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The Use of Accelerator Mass Spectrometry in Human Health and Molecular Toxicology

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Abstract

Accelerator Mass Spectrometry (AMS) has been adopted as a powerful bio-analytical method for human studies in the areas of pharmacology and toxicology. The exquisite sensitivity (10^{-18} mol) of AMS has facilitated studies of toxins and drugs at environmentally and physiologically relevant concentrations in humans. Such studies include: risk assessment of environmental toxicants, drug candidate selection, absolute bioavailability determination, and more recently, assessment of drug-target binding as a biomarker of response to chemotherapy. Combining AMS with complementary capabilities such as high performance liquid chromatography (HPLC) can maximize data within a single experiment and provide additional insight when assessing drugs and toxins, such as metabolic profiling. Recent advances in the AMS technology at Lawrence Livermore National Laboratory have allowed for direct coupling of AMS with complementary capabilities such as HPLC via a liquid sample moving wire interface, offering greater sensitivity compared to graphite-based analysis therefore, enabling the use of lower ^{14}C and chemical doses, which are imperative for clinical testing. The aim of this review is to highlight the recent efforts in human studies using AMS, including technological advancements and discussion of the continued promise of AMS for innovative clinical based research.

Introduction

Accelerator mass spectrometry (AMS) is an analytical measurement technique that can quantify rare, long-lived isotopes with attomole (amol) (10^{-18}) sensitivity for isotope-labeled drugs and toxicants. Unlike decay-based methods such as liquid scintillation counting, AMS counts atoms of a rare isotope of interest independent of decay events, and reports the ratio of the counted isotope to that of the total number of atoms of the element in

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a given sample. Because most biological materials contain carbon, radiocarbon (^{14}C, $t_{1/2}$ = 5730 years) is most commonly used to label the compound or drug of interest. Furthermore, most drugs and toxicants are amenable to radiocarbon labeling. The innate sensitivity of AMS enables analysis of very small amounts of compounds such that sub-therapeutic or environmental levels of analyte can be quantified. These low level attributes of AMS detection therefore allow for studies in cells, animals and humans without perturbing the biological system under investigation.

AMS has enabled a variety of biological applications including: studies investigating cell turnover,[1, 2] pharmacokinetics and bio-distribution of drug candidates[3, 4] and toxicants[5], bioavailability of nutrients such as B-12[6] and covalent binding (adduct formation) of compounds with DNA and proteins.[7, 8] AMS can also be coupled to other measurement capabilities such as high performance liquid chromatography, therefore enabling quantification of not only the compound of interest but also its metabolites.

AMS is the most appropriate technique to measure long-lived, low natural abundance radioisotopes. While ^{14}C is the most common radioisotope used, other studies have utilized other isotopes such as ^{3}H and ^{41}Ca for detection.[9, 10] Specific biological applications that are enabled by AMS include: long-term tracer measurements (weeks to months), low bioavailability or systemic distribution of compounds, highly potent drugs or toxic compounds and low quantity/specific activity compounds. Unlike other forms of mass spectrometry, AMS only measures the isotope of interest. AMS does not provide any structural information within the measurement itself; therefore, the analyzed sample must be properly characterized prior to measurement to ensure meaningful results. Fortunately, many sample preparation methods used in biological research are compatible with AMS analysis as long as sources of extraneous carbon are controlled. For example, volatile carbonaceous HPLC mobile phases should be considered to prevent the unwanted addition of carbon to the AMS sample. Additionally, the carbon in the biological sample must be sufficiently homogenized prior to the removal of the aliquot for AMS analysis to prevent sampling error.

The $^{14}C/C$ isotope ratio measured with AMS is determined by comparing to ratios of prepared standards; this ratio is expressed in units of fraction Modern. One Modern is equivalent to 13.56 dpm/g C or 97.89 amol/mg C. For AMS measurement, samples must be converted into a form that retains the isotopic ratio of the sample and provides chemical and physical equivalence for all measured atoms. Typical ion sources for routine quantitation of ^{14}C require samples to be thermally and electrically conductive solids. Consequently, biochemical samples for ^{14}C-AMS analysis are first combusted to CO_2, followed by a chemical reduction to graphite.[11, 12] This process eliminates any sample matrix dependence on the analyte quantititation.

The conversion of carbonaceous biochemicals to graphite has been extremely successful for the vast majority of AMS applications in the biosciences. However, significant manual sample processing is required and the whole process suffers from low sample throughput (~150 graphite samples prepared/day) and long turnaround times (~3 days minimum). The quantitative addition of carrier carbon to samples containing <0.5 mg carbon also limits sensitivity to ~2 amol ^{14}C/mg C.

Conversion of a sample to CO_2 gas, which is then fed directly into an AMS gas accepting ion source can overcome this bottleneck. CO_2 gas-accepting ion sources offer several advantages over solid sample ion sources. They make more efficient use of the sample; hence much smaller sized samples may be analyzed. Less sample handling is required, increasing throughput, while reducing turnaround. Finally, gas-accepting ion sources are amenable to the measurement of the continuous output of a gas stream, giving higher time resolution for flow separations.

Most applications involving the direct analysis of CO_2 are focused on naturally-labeled compounds. [13–17] Recently, van Duijn, et al., has adapted some of those methods for tracer biomedical applications.[18] Discrete aliquots are combusted using a commercial elemental analyzer with the resultant CO_2 captured and transferred to a gas-tight syringe for subsequent metering into a gas-accepting hybrid ion source on a 1 MV HVEE AMS spectrometer. A limit of quantitation of 0.52 amol of ^{14}C in biological samples containing 70 μg carbon was reported. Up to 200 samples may be measured automatically in sequence, limited by the capacity of the ion source.

