Journla of Molecular Pharmaceutics & Organic Process Research

ISSN: 2329-9053

Lingli et al., J Mol Pharm Org Process Res 2014, 2:1
DOI: 10.4172/2329-9053.1000112

Research Article

Open Access

Determination of Intracellular Concentrations of Nucleoside Analogues and their Phosphorylated Metabolites

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Abstract

Nucleoside analogues are broadly used in antiviral and anti-tumor therapy. The clinical response depends on the intracellular formation of the pharmacologically active mono-, di-, and tri-phosphates moiety. So it is not advisable to simply monitor plasma concentration without concerning the variation of concentration of active constituent in some particular effector cell apparently while this kind of drugs are applied to the clinical therapy. Therefore, determination of intracellular concentration of nucleoside analogues and their phosphorylated metabolites is of great significance. In this review, several methods including RP-HPLC, LC-MS/MS, radioimmunoassay and capillary electrophoresis (CE) for analysis of anti-viral and some anti tumor nucleoside analogs are discussed. Because of the complex biological matrices as well as the extremely low concentration of target analytes, cell lysis techniques and sample pretreatment methods such as protein precipitation (PP) and solid-phase extraction (SPE) are also discussed.

Keywords: Review; Nucleoside analogues; Phosphorylated metabolite; Intracellular concentration 3. Introduction

Nucleic acids are a kind of important biomacromolecules and the most important genetic materials of organism. The basic structural unit of nucleic acid is nucleotides, while the nucleoside is a prerequisite to the synthesis of nucleotide. People have been studying them for more than one hundred years since nucleotides were discovered in 1868. Now the researchers focus on two aspects: one is to decipher the genetic gene and the other is to synthesize various of nucleoside analogues based on the nucleic acids, which have gained great success and been applied into genetic research. Nucleoside analogues have also been broadly used in clinical practice, especially in antiviral and anti-tumor treatment. Antiviral drugs such as Lamivudine, Adefovir, Enticavir, Telbivudine, Zidovudine, etc, and antitumor drugs such as Fludarabine, Cladribine, Gemcitabine, Nelarabine, etc, are all nucleoside analogues.

After the first antiviral nucleoside analogue-idoxuridine was found, nucleoside analogues have been accounting for an important position in antiviral drugs. More than fifty percent of antiviral agents now used are nucleoside analogues. Nucleoside analogues are now among the most widely used drugs in antiviral treatment. Meantime, nucleoside analogues (NAs) are a family of anti-metabolites. As important anticancer chemotherapeutic agents, nucleoside analogues have been widely used in the treatment of solid tumors, malignant disorders of the blood, autoimmune disorders as well as multiple sclerosis and HIV infection. The antitumor nucleosides include several analogues of physiological pyrimidine and purine nucleosides.

As antiviral and anticancer drugs, nucleoside analogues have attracted more and more attention in recent years. As demonstrated in several studies, prototype drug and metabolite of nucleoside analogues often have no bioactivity in blood. Only after the drugs enter cells and are phosphorylated by cellular kinases (deoxycytidine kinase and other nucleotide kinases) to triphosphate [1], they can produce pharmacological activities [2-4]. In the anti-tumor therapy, nucleoside analogues induce cytotoxicity through its active phosphorylation product, which will result in inhibition of DNA synthesis and death of apoptotic cell. The mechanism of its anti-tumor activity can be described in three steps: being incorporated into and altering the DNA and RNA, interfering with various enzymes involved in synthesis of nucleic acids, and modifying the metabolism of physiological nucleosides [5,6]. When used in the antiviral therapy, nucleoside analogues need to be phosphorylated to their triphosphate derivatives in order to become active on their cellular target to inhibit DNA elongation and to exert their biological effects [7,8]. It is evident that intracellular phosphorylation is indispensable in the pharmacological actions of nucleoside analogues. Nucleoside analogues are different from other drugs both in the existence form of active ingredient and location of the active site.

