ALZET® minipumps maintained a constant drug level, resulting in a well-defined, consistent pattern of drug exposure throughout the period of drug administration."

Combination therapy with octreotide (a somatostatin analogue), galanin, and serotonin has shown promise as a potential treatment for colon cancer, more so than single or double therapy. Studies indicate that the effectiveness of this triple drug therapy is also dependent on the mode and route of administration (El-Salhy 2005). Nude mice bearing human colon cancer xenografts were treated with octreotide, galanin, and serotonin, either via daily bolus injections or continuous infusion with osmotic pumps. Drug treatments were maintained for 14 days and were given via the subcutaneous (SC) or intraperitoneal (IP) route. Regardless of the route of administration, triple drug therapy effectively induced apoptosis and reduced tumour volume, weight, and vascularisation. However, continuous IP infusion via osmotic pumps offered the most effective treatment, decreasing tumour weight and volume by 70% compared to only 20% in the IP injection group. Triple drug therapy was well tolerated, as indicated by stable animal weights throughout the study. These agents, like most neuroendocrine gut peptides, have short half-lives. Treatment success was attributed to the low but prolonged drug concentrations offered by the continuous administration method. On the contrary, intermittent injections provided high drug concentrations, but only during brief periods.

C. Antiobesity and Antidiabetic Effects of FGF21 Infusion

Researchers have found that continuous administration of fibroblast growth factor 21 (FGF21) may offer a safe and efficacious option for the treatment of metabolic disorders (Kharitonenkov et al. 2005; Coskun et al. 2008). Kharitonenkov et al. used ALZET® pumps to administer FGF21 to *db*/*db* mice for 8 weeks at an efficacious dose of 11 μg/kg/h. Compared to vehicle controls, continuous administration of FGF21 resulted in a significant and prolonged reduction of plasma glucose during the treatment period. FGF21 treatment, even at high doses, was found to be free of the typical adverse effects associated with other therapies. Coskun et al. demonstrated that FGF21 also exerts potent antiobesity effects, and that continuous administration is necessary to maximise its therapeutic action. Diet-induced obese (DIO) mice were treated for 2 weeks with either vehicle or increasing doses of FGF21 administered by daily SC injections or continuous infusion by ALZET® pump. Although both treatment regimens resulted in a dose-dependent reduction in total body weight, "a 10-fold greater [injection] dose of FGF21 was required to achieve an equivalent weight reduction compared with FGF21 administration via ALZET® pumps," as seen in **Figure 3** (Coskun et al. 2008). The researchers speculate that a continuous activation of FGF21 signalling is required to achieve maximal therapeutic effects. Administration via injections leads to a temporary rise in FGF21 blood levels due to its short half-life. However, "administration via infusion allows for the continuous presence of circulating bioactive FGF21 throughout the course of the study" (Coskun et al. 2008).

Figure 3.



Cumulative change in body weights of DIO mice following continuous FGF21 administration with ALZET® pumps (A), or daily bolus administration by injections (B). Note that a higher FGF21 dose is required with injections to achieve similar antiobesity effects produced following continuous infusion. (Reprinted, by permission, from Coskun et al. 2008)

D. Effective Epileptic Treatment Requires Continuous Drug Delivery

When evaluating the efficacy of antiepileptic drugs (AEDs) in rodent models of chronic epilepsy, researchers face the critical task of selecting the most appropriate dosing method for their study: one that is both convenient and effective at maintaining prolonged AED exposure at therapeutic levels.