Lawrence Livermore National Lab (LLNL) has developed a moving wire interface to enable the conversion of biochemical samples to CO_2 for direct injection into a gas-accepting ion source.[19–21] Figure 1 shows a schematic of the Liquid Sample AMS system (LS-AMS) at LLNL. The output from an HPLC is deposited onto a moving nickel wire as a coherent jet. The wire is then pulled through a drying oven to remove the solvent before entering a high-temperature combustion reactor where the carbon content of the sample is oxidized to CO_2. A helium gas stream is used to carry the combustion products to the AMS system for carbon isotope analysis. Although not depicted in Figure 1, microliter-sized drops for discrete sample analysis can alternately be placed onto the wire in the same location as the silica tip using a disposable pipet tip.

The 50 zmol ^{14}C limit of quantitation of our LS-AMS capability is based on a measurement precision of 5%, based on counting statistics. This lower limit can decrease if a lower precision can be tolerated (or, conversely, increase if a higher precision is required). The minimum carbon sample size for LS-AMS quantitation is 10 ng ^{12}C. These limits represent a 50,000-fold reduction in the minimum sample size and a 20-fold improvement in ^{14}C sensitivity over solid graphite AMS analysis, enabling the use of even lower ^{14}C and chemical doses.

Human studies enabled by AMS

A major concern when developing risk assessments for highly toxic chemicals or when investigating new drugs in development is extrapolating high dose experimental animal data to relevant real-world human exposure conditions. Traditionally, to assess bioavailability, metabolism and pharmacokinetics, animal models are exposed to high chemical doses, which do not always extrapolate well to human relevant exposure scenarios.[22] AMS can bridge this gap and provide a sensitive and accurate method to measure low level doses that can range from environmentally relevant concentrations of toxicants to sub-therapeutic and microdosing levels of drugs. Microdosing is the administration of a low sub-

pharmacological dose (up to 100 μg or 1% of the therapeutic dose, whichever is smaller) to study the effects of a drug or toxin at levels that are unlikely to produce an observable effect, but high enough for assessment of cellular and systemic response.[23–25] AMS allows for direct testing of toxicants in humans, allowing for a more accurate risk assessment. For drug microdosing, AMS allows pharmacokinetics, absolute bioavailability and metabolism studies of drugs with minimal risk to human volunteers or patients with relatively few animal studies prior to the initiation of clinical work compared to a traditional Phase I human study.[23, 26, 27]

AMS has enabled many human studies including: personalized medicine and earlier assessment of novel drug candidates and xenobiotics.[28–30] In this review, we focus on recent applications for AMS in human risk assessment and new applications of microdosing.

Platinum Drug-DNA adducts as a potential prognostic and predictive biomarker for chemotherapy response

The platinum-based drugs cisplatin, carboplatin and oxaliplatin are amongst commonly prescribed chemotherapeutic drugs and are used against a broad spectrum of cancers, such as bladder, lung, ovarian, and colon, see structures in Figure 2.[31–33] However, the overall efficacy of platinum-based chemotherapy is limited due to severe side effects and intrinsic or acquired drug resistance.[32, 34] Gene expression analysis and other genomics approaches have revealed many insights into the mechanisms of platinum-based drug resistance. However, the genomics approach has so far failed to develop clinically useful tests for predicting resistance prior to the initiation of chemotherapy—a critical unmet medical need.[33, 35]

The primary mode of action of platinum-based drugs is the covalent modification (damage) of genomic DNA, which initiates cell death via induction of in apoptosis or necrosis.[32] The levels of such DNA alterations (adducts) are the cumulative result of many factors that govern cellular responses to drug exposure including genetics, tumor microenvironment, kidney function, overall patient health and others.[34, 36] A positive correlation between therapy-induced levels of platinum-based drug-DNA adducts in peripheral blood mononuclear cells (PBMC) or other surrogate tissues, and good clinical outcome have been widely reported in literature for a variety of cancers.[37–49] For example, Schellens et al found a significant positive relationship of cisplatin induced adduct levels in leucocytes (area under the DNA-adduct time curve) with favorable clinical outcome in several different cancers, including NSCLC, melanoma and cervical cancer.[40] However, not all investigators have observed such correlations,[50, 51] possibly due to different study designs, drug regimes, analytical methods employed and small numbers of patients. Furthermore, limited access to tumor tissue for adduct analysis has precluded comparison of drug-DNA adduct levels to patient response, and to those measurements in surrogate tissues such as PBMC. Despite the previous contradictory conclusions, drug-DNA adduct levels as pharmacodynamic endpoints remain potentially more informative and useful than genomic analysis of drug response to cytotoxic chemotherapy agents. However, due to the insufficient sensitivity of available standard detection methods (i.e. ICP-MS, immunoassays), the adduct levels are commonly measured only after exposure to high, therapeutically relevant, drug concentrations.