In pharmacokinetic studies, plasma concentrations are usually used to indicate the drug effects. But according to the pharmacological mechanism of nucleoside analogues, it is obvious that the parameter for predicting the clinical efficacy of a given nucleoside analog is not the plasma drug concentration, but its active constituent concentration in particular effector cell. Therefore, pharmacokinetic evaluation of its active constituent will provide crucial information to optimize nucleoside analogues therapy. Most of the nucleoside analogues varied significantly individually in drug efficacy and serious toxicity, and their side effects are also frequently observed. Evidences show that serious toxicity and side effects, such as myelosuppression, thrombocytopenia, cardiac toxicity, etc, are mostly related to active phosphorylation product in the cells [9,10]. Simply monitoring plasma concentration without concerning the variation of concentration of active constituent in some particular effector cell apparently cannot meet the clinical monitoring demands of these drugs.

Most of nucleoside analogues are hydrophilic molecules and can't readily permeate the plasma membrane, so they need some specific nucleoside-specific membrane transport carriers to enter the cell, and then turn into active phosphate derivatives by related phosphokinase.

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Received February 24, 2014; **Accepted** April 28, 2014; **Published** April 30, 2014

Citation: Lingli Mu, Xingling Liu, Sanwang Li, Fang Tang, Peng Yu (2014) Determination of Intracellular Concentrations of Nucleoside Analogues and their Phosphorylated Metabolites. J Mol Pharm Org Process Res 2: 112. doi: 10.4172/2329-9053.1000112

It is generally considered that the step occurring between a nucleoside analogue drug and its monophosphate is the rate-limiting step of cellular metabolic processes. It's evident that determination of intracellular active metabolites concentrations is of great significance.

Clinical Significance

Nucleoside analogues have brought clear benefits to antiviral and anticancer treatments. Meanwhile, the side effects of this kind of drug can not be neglected [11]. Actually, early in 1995 it is reported that a potential anti-HBV nucleoside analogues, fialuridine, had severe toxic reactions including hepatic failure, lactic acidosis, serious muscle damage and nervous lesion in phase 2 clinical trials, and the phase 2 clinical trial of the drug was quickly terminated [12]. An earlier case can date back to 1970s and 1980s that adenine arabinoside monophosphate ever used for clinical treatment was inhibited in clinical application because of its neurotoxicity [13]. Clevudine, a thymidine L-nucleoside analogue, has a very strong effect on inhibition of hepatitis B virus replication, as well as low relapse rate after withdrawal, but Kim BK [14] reported that two chronic hepatitis B patients presented severe myopathy after administration of clevudine, and the clinical symptoms of myopathy developed after 12 and 10 months of clevudine treatment. Lok [15] discussed some myopathy and neuropathy associated with nucleos(t)ide analog therapy for hepatitis B in his review. He reported some cases of myopathy and mitochondrial toxicity after clevudine treatment, and he also mentioned that fialuridine had toxicity like mitochondrial damage and that telbivudine, especially when used in combination with pegylated interferon, could develop myopathy and neuropathy.

All these cases undoubtedly give us some enlightenment that nucleoside analogues obviously have some side effects and even severe toxicity. So, knowing how to control the risks and gain more therapy benefits in clinical applications of these drugs is very important.

Nucleoside analogues have their own particularity and special characters in drug-safety, intensity of drug effect, the occurring time and incidence of drug resistance, applicable persons and treatment methods for drug resistance and adverse reaction. Some rare adverse reactions in the long-term treatment should also be paid great attention to. Although every new drug has to be strictly tested in several clinical trials, not all toxicities are evident during trials. In the last several years, many drugs have been withdrawn from the market only after these drugs have been used in hundreds of thousands of patients in clinical practice. Establishing a causal relationship between an approved drug and a major adverse event is greatly demanded. It is evident that long-term safety of nucleoside analogues treatment and monitoring of toxicities during drug development are core points [15]. Under this condition, developing a validated method for determination of intracellular concentrations of nucleoside analogues and its phosphorylated metabolites is of fundamental significance.

