Real-time measurement of small molecules directly in awake, ambulatory animals

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The development of a technology capable of tracking the levels of drugs, metabolites, and biomarkers in the body continuously and in real time would advance our understanding of health and our ability to detect and treat disease. It would, for example, enable therapies guided by high-resolution, patient-specific pharmacokinetics (including feedback-controlled drug delivery), opening new dimensions in personalized medicine. In response, we demonstrate here the ability of electrochemical aptamer-based (E-AB) sensors to support continuous, real-time, multihour measurements when emplaced directly in the circulatory systems of living animals. Specifically, we have used E-AB sensors to perform the multihour, real-time measurement of four drugs in the bloodstream of even awake, ambulatory rats, achieving precise molecular measurements at clinically relevant detection limits and high (3 s) temporal resolution, attributes suggesting that the approach could provide an important window into the study of physiology and pharmacokinetics.

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The availability of versatile and convenient sensors supporting the continuous, real-time measurement of specific molecules directly in the body could prove transformative in research and in medicine. In the short term, for example, such an advance would allow the in vivo concentrations of drugs, metabolites, hormones, and other biomarkers to be measured with high precision in subjects as they undergo their normal daily routine, improving our knowledge of physiology, pharmacokinetics, and toxicology. On longer timescales, such an advance would facilitate “therapeutic drug monitoring,” in which dosing is personalized using a patient’s directly measured (rather than crudely and indirectly estimated) metabolism. By permitting the continuous monitoring of biomarkers (e.g., creatinine and hormones), such a technology would likewise provide a new and highly detailed window into health status (e.g., kidney or endocrine function). Finally, the real-time measurement of specific molecules in the body would advance drug delivery (1). Such a technology, for example, could easily support feedback-controlled dosing, in which the delivery of drugs is adjusted in real time based on their concentration in the body or on the body’s molecular-level response to treatment. This real-time, feedback-controlled drug delivery would provide new routes by which drugs with dangerously narrow therapeutic windows or complex optimal dosing regimens can be administered safely and efficiently.

Although technologies already exist for the continuous or near-continuous measurement of a small number of metabolites [e.g., glucose (2) and lactate (3)] and neurotransmitters [e.g., dopamine (4, 5), serotonin (6), glutamate (7), and acetylcholine (8)] in vivo, these approaches all rely on the specific chemical reactivities of their targets (e.g., the redox chemistry of the analyte or its ability to be oxidized by a specific enzyme). Because of their dependence on reactivity, these technologies are not generalizable to the detection of many other physiologically or clinically important molecules, and there remains an open, critical need for

strategies that support the continuous detection of specific molecules in the body irrespective of their reactivity. Unfortunately, however, serious technical hurdles stand in the way of realizing this goal (9, 10). First, to support continuous measurements, a sensor cannot rely on batch processing, such as wash or separation steps. Second, to support in vivo measurements, a sensor cannot use exogenously added reagents and must remain stable against prolonged exposure to blood or interstitial fluids in vivo. To date, the vast majority of molecular detection strategies have failed to meet one or both of these critical challenges. Chromatography, mass spectrometry, and immunochemistry, for example, are complex, multistep batch processes requiring wash steps, separation steps, and/or sequential reagent additions, hindering their ability to perform continuous measurements. Conversely, whereas biosensors based on surface plasmon resonance (SPR), quartz crystal microbalances (QCM), field-effect transistors (FET), and microcantilevers all support continuous, real-time operation, each fails when challenged in blood (much less in vivo) due to their inability to discriminate between the specific binding of their target and the nonspecific adsorption of proteins and cells (11–14). Here, in contrast, we demonstrate the ability of electrochemical aptamer-based (E-AB) sensors, a sensing platform adaptable to the detection of any of a wide range of molecular targets irrespective of their chemical reactivity, to support continuous, real-time measurements directly within the body.

Significance

The ability to monitor arbitrary molecules directly in living subjects as they undergo their daily routines remains one of the “holy grails” of bioanalytical chemistry. Such a technology would, for example, vastly improve our knowledge of physiology, pharmacokinetics, and toxicology by allowing the high-precision measurement of drugs and metabolites under realistic physiological conditions. Real-time molecular measurements would also provide an unparalleled window into health status (e.g., kidney function) and would facilitate “therapeutic drug monitoring,” in which dosing is personalized to the specific metabolism of each individual patient. Finally, the ability to measure molecules in the body in real time would provide unprecedented new routes by which drugs with dangerously narrow therapeutic windows could be safely and efficiently administered.

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Conflict of interest statement: K.W.P. discloses service on the scientific advisory boards of Diagnostic Biochips Inc., Illumi Health, and Ecce Systems. N.A.-C., J.S., and K.W.P. have filed a provisional patent based on the work presented in this paper.

