Journal of Nuclear and Radiochemical Sciences, Vol. 2, No.1-2, pp. R9-R12, 2001

Applications of ^{14}C-AMS for Carcinogen, Pharmaceutical and Nutrient Studies

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Received: November 2, 2001

Accelerator mass spectrometry (AMS) is an ultrasensitive measure for tracing ^{14}C labeled molecules *in vivo* or detecting the biomarker for assessment of carcinogenesis. Basic principles, wide applications and new progresses of bio-^{14}C- AMS have been presented. It is a state-of-the-art tool for measuring the adduction of biological molecules with xenobiotics, including carcinogens, drugs, agrochemicals, nicotine etc.. We have studied the genotoxicity and proteins adduction of smoking specific nicotine and its nitrosamine derivative NNK since 1994. The successful applications have proven the effectiveness of AMS in assessment of cancer risk, screening of drug toxicity and studies of nutrient uptake. In particular, AMS is characterized by measuring xenobiotics at very low dose levels relevant to human environmental exposure. It is sensitive and precise to an attomole (10^{-18} mole) of ^{14}C per mg carbon. Although it has some shortcomings, undoubtedly, AMS possesses an evident merit of high sensitivity and will have widespread applications in the biomedical sciences.

1. Introduction

AMS is usually built up based on a tandem Van de Graaff accelerator or other specific accelerator, which consists of ion source, injection system, acceleration facility, high-energy analysis system, detectors and computer controlled system. In general, AMS quantitates long-lived isotopes by counting isotopic nuclei directly through a variety of physical instrumentations.[1] It provides much greater sensitivity for isotope detection than other decay counting methods, e.g., liquid scintillation counting (LSC). The sensitivity is at attomol of ^{14}C per mg carbon. The detection limit of DNA adducts in AMS measurement reaches 1 adduct/10^{12} nucleotides.[2] Besides the very high sensitivity, the merits of AMS also include smaller sample size, lower specific activity and less measuring time allowed in the measurement.

At present, the major application of AMS is in the fields of geoscience, oceanography and archeology based on the chronological evaluation and dating principle. The early pioneering work of bio-AMS (AMS in biomedical sciences) was done by LLNL in 1989.[3] They measured adduction of DNA with a food carcinogen MeIQx in mouse liver by AMS. In 1994 Liu et al.[4] published a review paper on the non-dating applications of AMS, including various biomedical applications. Nowadays, a variety of studies in bio-AMS field have been done and this sophisticated technique has drawn much more attentions in the biomedical community.

The long-lived isotopes can be used in bio-AMS studies are ^{14}C, ^{3}H, ^{26}Al, ^{36}Cl, ^{41}Ca, ^{99}Tc, ^{129}I etc.. However, the most studies have been carried out with isotope ^{14}C, an isotope of the most essential element in the living organisms. This review paper deals with the studies of carcinogens, pharmaceuticals and nutrients using ^{14}C- AMS.

2. Carcinogens

The carcinogenesis of a xenobiotic is a function of the dose delivered to an organ, as the dose remains in the tissue inducing the adduction or affinity of the xenobiotic to a key biomolecule. The reaction of chemical xenobiotic with DNA appears to be one of the most important and easily happened events in the initiation phase of cancer. The nature of binding is mainly forming a covalent bond between the small toxicant molecule and the macromolecule. Also there are weak noncovalent bindings, such as groove interaction and electrostatic interaction around DNA helix. Thus, a dose and a time response curve for each organ are required for assessing the cancer risk of organs damaged from a xenobiotic. In the past, the relationship between adduct formation and exposure was primarily established at high dose levels of carcinogen due to the limitation of sensitivity in assay. Bio-AMS may offer a distinct advantage of detecting extremely small amounts of ^{14}C-carcinogen bound to DNA.

Table 1 shows ^{14}C-AMS study of carcinogens either to human or animal by researchers of Lawrence Livermore National Lab (LLNL) and their collaborators in U.S. or U.K.. MeIQx, a hetrocyclic amine carcinogen found at the ppb level in cooked beef, is the first xenobiotic studied by AMS.[3] A linear relationship of DNA-MeIQx adducts and doses over a range of 500 ng/kg b.w. (body weight) to 5 mg/kg b.w. in mouse liver was observed. The 500 ng/kg b.w. dose level, corresponding to 1 adduct/10^{11} nucleotides, is the lowest dose the authors can statistically discriminate from the control. This is ten to hundred folds improvement over the best sensitivity offered by the ^{32}P-post-labeling assay, and 3 to 5 orders of magnitude better than other techniques used for quantitative assay of DNA adducts.