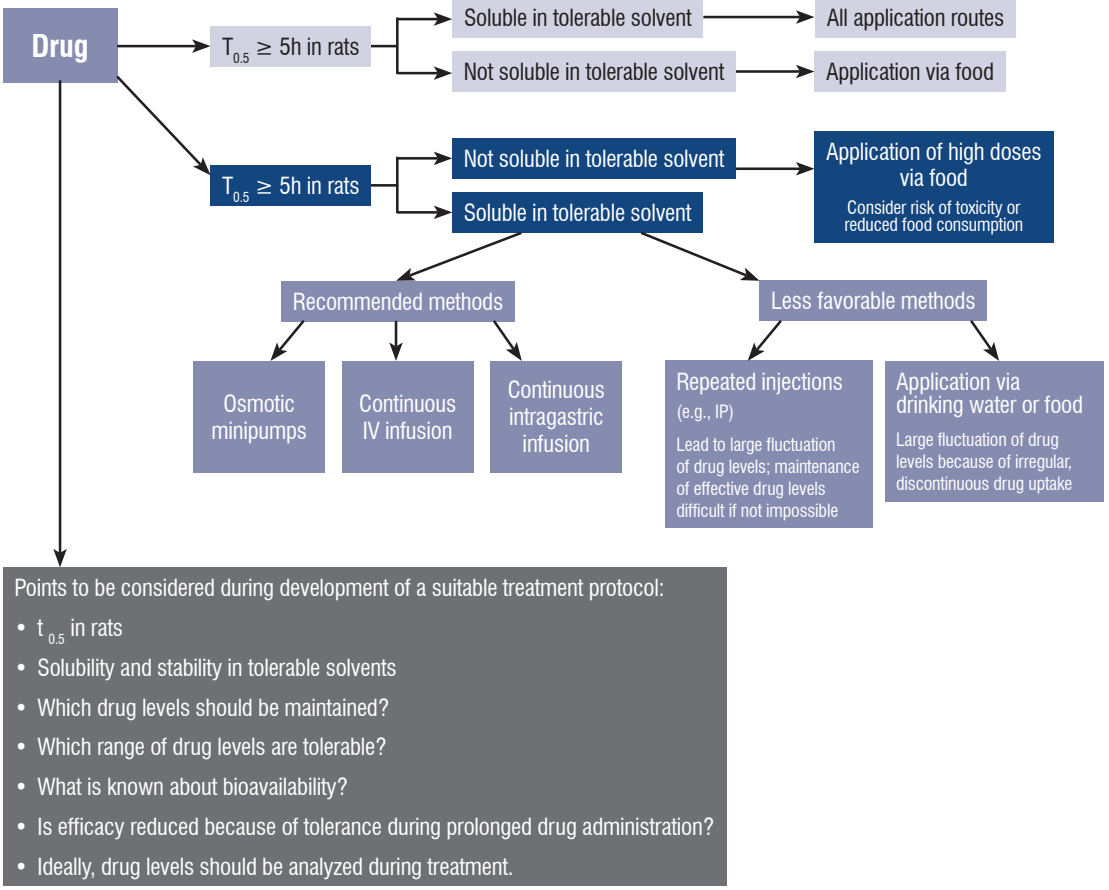
For AEDs with relatively long half-lives (> 5 hours), conventional methods of drug administration, such as daily injections or oral dosing, are viable options for maintaining effective drug levels. However, most AEDs have short elimination half-lives in rodents (< 5 hours), making these methods inadequate for effective dosing. Furthermore, these methods are labour-intensive and often lead to higher drug toxicity and stress during chronic treatment. Administration via drinking water or food is not a viable alternative either, given that rodents drink or eat mostly at night; thus, drug levels fluctuate widely according to the animal's feeding and drinking behaviour. Further, administration via drinking water is not an option for water-insoluble AEDs, or for those that alter the palatability of the water.

For AEDs that are rapidly eliminated, effective levels can be sustained by continuous administration. Tethered infusion systems consisting of external pumps and chronically implanted intravenous catheters can be used for continuous administration of AEDs. However, these methods are costly and carry increased risk of catheter clotting, infection, and animal stress, thus jeopardising experimental results. On the other hand, ALZET® osmotic pumps have successfully been used as a convenient method for chronic AED dosing in laboratory animals. Importantly, ALZET® pumps are specifically designed to deliver a continuous and automatic dose for up to 6 weeks, thus ensuring constant exposure of short half-life AEDs over the course of the study.

The various dosing methods available for AED administration are summarised in a review by Wolfgang Loscher (Loscher 2007), and a set of experimental recommendations for chronic AED administration in rats is summarised in **Figure 4**.

Valproic acid (VPA) is an AED with short half-life (0.8 hr in mice; 1-5 hr in rats, depending on the dose), and chronic dosing by conventional methods often leads to significant toxicity. Serralta et al. studied the schedule-dependent effects of VPA in a rat model of kindled epilepsy. VPA was administered either by continuous intracerebroventricular (ICV) infusion, ICV injections, or IP injections to evaluate anticonvulsant efficacy, hepatotoxicity, and neurotoxicity. VPA levels in the brain, plasma, cerebrospinal fluid (CSF), and liver were also determined.