Henderson and coworkers recently reported progress on developing a microdosing strategy via accelerator mass spectrometry (AMS), with the goal of predicting individual tumor responses to platinum-based chemotherapy before cytotoxic chemotherapeutic intervention.[29, 52, 53] This approach, called “diagnostic microdosing”, allows study of pharmacokinetics (PK) and pharmacodynamics (PD) of carboplatin or oxaliplatin via the administration of sub-therapeutic drug doses that are unlikely to produce whole-body effects.[53] To date, most microdosing efforts have focused on PK predictions of new compounds to accelerate drug development; it is now extending to medical diagnostics applications. AMS can easily measure one ^{14}C-labeled drug molecule bound to DNA per 10^8 nucleotides,[8] with a limit of detection of one adduct per 10^{12} nucleotides,[54] and has been used to quantify radiocarbon-labeled drug or metabolite concentrations in urine, plasma or purified DNA samples.[55, 56]

In order to measure microdose induced drug-DNA adduct levels the compound needs to contain at least one carbon atom that can be labeled with ^{14}C. Cisplatin does not contain a carbon atom in the molecule and cannot be detected by AMS. Although less potent, carboplatin forms the same final drug-DNA diadduct crosslink structure as cisplatin and clinical cross-resistance is common.[57, 58] Consequently, these two drugs are sometimes used interchangeably in clinical practice and the microdosing approach to identifying carboplatin resistance can possibly be applied to cisplatin. AMS specifically detects carboplatin-DNA monoadducts (a precursor of the final diadduct), since the ^{14}C-labeled cyclobutane dicarboxylate (CBDCA) group is released once the diadduct is formed.[32] Initial preclinical microdose AMS studies inversely correlated induced drug-DNA adduct level in various cancer cell lines with cell line sensitivity toward the drug.[29, 52, 59]

In ongoing clinical diagnostic microdosing trials lead by Accelerated Medical Diagnostics Incorporated in collaboration with UC Davis and LLNL, induced carboplatin-DNA adduct levels are being correlated with clinical outcome of bladder and lung cancer patients (Figure 2A). Patients are administered a diagnostic microdose of ^{14}C label carboplatin at approximately 100-times lower than the anticipated therapeutic dosage (Clinicatrials.gov identifier NCT01261299). Blood or tissue samples are collected over a period of 24 hours and carboplatin-DNA adduct level are then quantified via AMS. Within four weeks each patient starts full dose platinum-based neoadjuvant chemotherapy, and drug-DNA adduct levels are correlated with tumor response. Our preliminary data show that patients with the highest PBMC drug-DNA adduct levels positively responded to the therapy (Figure 2B). However, not all responders had high levels of carboplatin-DNA adducts (not shown). The possibility that other drugs in the treatment regimen caused the response is being tested with bladder cancer patient derived tumor xenografts (PDX) in mice. Mice are subjected to microdoses of [^{14}C]carboplatin or [^{14}C]gemcitabine (a commonly used drug combination) and to platinum-/gemcitabine-based therapy in order to dissect the role of each individual drug on tumor response. Another pilot clinical trial aims to correlate the level of microdose-induced [^{14}C]oxaliplatin-DNA adducts with clinical outcomes in colorectal cancer patients (clinicaltrials.gov identifier NCT02569723). Collectively, these efforts support the feasibility of diagnostic microdosing to improve patient outcomes by personalizing chemotherapy.

Drug development

AMS was initially considered promising for lead candidate selection in the drug development process, which could potentially improve success rates and reduce drug development costs.[60] Early efforts using AMS for drug development have focused on: 1) determining the dose linearity between a microdose and a pharmacological dose[61, 62] and 2) using microdosing to evaluate the potential of new chemical entities.[63–65]

Linearity must exist between a microdose and therapeutic dose of a drug in order for microdosing to be of value in predicting clinically relevant effects. Recent efforts have focused on determining the dose linearity of several drugs to evaluate the feasibility of using AMS microdosing for drug development.[61, 62, 66]

Key validation studies for microdosing in humans have been reported from the CREAM (Consortium for Resourcing and Evaluating AMS Microdosing), EUMAPP (European Microdosing accelerator mass spectrometry [AMS] Partnership Programme), and the NEDO (New Energy and Industrial Technology Development Organization) trials. These studies evaluated PK and bioavailability correlations between micro and therapeutic doses of drugs.[61, 67, 68] In example, in the study by Lappin and colleagues, correlations between microdoses and therapeutic doses were determined for five drugs (diazepam, midazolam, ZK253, warfarin and erythromycin) in human subjects.[61] Good agreement for PK parameters (i.e. $t_{1/2}$, CL, F) was observed for all drugs except warfarin and erythromycin. While a microdose of warfarin predicted clearance, it did not accurately predict distribution pharmacokinetics. Erythromycin data could not be correlated due to possible acid lability from the oral dose within the stomach. The authors concluded that while some limitations may exist, microdosing has the potential to gain insight into early candidate selection.