TCDD (Dioxin) is an extremely toxic and potent carcinogen. Particularly, in recent years it was recognized as a notorious carcinogenic toxin. In the past, TCDD-DNA adducts were not detectable following 6 months of repeated weekly high dose exposure using the most sensitive assay available at that time.[5] Because of very high-dose taken in the previous work the extrapolation of the effects to lower relevant exposures was suspected. Compatibly, the present AMS measurement has showed that no TCDD related adducts were found except for very small amounts of adduct observed at the lethal dose level.[6] As a very rare exception, these results suggest that the potent carcinogenesis of TCDD is not originated from the effects of initial DNA adduction.

We have measured DNA adduction with one major nitrosa-

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Published on Web 12/28/2001

TABLE 1: Carcinogens Studied by ^{14}C-AMS

Carcinogen	Biomolecular research
MeIQx, 2-amino-3, 8 -dimethylimidazo[4,5-f] quinoxaline	DNA adducts in mouse liver[3] DNA adducts in rat liver and distribution[9] Hemoglobin (Hb), albumin adducts in rat and human[10] Metabolism in human urine[11] Distribution and DNA adducts in human and rodent colon[12] DNA adducts in rodent and human colon[13]
TCDD, 2,3,7,8-tetra-Chlorodibenzo-p-dioxin	DNA adducts in mouse liver, not observed[6]
PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine	DNA adducts in mouse liver, lung, heart and metabolites; fate and distribution in mouse[14] Distribution and metabolism in female rats and their pups[15] DNA and protein adducts in human colon and blood[16] Colon DNA adducts and metabolites in urine of human and rat[17]
Benzene	DNA and protein adducts in mouse mallow chromosome; distribution and liver DNA adducts in mouse[18] DNA adducts in liver and mallow of rat and mouse[19]
Trichloroethylene	DNA and protein adducts in mouse liver[20] Initial uptake kinetics in human skin[21]
NNK, 4-(methylnitrosamino) -1-(3-pyridyl)-1-butanone	DNA adduct in mouse liver[7] Decay kinetics of adducts[8]

tion derivative of nicotine, 4-(methylnitrosamino-)-1-(3-pyridyl-)-1-butanone (NNK). It is a well-known potent and predominant carcinogen and widely present in cigarette smoke as well as unburned tobacco. Its content in cigarettes is roughly 1/10,000 of nicotine content. A good linear dependence of DNA-NNK adduct number on the exposure dose levels is shown.[7] The decay kinetics of DNA-NNK adducts measured shows a slow decay process.[8]

3. Carcinogenic Suspects

It is very important to identify whether an organic compound is carcinogenic or not in the cancer toxicological study. In particular, some organic compounds have proven no property of carcinogenesis by conventional medical tests, but they could be genotoxic that indicates they could finally become carcinogenic. AMS has shown a greater potential than the conventional medical methods to solve the identification problem.

3.1. Nicotine. Nicotine, 3-(1-methyl-2-pyrrolidinyl)-pyridine is a major alkaloid in tobacco products, typically comprising 1-2 % weight of tobacco. Nicotine related to smoking is a dominant factor for tobacco addiction. So far it is unclear whether it is carcinogenic to humans.

Since 1994, our group has carried out a series of genotoxicity studies of nicotine in mouse by ^{14}C-AMS.[7, 8, 22–24] We found that mouse liver/lung DNA adduction as well as histones(H1 & H3)-nicotine adduction increased with increasing dose level of nicotine, which was equivalent to the level of human exposure to cigarette smoking. Very good linear correlations in the dose response were given. Our findings demonstrate clearly that nicotine damages DNA as well as its closely related significant nucleoprotein histones through the chemical binding in-between. Besides, we have measured the decay kinetics of the adducts and the effect of $NaNO_2$ on liver DNA adduction. By these ^{14}C-AMS studies we have drawn to an important point that nicotine should be no longer only recognized as an addiction factor in smoking, but also a potent carcinogen *per se*.