Cell Lysis Techniques

Accurate detection of intracellular drug concentration needed to lyses the cells fully. There are different degrees of extraction when using different cell lysis methods.

Chemical cleavage

Move the cell culture plate to the super clean bench, discard the supernatant, and add cell lysate and oscillating adequately. Then stand at the room temperature for fully lysis. After standing, collect the cell lysate in ethyl pyruvate tube (EP) for the detection of intracellular drug concentration. Chemical cleavage can lyse the cells completely, but experiments [1] have confirmed that the chemical cleavage method has large effect on the activity of ALP (alkaline phosphatase) in the cells. There might be a chemical reagent which affected the protein spatial structure. It is also possible that using chemical cleavage to broken cells, and dissolute ALP, to produce more broken objects of cells that have inhibitory effect on the enzyme activity.

Ultrasonic

Move the cells in the culture plate to the ultrasonic cells grinder soundproof box, select the appropriate conditions for ultrasonic, then move to the super clean bench, beat repeatedly and collect the cell lysate for the detection. Or let part of sample under the water while handling the Eppendorf tube vertically in ultrasonic instrument. The energy produced by the ultrasonic grinding can increase the liquid temperature and then damage the activity of the cells enzyme [2]. It was found that ultrasonic granding cannot completely lyses cells.

Freeze-thawing

Remove culture plate, discard the supernatant and add the quantitative PBS (phosphate buffer) in teach hole. Move cells to -80° C for freeze-thawing, frozen melt at room temperature. While melting, appropriately shocks and vortex are acceptable. Repeated freezing and thawing three times and collect the cell lysate. Pure physical methods repeated freezing and thawing cannot completely lyses cells or fully release the enzyme which has certain effect on the detection. In addition, the value variation coefficient of ALP activity detection increase significantly in the cell lysate processed by single freeze-thawing, which might be related with to freezing and thawing conditions. Therefore, freezing and thawing make people question about the reliability of the detection.

Combination of ultrasonic and freeze-thawing

Remove culture plate, discard the supernatant and add the quantitative PBS (phosphate buffer) in teach hole. Move cells to -80° C for freeze-thawing, frozen melt at room temperature. While melting, appropriately shocks and vortex are acceptable. Move to the ultrasonic cells grinder soundproof box after repeating freezing and thawing twice, select the appropriate conditions for ultrasonic, then move to the super clean bench, beat repeatedly and collect the cell lysate for the detection. Ultrasonic after freezing and thawing can avoid high temperature; also reduce the repeat times to improve the precision of the experiment. It can release the ALP completely from cells with the less or none factors affecting enzyme activities.

Other methods

Bead mill homogenization refers to add sterilized glass beads in an Eppendorf tube and vortex, so it cannot lyses cells completely, and leads to the fracture of DNA. Liquid nitrogen method refers to guide into the proper amount of liquid nitrogen to make samples frozen, grind to the gray powder and then process. It cannot completely lyses cells, which affects the detection of the intracellular drug concentration. High temperature extraction refers to bath in high temperature water, which produces less broken cells and low lysis efficiency. At the meantime, the activity of enzyme in cells is easy to be damaged by high temperature, which brings a certain impact on the accuracy of the results.

Sample preparation

Sample preparation is an essential stage in the analysis process. It takes place between sample taking and measuring the prepared

sample. Sample preparation is a very important step in most analytical techniques because the techniques are often not responsive to the analyte in its prototype form, or impurity substances in samples interfere with the results. Because of the complexity of the matrix, biological samples such as urine, serum or plasma, tissues, etc., cannot be directly injected into a separation system. Therefore, corresponding sample treatment steps are required prior to analysis. The main goals of sample pretreatment are to reduce matrix effects, enhance selectivity and transform the analytes to a compatible form for analysis depending on which technique we choose [16].