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Fig. 3. High-precision pharmacokinetics. Shown are high-resolution pharmacokinetic profiles for the drugs DOX (A) and gentamicin (B) upon i.v. injection of 50 mg/m² and 20 mg/kg doses, respectively. As is easily seen, the resolution of in vivo E-AB sensors is sufficient not only to define the slower β phases of these drugs (red dots) but also to define their much more rapid α phases (blue dots) with excellent statistical significance. These measurements constitute a precise determination of the i.v. distribution phase of a small-molecule drug.

detection of new, chemically unrelated molecules via the simple expedient of replacing their aptamer recognition element. To demonstrate this modularity, we fabricated sensors using an aptamer recognizing the aminoglycoside antibiotics (32, 33). Using these sensors, we first followed monotonically increasing i.v. doses of kanamycin spanning the therapeutic ranges used in humans (34) (10–30 mg/kg) and animals (35) (25–30 mg/kg). The sensor responded rapidly to each injection, measuring maximum concentrations between 34 and 400 μM depending on the delivered dose (Fig. 2B). The 200 μM maximum concentration observed after a 10 mg/kg dose was in agreement with peak plasma concentrations determined previously (using cumbersome, poorly time-resolved ex vivo radioimmunoassays) after similar doses were injected into multiple animal species (36). The sensor can likewise monitor in real time the in vivo concentrations of the aminoglycosides gentamicin (Fig. 2C) and tobramycin (Fig. 2D and *SI Appendix*, Fig. S3) following either i.m. or i.v. injections, applications in which it once again achieves excellent precision and time resolution.

The ability to perform the continuous measurement of specific molecules in the body opens the door to many potentially transformative applications in the study of physiology and pharmacokinetics. For example, the few-second time resolution of E-AB sensors (Fig. 1F), which reflects orders of magnitude improvement over the time resolution of traditional pharmacokinetic methods, is sufficient to measure the kinetics with which drugs distribute following i.v. injection (Fig. 3 and *SI Appendix*, Fig. S4), a pharmacokinetic phase that has rarely if ever been previously measured (e.g., refs. 37–39). Indeed, the precision of E-AB measurements is sufficient not only to robustly identify animal-to-animal pharmacokinetic variability, but even variability within a single animal over the course of a few hours. To explore this ability, we monitored the pharmacokinetics of tobramycin following sequential 20 mg/kg i.v. injections conducted 2 h apart in each of three rats. Fitting the resultant data to a two-compartment model, we easily observe statistically significant inter- and even intraanimal variability (Fig. 4). The distribution phase (α phase) of this drug, for example, is defined largely by blood and body volume and thus, although the distribution differs between animals, it differs much less as a function of time within individual animals. The elimination kinetics of tobramycin (β phase), in contrast, not only vary significantly between animals but also exhibit variations within a single individual over the course of a few hours that are easily measurable using our approach (Table 1). For example, although the kinetics of the α phase remain relatively constant for a given animal, the β phase invariably slows with time. This change presumably occurs because, whereas drug absorption (captured by the α phase) is defined by body volume, which remains fixed, the elimination of tobramycin (captured in the β phase) is predominantly via excretion from the kidneys (40, 41), the function

of which likely changes due to alterations in the animal's blood pressure (42) and/or hydration after several hours under anesthesia.

The ability of E-AB sensors to reject false signals arising from background interferents is excellent; none of the many

Fig. 4. The measurement of inter- and intraanimal pharmacokinetic variability. The precision of E-AB measurements is sufficient to measure not only interanimal pharmacokinetic variability but also variability within an individual animal over time. Shown are the pharmacokinetic profiles of the drug tobramycin following two sequential 20 mg/kg i.v. injections in three different rats (A, B, and C). These high-precision measurements reveal a decrease in the rate of drug elimination kinetics (β phase) in the second injection with respect to the first in all three animals, an effect that presumably arises due to changes in the animal's blood pressure and/or hydration after several hours under anesthesia. The bold black lines represent the mathematical fit of each injection dataset to a two-compartment pharmacokinetic model.

Table 1. Pharmacokinetic parameters corresponding to repeated i.v. injections of tobramycin in three Sprague-Dawley rats

Rat no.	Injection no.	A, * μM	α , min	B, μM	β , min	C_{max} † μM	AUC, $\mu\text{mol}\cdot\text{min}\cdot\text{L}^{-1}$	Cl_T , $\text{mL}\cdot\text{min}^{-1}$
1	1	255 $\pm$ 82	2.2 $\pm$ 0.4	71 $\pm$ 2	51 $\pm$ 3	326 $\pm$ 82	117 $\pm$ 58	129 $\pm$ 64
1	2	267 $\pm$ 56	1.6 $\pm$ 0.3	80 $\pm$ 4	57 $\pm$ 4	347 $\pm$ 56	168 $\pm$ 61	90 $\pm$ 32
2	1	113 $\pm$ 38	1.6 $\pm$ 0.4	82 $\pm$ 2	32 $\pm$ 1	195 $\pm$ 38	73 $\pm$ 39	208 $\pm$ 100
2	2	109 $\pm$ 22	2.7 $\pm$ 0.6	50 $\pm$ 2	71 $\pm$ 7	159 $\pm$ 22	41 $\pm$ 15	370 $\pm$ 139
3	1	284 $\pm$ 34	1.2 $\pm$ 0.1	46 $\pm$ 2	38 $\pm$ 3	330 $\pm$ 34	237 $\pm$ 49	63 $\pm$ 13
3	2	138 $\pm$ 32	1.7 $\pm$ 0.1	35 $\pm$ 2	67 $\pm$ 3	173 $\pm$ 32	82 $\pm$ 28	186 $\pm$ 63

Confidence ranges reflect 95% confidence intervals.