3.2. Insecticides and Herbicides. We began studying the genotoxicity of insecticide pirimicarb by AMS early in 1994.[25] Pirimicarb is a selective and effective antiaphid insecticide widely used in China and other tens countries. By using AMS we found DNA adduction with pirimicarb in mouse liver after exposure to environmental dose of pirimicarb. DNA adducts increased linearly with increasing exposure doses in a log/log plot. However, the number of adducts greatly reduced as the survival time elapsed. In another word, pirimicarb is an insecticide of low genotoxicity, probably owing to the potent repair mechanism occurred in the damaged DNA.

Other groups also studied insecticides exposed in environment, such as atrazine (triazine herbicide), which has widespread use in fields with low toxicity to mammals. The experiment was designed to examine metabolites of atrazine in human urine by AMS.[26] A mean excretion half-life of atrazine in urine of 24 ± 3 h was measured by AMS.

As a special case, the aim of studying the uptake decrease of pyrethroid insecticide ^{14}C-permethrin, which is taken as an analogue of a warfare toxin, in rat central nervous system (CNS) using AMS is to evaluate the medication effect of a pyridostigmine, a pretreatment nerve agent for "Gulf War Syndrome".[27]

3.3. Therapeutic Drugs. Tamoxifen, Z-2-[4-(1,2-diphenyl-1-butenyl)-phenoxy]-N,N-dimethylethanamine, is widely used in adjuvant therapy of breast cancer as a cancer chemopreventative agent. By AMS study carried out at LLNL, tamoxifen was shown to bind to liver DNA of female rats in a linear dose-dependent relationship over a range of 0.1-1 mg/kg b. w., compatible with the therapeutic dose used in women (20 mg/person per day).[28] The genotoxicity of analog of tamoxifen, toremifene (Z-2-[(4-chloro-1,2-diphenyl-butenyl) phenoxy]-N,N-dimethylethanamine) was also measured by AMS.[28] Toremifene binding to rat liver DNA was one order of magnitude lower than that seen with tamoxifen. These results remarkably show that some effective anticancer drugs might be genotoxic *per se*, thus, probably becoming carcinogenic and injurious towards the cancer patients administered drug.

4. Pharmaceuticals

In the pharmaceutical tracing studies the quantitation of ^{14}C-radioactivity is usually made by decay counting with a liquid

scintillation counter (LSC). Compared with LSC, AMS has a large attraction for its much higher sensitivity, in 10^5–10^6 fold enhancement.[26] Consequently, the radioactivity as well as the sample size required for AMS is much smaller than that for LSC. Garner et al.[29] of the CBAMS of York, UK have made a validation study comparing AMS with LSC measurements. In this experiment they used human plasma *in vitro* spiked with ^{14}C-fluconazole, an antifungal drug. An excellent correlation between the two methods (correlation coefficient 0.999) was resulted.

^{14}C-AMS has shown the great potential in studies of absorption, distribution, metabolism and excretion (ADME) of pharmaceuticals in human. It is surely recognized by the medical scientists that ADME studies in humans are absolutely indispensable for developing a new drug before it enters the phase of clinical trials in hospital.

Garner et al.[29] also have analyzed the pharmacokinetic excretion of drug X (anonymous for the drug company's commercial profit) in human volunteers administered orally 300 nCi of ^{14}C-labelled drug X. Each tablet contained 30 mg of cold drug. This dose was selected as it equated to 0.9 μSv i.e., below the British official regulatory standard. Percentage excreted of drug X in total faeces and urine were measured in terms of mass balances. The authors believe that this kind of early clinical studies by using AMS can shorten largely the drug development times, e.g. 1 yr, thus ensuring that drugs come to market earlier.

We are planning to conduct the ADME studies of the bioactive extracts of some Chinese medicinal herbs using AMS technique. These studies may find access to the discovery of the receptor targets of the extract molecules in humans.