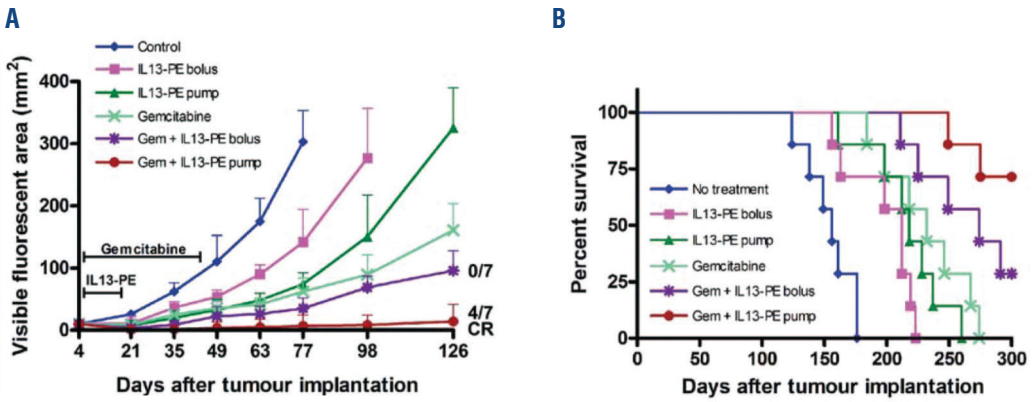
Figure 4.



Decision flow chart for researchers planning studies with prolonged AED administration in rats. [Reproduced by permission of Blackwell Publishing Ltd. Wolfgang Loscher. Epilepsia 2007;48(7):1245-1258]

The study revealed that ICV injections control both generalised and focal seizures, but only with considerable levels of ataxia and sedation. VPA concentrations and efficacy declined rapidly within 5-15 minutes post-injection. IP injections showed less control of seizures, required a higher dose of VPA, and were also associated with high levels of ataxia and sedation. In contrast, continuous ICV administration controlled both generalised and focal seizures with low neurotoxicity, and very low plasma and hepatic concentrations. Continuous infusion led to a progressive decrease in seizure severity with increasing dosages and infusion times. By day 4, all animals were seizure-free. Toxicity was low to non-existent during and after the infusion period, with no sedation in any animals and low (level 1) ataxia in only 4 of 6 high-dose animals.

Figure 5.



Quantification of tumour growth by real-time whole-body imaging (A) and mice survival curves (B) in an early pancreatic cancer model using HS766T cells. Reprinted with permission from Fujisawa et al. Int. J. Cancer 2011;128:1221–1231

E. IL-13-PE and Gemcitabine Combination Therapy for Pancreatic Cancer

Researchers at the U.S. Food and Drug Administration and Yokohama City University evaluated a novel therapeutic approach for treating prostate cancer by combining standard chemotherapy with specific immunotherapy to tumour cell surface receptors. Fujisawa et al. identified IL-13Rα2 as an ideal target for tumour immunotherapy since it is overexpressed in many human cancers, including 71% of pancreatic ductal adenocarcinomas (PDAs). To target the IL-13 receptor, the researchers developed a recombinant immunotoxin, named IL-13-PE, by linking IL-13 to a mutated form of the Pseudomonas exotoxin (PE). Fujisawa et al. then evaluated the efficacy of IL-13-PE and gemcitabine in various mouse models of human PDA.

Nude mice were implanted with orthotopic pancreatic tumours derived from HS766T and MIA-PaCa2 cancer cells. Once tumours were visualised, treatments were initiated on day 5 for the early pancreatic cancer model or day 29 for the advanced cancer model. Mice were treated with gemcitabine, IL-13-PE, or a combination of both agents. IL-13-PE was administered IP at a dose of 100 μg/kg/day (or 25 μg/kg/day for the low-dose study) for 14 days, either by continuous infusion (IL-13-PE pump) or twice-daily bolus injection (IL-13-PE bolus). In general, continuous administration was superior to chronic injections for both combined and monotherapy regimens. Infusion groups showed reduced tumour growth and enhanced survival, with combination therapy being most effective. In the early pancreatic cancer model, combination therapy was the only treatment that resulted in complete eradication of established pancreatic tumours. Most mice (6/7) had no detectable tumours on day 21, compared with 4/7 mice from the gemcitabine + IL-13-PE bolus treatment group. Notably, 4/7 mice from the gemcitabine + IL-13-PE pump group remained tumour-free throughout the course of the study (**Figure 5A**). These animals also survived much longer, with a mean survival time (MST) of > 300 days, compared to 156 and 274 days in the no-treatment and gemcitabine + IL-13-PE bolus groups, respectively (**Figure 5B**). Single therapy also decreased tumour size and increased survival, but to a lesser extent (**Figure 5**). The antitumour effect of IL-13-PE was further evaluated in an advanced pancreatic tumour model with treatment initiated on day 29; an early pancreatic cancer model at a suboptimal dose of IL-13-PE (25 μg/kg/day for 14 days); and a cancer model using MIA-PaCa2 cells, which express lower levels of IL-13Rα2 compared to HS766T cells. In all cancer models, continuous infusion of IL-13-PE combined with gemcitabine was most effective at reducing tumours.