*A, α , B, and β are derived from the fit to a two compartment model: $[\text{target}] = Ae^{-t/\alpha} + Be^{-t/\beta}$, where α and β are the half-lives for distribution and elimination, respectively.

[†]C_{max}, AUC (area under the curve), Cl_T (drug clearance), and their associated confidence intervals propagated from the kinetic parameters A, α, B, and β.

endogenous metabolites and hormones in rat blood activates the sensor, as evidenced by their performance in vivo. The platform's ability to distinguish between structurally similar molecules, in contrast, can be problematic due to the sometimes [although not always (43, 44)] limited specificity of aptamers because, of course, the sensor cannot be more specific than the aptamer from which it is constructed. E-AB specificity is nevertheless sufficient for many research and clinical applications. For example, although the aminoglycoside-binding aptamer recognizes multiple members of this closely related family of drugs (Fig. 2 B–D), coadministration of more than one of these highly toxic drugs is clinically contraindicated, and thus the inability to distinguish between them is of little medical relevance. The therapeutic action of DOX is driven by its ability to bind DNA, and thus the aminoglycoside sensor also exhibits cross-reactivity to this drug (*SI Appendix, Fig. S5A*). Here too, however, the coadministration of the two is so rare as to limit the clinical impact of this effect. The DOX-detecting sensor, in contrast, exhibits no significant cross-reactivity with the aminoglycosides (*SI Appendix, Fig. S5B*), nor does it exhibit

significant cross-reactivity with other chemotherapeutics that are commonly coadministered with DOX in clinical applications (26).

In addition to studies, as those above, performed on anesthetized animals, the simplicity, physical robustness, and small size of E-AB sensors also renders it possible to perform measurements on awake, ambulatory animals. To illustrate this ability, we surgically implanted permanent catheters in the jugular veins of rats and allowed the animals to recover from this surgery for 2 wk before using the catheter to insert a flexible E-AB sensor under light anesthesia. The sensor connects to its supporting electronics via flexible wire leads that allow the awake animals to move largely unimpeded (Fig. 5A and Movie S1). Aminoglycoside sensors used under these conditions support run times of up to half a day as the animal feeds, drinks, and explores its environment (Fig. 5B and C), producing pharmacokinetic data that avoid potentially confounding factors associated with measurements based on (repeated) blood draws, which require anesthetized or otherwise immobilized (and thus stressed) animals.

Here, we demonstrate the ability of E-AB sensors to track specific small molecules continuously and in real time in awake,

Fig. 5. Continuous, *in vivo* molecular measurements on awake, ambulatory animals. (A) The small size and physical robustness of E-AB sensors renders it possible to use them in animals as they eat, drink, and explore their cage (Movie S1). This robustness, in turn, enables the measurement of specific molecules in the blood of animals as they undertake their normal daily routine, conditions perhaps more relevant to human health than those traditionally used for the collection of metabolic and pharmacokinetic data. Shown are blood levels of the drug tobramycin after a 25 mg/kg *i.m.* injection (thigh) (B) or sequential 40 mg/kg *i.v.* (juvular vein) injections (C) in awake, freely moving animals.

ambulatory animals, a capability that could provide an important tool for understanding physiology and pharmacology. By allowing arbitrary molecules (limited only by the availability of an aptamer of appropriate specificity and affinity) to be monitored with high resolution in animals undergoing their normal daily routine, for example, the ability to perform such measurements could improve our knowledge of metabolism, pharmacokinetics, and toxicology. The few-second time resolution of our approach likewise suggests that it could improve our understanding of rapidly fluctuating physiological events, such as uptake and distribution pharmacokinetics, hormone and neurotransmitter release, and the movement of drugs and metabolites across the blood–brain barrier and within the central nervous system. Finally, the ability to perform the measurement of specific molecules in the body in real time could enhance the efficiency and accuracy with which drugs are dosed, in applications ranging from personalized, patient-specific pharmacokinetic measurements as a means of precisely tailoring dosing to long-term feedback-controlled drug delivery in which the dosage of a drug is varied in real time in response to minute-to-minute changes in a patient's physiological status. In short, the technology

demonstrated here could enhance not only our understanding of health but also our ability to detect, monitor, and treat disease.

Materials and Methods

All animal procedures were consistent with the guidelines of the NIH *Guide for the Care and Use of Laboratory Animals* (47) and approved by the Institutional Animal Care and Use Committee of the University of California, Santa Barbara.

A detailed description of the materials and methods used in this work can be found in *SI Appendix*, which includes a descriptive list of chemicals and materials used, E-AB sensor fabrication, calibration and surgical emplacement, a description of the electrochemical methods (including KDM) and data analysis software used, and details on the experimental setups used to carry out in vitro and in vivo measurements.

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