The utility of microdosing using AMS in drug development has also been illustrated in children.[62, 66] Higher risks for drug toxicity and therapeutic failure exist for children compared to adults; accurate dose selection in children is often difficult due to developmental changes in drug pharmacokinetics (absorption, distribution, metabolism and excretion). The potential for microdosing using AMS in children was first reported in a review by Vuong et al in which a ^{14}C-labeled ursodiol microdosing study was conducted in infants.[69] Recent efforts to demonstrate microdosing feasibility in children have included PAMPERS (Paediatric Accelerator Mass Spectrometry Evaluation Research Study) and PEDMIC (Pediatric Microdosing: elucidating age-related changes in oral absorption to guide dosing of new formulations)[66]. The PAMPERS project is evaluating the feasibility of both microdosing and microtracer studies in children using ^{14}C-paracetamol and ^{14}C-midazolam, two drugs that are well characterized and commonly prescribed to children. The PEDMIC study is aimed towards developmental pharmacology; oral microtracers of ^{14}C-paracetamol and ^{14}C-midazolam are administered with an intravenous therapeutic dose. Both projects have preliminary data, which support acceptable dose linearity and feasibility of microdosing/microtracer studies for drug development in children.[62, 70]

In example, in the work by Mooij et al., the feasibility of utilizing microdosing to study developmental pharmacokinetics in children was assessed using ^{14}C-paracetamol (acetaminophen, AAP).[62] Children (0–6 years of age) were given both a single oral

microdose of ^{14}C-AAP (3.3 ng/kg, 60 Bq/kg) and intravenous therapeutic doses of AAP (15 mg/kg) every 6 hours. LC-MS/MS was used to measure AAP blood concentrations and AMS was used to measure both ^{14}C-AAP and metabolites (^{14}C-AAP-Glu and ^{14}C-AAP-4Sul). ^{14}C-AAP and metabolites were detectable at expected concentrations in blood of nine out of ten patients tested; this pilot study demonstrated feasibility of using a microdose to study PK and metabolite disposition in children.

The combination of technologies such as AMS and positron emission tomography (PET), a non-invasive nuclear imaging technique, has shown promise to maximize data output from clinical microdosing studies.[22, 71, 72] PET is capable of measuring tissue distribution and pharmacokinetics of drugs and is enabled using short-lived positron emitting radioisotopes (i.e. ^{11}C $t_{1/2}$ = 20.4 min, ^{18}F $t_{1/2}$ = 109.8 min).[73, 74] Combining the technologies of PET and AMS allows identification of tissue distribution and plasma PK information beyond the short examination periods using PET. In example, microdosing using PET/AMS was evaluated by Wagner et al using a mixture of (R/S)-^{14}C-verapamil and ^{11}C-verapamil as a model drug in six healthy volunteers.[72] For this combined PET/AMS study, verapamil was chosen due to its well-characterized safety profile and frequency of use. Pharmacokinetic parameters were measured by both AMS and PET. Plasma PK of ^{11}C-verapamil was measured using gamma counting and corrected for metabolites using HPLC, brain PK of both ^{11}C-verapamil and its metabolites were measured with PET. Plasma PK was also measured using AMS after chiral HPLC for the (R/S)-^{14}C-verapamil mixture. The power of combining these two technologies was highlighted in this study. If only using PET, the drug's elimination phase would not have been captured given the longer half-life of verapamil ($t_{1/2}$ = 6–8 hours) and the shorter time frames used for PET experiments. Therefore, utilizing PET imaging for drug tissue distribution with AMS for PK analysis is a powerful combination for early clinical drug assessment.

Novel drug candidates have also been assessed with microdosing.[4, 63–65, 75] These have included potential therapeutics for Alzheimer's disease,[65] *Staphylococcus aureus,*[63] *Streptococcus pneumonia*[64] and cancer.[65] The pharmacokinetics, absolute bioavailability and lung distribution of AR-709, a novel diaminopyrimidine antibiotic for *S. pneumonia*, was assessed in healthy volunteers in a recent study by Lappin et al.[64] While appreciable concentrations of AR-709 were measured in the lung, absolute oral bioavailability was low (2.5%), indicating the need for an alternative route of administration if the drug was further developed for clinical use. In another study, Kaplan et al compared ADME properties of an oral and intravenous dose of AFN-1252, a novel inhibitor of the bacterial fatty acid biosynthesis pathway.[63] Similar pharmacokinetics were observed for both dosing routes, including terminal half-lives (~7 hours) and bioavailability (83%) and adequate distribution in skin-related samples were also consistent demonstrating the potential use of the novel drug for *Staphylococcus spp*.

Prior to initiating human studies, Malfatti et al. examined whether a novel GyrB/ParE inhibitor pre-clinical drug candidate displayed linear kinetics across a sub-pharmacological and a pharmacological dose range in an animal model.[4] The plasma concentration of the ^{14}C-labeled inhibitor was quantified by AMS and the pharmacokinetic parameters from both a microdose and a pharmacologic dose were evaluated. Dose linearity across a 300-fold

dose range for the novel inhibitor was observed; PK properties for a 0.01 mg/kg microdose were similar to those given a 3 mg/kg pharmacologic dose (R^2=0.989). Based on these data, the PK from the microdosed animals was considered to be predictive of the PK from the pharmacologically dosed animals.

Although AMS has clear utility in PK and ADME studies, it has not been widely adopted by the pharmaceutical industry for lead candidate selection owing to several factors, including skepticism of the value of extrapolation of microdose PK to physiologically relevant doses, especially for oral medications, and a conservative culture for adopting new technologies. Most pharmaceutical companies are aware of AMS, and are developing internal guidelines on when a pharmaceutical project requires the limited utilization of AMS studies in order to de-risk a problematic PK issue. For example, Genentech extensively used AMS to better understand the complex pharmacokinetics and metabolism of the drug Vismodegib (GDC-0449).[76] The purpose of the study was to determine routes of elimination and the extent of vismodegib metabolism, including assessment and identification of metabolites in plasma, urine, and feces in six healthy female subjects. More recently, industry has focused resources on the use of AMS for absolute bioavailability studies to support regulatory filings, which is the predominant use of AMS for pharmaceutical research currently.[77] Reasons for this development include advantages related to concomitant administration of oral and intravenous doses, which reduces within-subject variability, reduced clinical costs due to fewer visits/time spent at the clinic, and the use of such low specific activities that samples are treated as non-radioactive and may be processed in any laboratory.