5. Nutrients

Around 1998 Vogel et al.[30] created a new application field of AMS studying the distribution and metabolism of nutrients, some vitamins as folic acid and β-carotene. These studies could be largely extended to a great deal of other common nutritives, which are very important to ensure the healthy life of humans. In the experiments, 100–200 nCi doses of ^{14}C as molecular labels in nutrients were given to the testing volunteers without any radiation damage. In fact, a 70 kg person contains ~ 90 nCi of naturally produced ^{14}C in body. A 100 nCi dose yields at least a 20:1 signal to background level for tracing labelled compounds that may provide satisfactory AMS measurements.[31]

Since the ^{14}C-triolein, glycerol tri[1-^{14}C]oleate tests are widely used in the clinical medicine for the determination of fat malabsorption, AMS has been applied in the long-term turnover study of this fatty acid ester in humans at University of Lund, Sweden.[32] The measurements were carried out up to 4.5 yr. after administration. ^{14}C contents in exhaled air, fat, muscles, bones, faeces and urine were measured. 60% ^{14}C activity was recovered and the major part of the missing fraction was owing to the uncertainties connected to the large amount of the endogenous production of CO_2.

Very recently, AMS was used in an exotic way to distinguish fraudulent synthetics from the naturally products.[33] Nowadays, the public consumer trend towards foods and beverages labelled " natural " has created a growing demand. However, in the market so many "natural" foods are blended with inexpensive synthetics. The source of synthetics is mostly petrochemically derived compounds, which contain a low level ^{14}C from the "dead carbon". Through the AMS measurement it is easy to find the fake nutrient blended in the real natural products which originally contains a higher level of ^{14}C equilibrated with the ambient ^{14}C in the natural environment.

6. Important Progresses of Bio-^{14}C- AMS

6.1. AMS Tests in Human Subjects. LLNL began the studies of uptake and metabolism of anticancer drugs in cancer patients early in 1990.[6] Nevertheless, even though the ^{14}C radioactive dose administrated to human host was very low, far apart from the radiation limit standard, it is hard to recruit testing patients or volunteers. In the preceding paragraphs, we presented the fat metabolism studies in humans by Swedish group and the metabolism studies of folic acid and β-carotene in volunteers by LLNL group.[30, 32] In 1999 LLNL also measured DNA-MeIQx adducts and DNA/protein-PhIP adducts in 5 colon-cancer patients.[16] In all the tests, the radioactivity of ^{14}C was limited to only a max. < 1 μCi administered per person. The poor availability of the testing bodies is an inevitable bottleneck of this study.

6.2. AMS Coupled with HPLC and Other Techniques. High performance liquid chromatography (HPLC) is a popular technique for the chemical separation of complex mixtures, whose profile can afford information of chemical mechanism and estimation of the biomedical pathways of metabolism. Compounds elute from the HPLC systems with characteristics retention times and can be collected in fractions, which can be subsequently graphitized for AMS measurement.[34] A combination of AMS with this sophisticated method has largely benefited the studies of xenobiotics PhIP, trichloroethylene, benzene, atrazine, β-carotene, drug X etc..

Besides, gas chromatography (GC), thin layer chromatography (TLC) and radioimmunoassay have also been coupled with AMS that also makes AMS more efficient and satisfactory in the biomedical applications.

7. Prospect of Bio-^{14}C- AMS Studies

Bio-^{14}C-AMS offers the quantitative measurement with ultra-high sensitivity of low-frequency biomolecular events following exposure to environmental dose level of xenobiotics. Samples of 1 mg or less are easily handled and absolute resolution can be 1 attomole per mg of carbon that has priorities over all the other methods. Of course, it has been a new advanced tool in the biomedical, toxicological, pharmaceutical and nutritional sciences. Particularly, it will become increasingly important in cancer risk assessment, pharmaceutical screening and ADME studies of drugs and nutrients.

However, there are some remarkable disadvantages or limitations in AMS method: the very large size and high investment of accelerator, the special radiolabelled compound and the troublesome preparation procedure of the graphite sample all required for AMS measurement. Moreover, the large community of biomedical researchers is largely unaware of this new technology and the term AMS is often a confusing glossary to them.

But we believe that the merits and advantages of AMS method far outweigh its defects and disadvantages. The researchers of AMS are gearing up to meet the foreseeing needs of the biomedical sciences.

Acknowledgement. This work was supported by the National Natural Science Foundation of China (Grant Nos. 19935020 and 29871004).

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