The study demonstrated that IL-13-PE and gemcitabine work synergistically to achieve greater therapeutic effect in pancreatic cancer models. Continuous administration of IL-13-PE produced a stronger response compared to bolus injections. Furthermore, this treatment was also well tolerated, with no evidence of organ toxicity or other adverse effects.

F. Continuous Administration Improves *In Vivo* Imaging Evaluation

Real-time *in vivo* imaging of small laboratory animals is feasible with the use of luciferase reporters and bioluminescence imaging (BLI) technologies, such as IVIS® imaging systems. The technique is highly sensitive, noninvasive, and can reduce the number of animals used for experimentation since data can be acquired from the same animal over time. BLI is based on the detection of visible light produced during luciferase-mediated oxidation of the molecular substrate, luciferin, when the enzyme is expressed *in vivo* as a molecular reporter. The luciferase reaction is highly dependent on substrate availability. In fact, studies show that if "exogenously administered luciferin is not abundantly present, light emission might not be a true representation of luciferase activity" (Sadikot 2005). This concern can be addressed with the use of implantable infusion pumps, which can facilitate steady-state and prolonged delivery of bioluminescent substrates.

A study by Gross et al., published in *Nature Methods*, explored the benefits of continuous administration of D-luciferin to enable real-time imaging of Iκ kinase (IKK) inhibition in tumour xenografts (Gross 2005). The study also evaluated the pharmacodynamic properties of the drug candidate PS-1145, a selective IKK inhibitor. Nude mice bearing tumours expressing the IκBα-firefly luciferase fusion reporter (IκBα-Fluc), or unfused luciferase, were implanted with 7-day ALZET® pumps delivering D-luciferin. Animals were also treated with increasing doses of PS-1145 and imaged with the IVIS® imaging system at various time points before and after treatment. The authors found that PS-1145 induced a time-dependent increase in tumour bioluminescence that peaked 8-12 hours after drug administration, followed by a gradual decrease over 32 hours to levels of vehicle treated mice.

The reporter provided continuous, noninvasive monitoring of target-specific IKK activation in real time. This approach allowed a complete time- and dose-dependent pharmacodynamic analysis of the IKK inhibitor using less than 30 animals. The study suggests that "the utility of the reporter was further enhanced through innovative use of an implanted micro-osmotic pump for persistent and constant delivery of the bioluminescent substrate D-luciferin . . . By eliminating constraints of intraperitoneal bolus reinjections of substrate, the implanted pump allowed continuous real-time molecular imaging of reporter activity throughout the time course of a multi-day experiment, while simultaneously allowing rapid analysis of drug action" (Gross 2005).

Conclusion

Data derived from many experiments can be misleading as to the nature of compound effects and the dose required to elicit them. Through continuous infusion, ALZET® pumps maintain a well-defined, consistent pattern of drug exposure throughout the duration of the experiment and ensure that test compounds are present in plasma and tissues for a sufficient duration to allow their biological effects to develop fully and reproducibly. Additional benefits of the pumps include reduced stress arising from both injections and repeat handling, as well as the convenience derived from not having to prepare and plan repeat injections.

Charles River is the exclusive distributor of ALZET® Osmotic Pumps for Austria, Belgium, Czech Republic, France, Germany, Hungary, Ireland, Italy, Luxembourg, Netherlands, Portugal, Spain, Switzerland, and the UK. For further information on ALZET® Pumps please visit www.criver.com/ALZET.

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