Low dose pharmacokinetics and toxicology

The advent of AMS allows studies to determine the xenobiotic bioavailability and metabolism in animal models and directly in humans at very low non-toxic human relevant doses. The combination of HPLC separation coupled with AMS analysis has allowed for metabolic profiling for such exposures.[5, 78] These approaches have led to the use of AMS to determine the pharmacokinetics and pharmacodynamics of a number of specific agents within humans at physiological doses.[7, 54, 79] Incorporation of these labeled compounds into specific cellular pools has been used to characterize cellular targets and kinetics.[80] The sensitivity of AMS measurement gives this technique a number of major advantages over other methods for the detection of isotopes. Importantly, because of the extreme sensitivity, PK studies utilizing AMS for detection have the ability to measure long-term kinetics and metabolism using low doses for several months after isotope administration. Furthermore, since only low doses of chemicals and radioactivity are required, studies can be performed with levels of chemicals equivalent to environmental exposures. In addition, detailed pharmacokinetic data require frequent sampling, which is made possible with AMS detection, by virtue of the small sample sizes needed for analysis. AMS has been used to establish the kinetics of β-carotene uptake and plasma clearance in a human volunteer that received a single dose of ^{14}C-β-carotene obtained from ^{14}C-spinach.[81] Plasma concentrations of β-carotene and its metabolites were determined at intervals over a 7-month period and required just 30 μL of plasma for analysis. Such complete investigations would not be possible using other methods that lack the necessary sensitivity to detect compounds and metabolites months after dosing.

AMS has also been used in low dose toxicology to establish the kinetics and biodistribution of silica nanoparticles over eight weeks after a single exposure in rodents[82] and to determine the toxicokinetics of the carcinogenic polycyclic aromatic hydrocarbon dibenzochrysene in humans.[5] Additionally, AMS has been used to characterize the extent of DNA adduct formation in humans after exposure to carcinogenic heterocyclic amines at relevant environmental exposure levels.[83, 84]

In the nanoparticle study, the pharmacokinetic (PK) properties and quantitative long-term tissue distribution of ^{14}C-labeled amorphous spherical silica nanoparticles (^{14}C-SiNPs) was determined. Mice were exposed to a single dose of ^{14}C-labeled silica dioxide nanoparticles (0.113 nCi) and blood and tissues were collected over eight weeks.[82] The concentration of SiNPs in tissue and blood over time was determined by quantifying the amount of ^{14}C-SiNPs equivalents at each time point by AMS, and constructing concentration versus time curves. Mean plasma pharmacokinetic parameters showed the SiNPs were rapidly cleared from the central compartment following first order processes with a mean distribution half-life ($t_{1/2}\alpha$) of 0.38 h and an elimination half-life ($t_{1/2}\beta$) of 78.4 h. The long $t_{1/2}\beta$ indicated that not all the nanoparticles were cleared from the plasma within the 48 h sampling time. This was consistent with plasma measurements taken over the study period revealing appreciable levels of SiNPs in the plasma over the eight weeks. The sensitivity afforded by AMS also allowed for quantitative tissue distribution of SiNPs to be evaluated over the eight-week- time course. Initial biodistribution of SiNPs was rapid and was confined primarily to tissues of the reticuloendothelial system (RES) including the spleen, liver, kidney, lung, and cervical lymph nodes; peripheral tissues showed less accumulation of SiNPs. Analysis by AMS revealed that the concentrations of SiNPS varied from tissue to tissue with the highest concentrations occurring in the spleen and liver, followed by lung and kidney. In all tissues examined, the C_{max} concentration of SiNPs occurred within 2 h after dosing indicating a rapid distribution to the tissues. The use of AMS enabled long-term quantitation of SiNPs in tissue following a single low dose administration. This study highlights the utility of using AMS for long-term low dose experiments, which can be applied to assess the long-term fate of xenobiotics at environmentally relevant concentrations in human.

In a study to determine the human pharmacokinetics of the environmental toxicant/carcinogen dibenzo[def,p]chrysene (DBC), a nontoxic microdose of ^{14}C-DBC was administered to human volunteers.[5] The pharmacokinetics of DBC was determined in three female and six male human volunteers following an oral microdosing of ^{14}C -DBC (29 ng, 5 nCi) that was considered of *de minimus* risk to human health. Following exposure, plasma and urine were collected over 72 h and ^{14}C-DBC equivalents in plasma and urine were quantified by AMS. The mean plasma C_{max} was 68.8 ± 44.3 fg·mL^{-1} with a T_{max} of 2.25 ± 1.04 h. Elimination of DBC occurred in two clear phases: a rapid (α)- phase, with a $t_{1/2}$ of 5.8 ± 3.4 h followed by a slower (β)- phase, with a $t_{1/2}$ of 41.3 ± 29.8 h. The pharmacokinetics were notably consistent when body mass index and polymorphisms in metabolism are considered. Elimination in the urine yielded only 1.24% ± 0.49% of the administered ^{14}C dose over the first 72 h post dose. This was presumably due to the high hydrophobicity of DBC. The sensitivity provided by AMS allowed for the safe microdosing of human volunteers with DBC, at environmentally relevant doses, providing

pharmacokinetic parameters for risk assessment that do not rely solely on high-dose animal studies. The ability to determine human pharmacokinetics of carcinogenic chemicals found in the environment and their metabolites is an advancement in risk assessment.

The analysis of potential genotoxins for DNA binding was one of the first uses of AMS in biology. DNA binding has been measured directly in humans to help elucidate the relevance of animal models used to initially assess its genotoxic effects[7, 84]. The genotoxic heterocyclic amine 2-amino-1- methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) was labeled with carbon-14 and given orally (70 μg/person; 17 μCi/person) in a capsule to adult cancer patients who where scheduled to undergo surgery to remove colon tumors. The dose received was equivalent to a real-world exposure.[85] After harvesting colon tissue during surgery, DNA was also isolated from histologically normal colon tissue surrounding the tumor as well as the tumor itself. DNA was also isolated from the colon of rats which where given ^{14}C-PhIP using a regimen that provided a similar dose and exposure time compared to the humans. The results showed that humans produce higher levels of colon DNA adducts than the rats given an equivalent dose, showing that PhIP is bioactivated to a greater degree in humans or has different DNA repair capacity in these two systems. Binding of PhIP to albumin and hemoglobin was also greater in humans, favoring the hypothesis that people have a greater capacity to bioactivate these compounds than rodents.[27]

More recently, to understand the etiology of pancreatic cancer, human volunteers diagnosed with pancreatic cancer were dosed with a microdose of ^{14}C-2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx); a similar heterocyclic amine to PhIP.[30] Blood and urine was collected at several time points after exposure for pharmacokinetic and metabolite analysis, as well, as pancreatic tissue during surgery for DNA adduct profiling. Pharmacokinetic analysis of plasma revealed a rapid distribution of MeIQx with a plasma elimination half-life of approximately 3.5 h. In two of the four cancer patients, very low levels of MeIQx were detected in plasma and urine suggesting low absorption from the gut into the plasma. Urinary metabolite analysis revealed five MeIQx metabolites indicating extensive biotransformation of MeIQx in these volunteers. AMS analysis of isolated DNA revealed very low DNA adducts levels in pancreatic tissue. The results from the this study showed that MeIQx is rapidly cleared from the plasma and is extensively metabolized with less than 5% of unchanged MeIQx remaining in the urine. Under the experimental conditions, MeIQx DNA adducts were detected at or near background levels suggesting that adducts did not readily form in this tissue. These results suggest that exposure to MeIQx alone may not be a significant risk factor for pancreatic cancer. However, other meat-derived HCA mutagens have been shown to covalently bind DNA in pancreatic tissue.[86] These studies may allow for a more accurate prediction of the risk that potential carcinogens pose to humans with the ability to develop human relevant mechanistic data.

AMS has also been utilized to assess the effects of chemopreventative agents at low doses. In a study by Jubert et al. the effects of chlorophyll and chlorophyllin on the pharmacokinetics of Aflatoxin B_1 after low dose exposure in humans was evaluated. Chlorophyll and chlorophyllin have been shown to reduce carcinogen bioavailability in animal models. [87, 88] In addition, a study in China showed that chlorophyllin reduced alfoatoxin-DNA adducts in a population at high risk for liver cancer.[89] In the Jubert study,

the pharmacokinetics of Aflatoxin B_1 were assessed in humans after an oral microdose of ^{14}C-Alfatoxin B_1 with either chlorophyll or chlorophyllin. Results showed that both chlorophyll and chlorophyllin significantly decreased the absorption of Alflatoxin B_1 and reduced the C_{max} and AUCs in both plasma and urine when compared to individuals without treatment. The use of AMS enabled human pharmacokinetic data to be obtained at safe low dose exposures, and confirmed the animal model studies showing that chlorophyll and chlorophyllin may limit Alfatoxin B_1 bioavailability.

More recently, the chemoprotective effects of resveratrol were interrogated using AMS[90]. Cai et al. reported that when the pharmacokinetics and activity using a dietary equivalent dose and a dose 200 times higher were compared a nonlinear response was observed.[90] In rodents on a high fat diet the low resveratrol dose suppressed intestinal adenoma development more efficiently than animals receiving the higher dose. In human colorectal tissue exposed, low concentrations of resveratrol ex vivo and in cancer patients from a ^{14}C-resveratrol trial, enzyme expression profiles indicated better efficacy at the lower doses. These findings highlight the importance of dosimetry for dietary chemopretective agents and the utility of AMS for low dose human studies to define the proper dose levels for optimal efficacy.

Conclusions

AMS applications for human relevant research continue to grow in areas of drug development, risk assessment of xenobiotics and in new areas such as personalized medicine. AMS allows for quantification of low concentrations of analytes and metabolites directly in humans including sensitive subjects such as elderly patients and children. Combining AMS analysis with other highly sensitive techniques such as positron emission tomography can provide complementary information and maximizes clinical data within the same study including: long-term pharmacokinetic information, cellular binding and metabolite profiling. These measurements may provide critical information when assessing new drug candidates or potential toxicity of environmental materials and chemicals.

While AMS provides an isotope ratio measurement with no intrinsic structural information within its measurement, advances in sample fractionation and direct coupling to AMS instrumentation are helping to overcome this limitation. For example, liquid sample online coupling with chromatographic interfaces allows for identification of parent compound and its metabolites within the same sample[19–21] and also reduces the need for highly experienced personnel for sample processing. The unprecedented sensitivity, precision and recent advances in instrumentation highlight the power and increasing importance of AMS based studies in clinical research.

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Abbreviations

AAP	acetaminophen
ADME	absorption distribution metabolism excretion
AMS	accelerator mass spectrometry
AUC	area under the curve
CBDCA	cyclobutane dicarboxylate
CREAM	Consortium for Resourcing and Evaluating AMS Microdosing
C_{max}	maximum concentration
DBC	dibenzo[def,p]chrysene
DPM	disintegrations per minute
EUMAPP	European Microdosing accelerator mass spectrometry Partnership Programme
HCA	heterocyclic amine
HPLC	high performance liquid chromatography
LLNL	Lawrence Livermore National Lab
LS-AMS	Liquid Sample accelerator mass spectrometry
MeIQx	2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline
NEDO	New Energy and Industrial Technology Development Organization
PAMPERS	Paediatric Accelerator Mass Spectrometry Evaluation Research Study
PBMC	peripheral blood mononuclear cells
PD	pharmacodynamics
PEDMIC	Pediatric Microdosing
PET	positron emission tomography
PhIP	2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine
PK	pharmacokinetics
RES	reticuloendothelial system
SiNPs	silica nanoparticles

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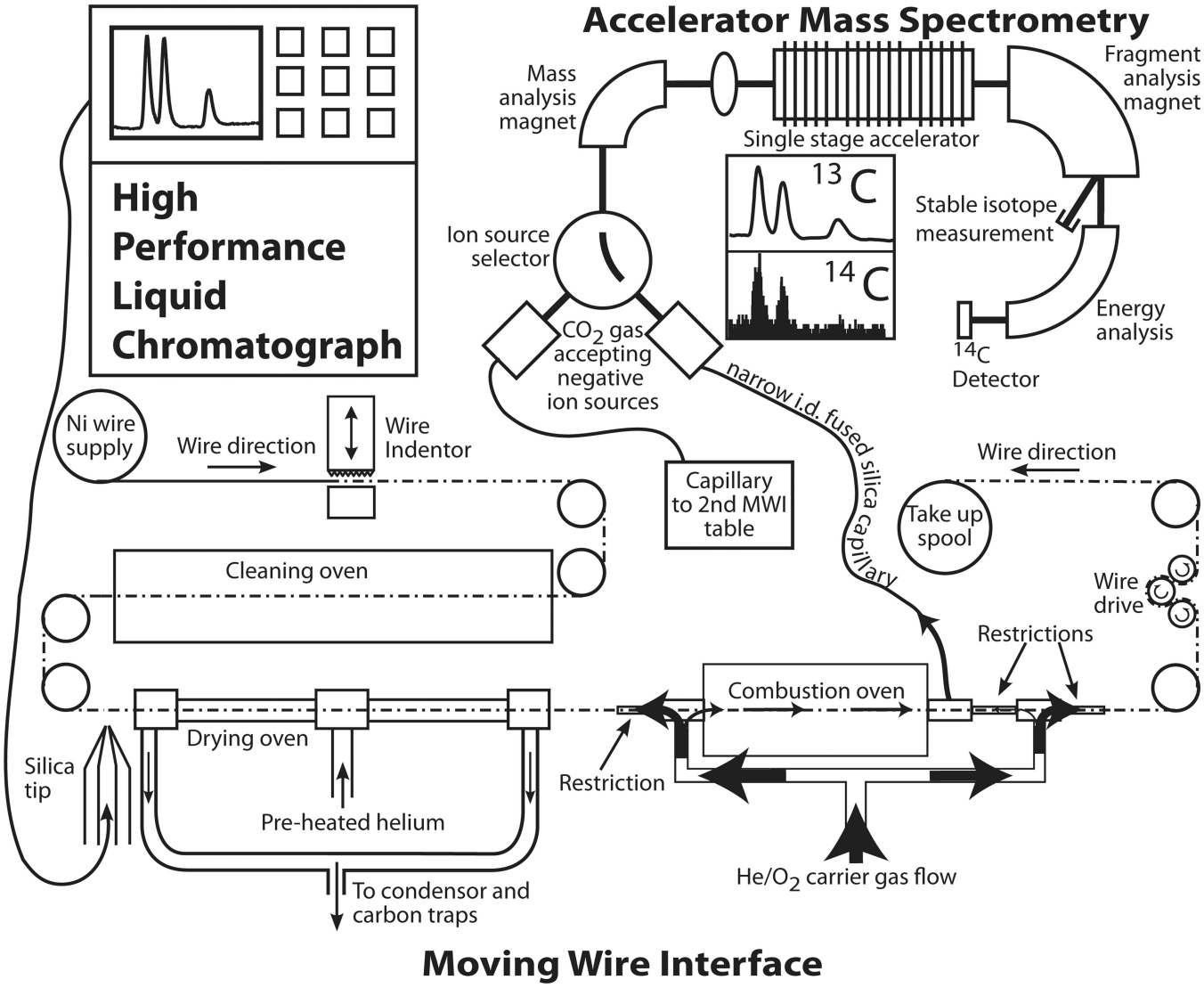

Figure 1.

Schematic of the Liquid Sample AMS system in which a moving wire device is used to enable directly-coupled ^{14}C-AMS quantitation of small liquid samples.

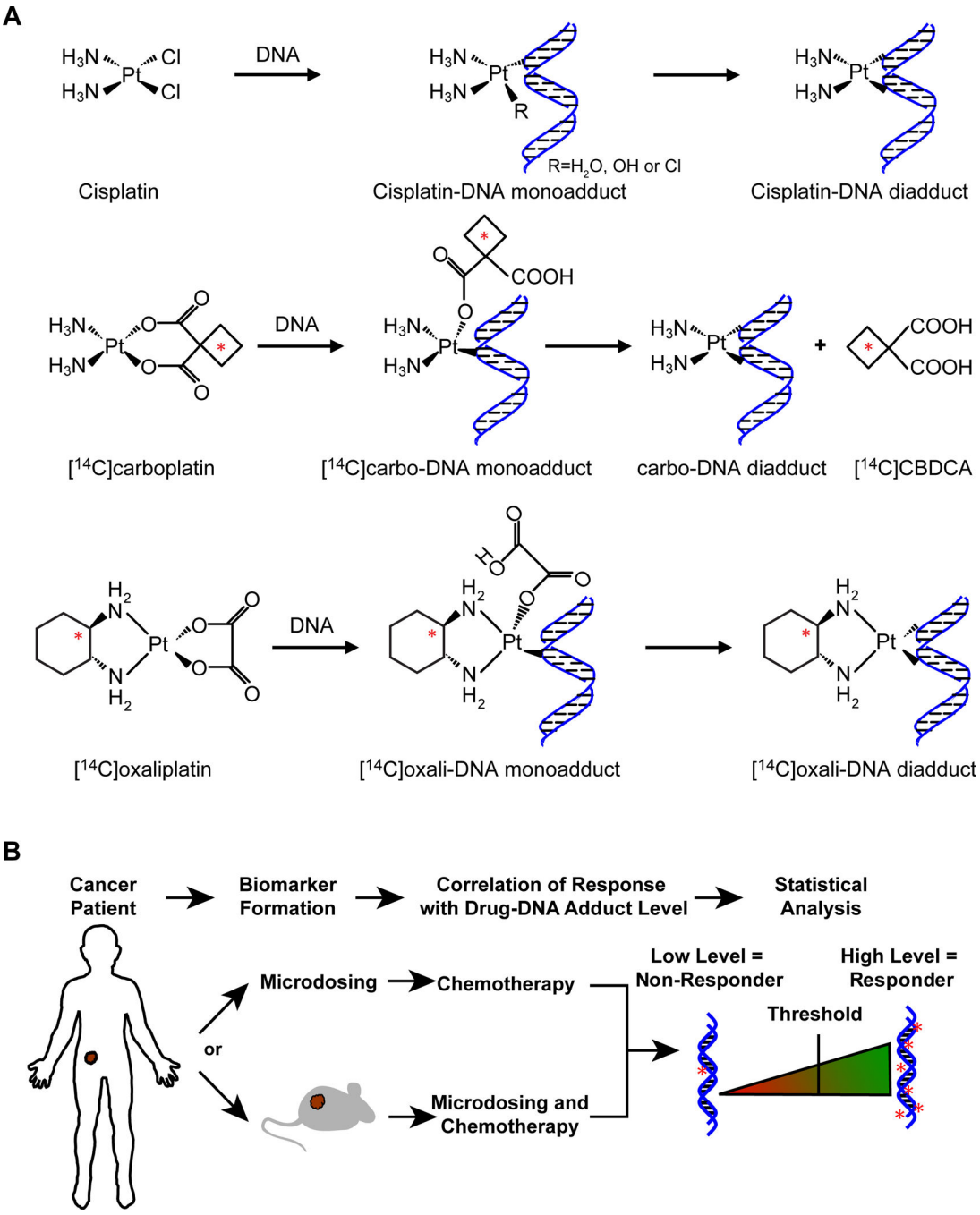

Figure 2.

Simplified schematic, summarizing the strategy for evaluating the feasibility of predicting response to cytotoxic chemotherapy using diagnostic microdosing. **A)** Diagram showing the formation of cisplatin, carboplatin and oxaliplatin-DNA adducts. Cisplatin does not contain carbon atoms and cannot be ^{14}C labeled. The ^{14}C label in carboplatin is included in the cyclobutane dicarboxylate (CBDCA) ligand, which is released upon diadduct formation, therefore only carboplatin-monoadducts can be measured via AMS. Note that cisplatin and carboplatin form the same final diadduct product. The ^{14}C label in oxaliplatin is positioned

with in diaminocylcohexane (DACH) ligand. Because the radiocarbon is located in the DACH carrier group, oxaliplatin-DNA monoadducts as well as diadducts can be detected by AMS. The location of the ^{14}C atom is asterisked. **B)** Experimental design overview scheme. Bladder cancer patients are administered one ^{14}C-labeled carboplatin microdose prior to blood sampling and tumor biopsy. DNA is then isolated from PBMC and tumor tissue and assayed for drug-DNA damage using AMS. Within four weeks patients begin a standard platinum-based regimen with collection of objective cancer response and patient toxicity. The drug-DNA damage levels are being evaluated for correlation to response and to patient toxicity. Or a biopsy portion from patients with myoinvasive bladder cancer are directly propagated in nod scid gamma severe combined immune deficient (NSG) mice in order to establish a sufficient population of NSG-PDX mice for subsequent drug-DNA adduct and treatment response studies.