The analysis of nucleosides and nucleotides have been performed in various matrix such as whole blood [17,18], erythrocytes [19], peripheral blood mononucleous cells (PBMC) [20-22], cultured cells [23], cerebrospinal fluid (CSF) [24] or tissues [25,26]. We intend to investigate the intracellular concentrations of nucleoside analogues and their phosphorylated metabolites, but there are lots of enzymes and other bioactive substances in cell matrices [27-29]. Therefore, we have to lyse the cells firstly to extract the target compound. Nucleoside analogues and the mono-, di-, and tri-phosphates are not that stable. So, the extraction is generally performed at low temperature or in a dark place. Since nucleoside analogues has its own particularity in structure and physicochemical property, the sample preparation methods depend partially to the detection mode and sample matrix [30]. In general, the common sample preparation methods used for determination of nucleosides and nucleotides and their phosphorylated metabolites are protein precipitation (PP) and solid phase extraction (SPE).

Protein precipitation

Precipitation is widely used for product recovery of biomolecules especially proteins. Precipitation is usually induced by addition of a salt or an organic solvent or by changing the pH to alter the nature of the solution. Protein precipitation (PP) is the simplest procedure to remove proteins in plasma and other biological matrices. The inorganic acid, organic acid or organic solvent such as perchloric acid (PCA) [17,31], trichloroacetic acid (TCA) [32], acetonitrile [ACN] [33-38] and methanol [39,40] were commonly used reagents for precipitating proteins in biological samples. Subsequently the mixture was centrifuged to remove the denatured proteins [41].

In protein precipitation, compared with perchloric acid (PCA), trichloroacetic acid (TCA) was less frequently used due to its poor compatibility with detection by MS [42,43]. While for analysis of gemcitabine phosphate deratives, TCA was preferred to PCA due to its negative effect on the retention behavior of nucleotides in ion-pair chromatography [44,45]. Losa R., et al. [32] isolated gemcitabine di- and triphosphate in peripheral blood mononuclear cells (PBMC) by using TCA as PP reagent. 10mL of patient blood samples were firstly collected into heparin containing tubes to which 50μL of tetrahydrouridine (inhibitor of plasma cytidine deaminase) were added immediately before use. Then the blood samples were kept at 4°C and diluted (1:1, v/v) with PBS and consecutively the 10mL of mixture was layered and isolated over 5mL Lymphoprep contained in 15mL conical tubes. Before analysis, the samples were treated with 80μL 40% trichloroacetic acid, mix-vortexed and placed in an ice bath for 20 min. After centrifugation at 16,000×g during 10 min at 4 , the supernatant was collected and 640μL of freshly prepared trioctylamine-freon (1,1,2-trichlorotrifluorethane) mixture (1:4, v/v) were added. After centrifugation (2 min, 16,000×g, 4), the organic layer was carefully removed. This extraction procedure was repeated for three times to allow the concomitant cleaning and neutralisation of the sample. Finally, 200μL of the aqueous layer was injected into the HPLC system.

Solid phase extraction

Solid phase extraction (SPE) is a separation process by which compounds that are dissolved or suspended in a liquid mixture are separated from other compounds in the mixture according to their physical and chemical properties. The extraction is most often accomplished by passing the sample through a column that is packed with a sorbent, which allows the liquid to pass through the apparatus while the solid remains are collected above. The purpose of solid phase extraction is to concentrate and purify samples for further study. These analytes might be contained in the solid or liquid phase. If the material to be analyzed is with the solid phase, then it can be purified by a solvent rinse. The general procedure is to load a solution onto the SPE phase, wash away undesired components, and then elute the desired analytes using another solvent into a collection tube. Solid phase extraction can be used to isolate analytes of interest from a wide variety of matrices, including urine, blood, water, beverages, soil, and animal tissues. SPE is similar to chromatography in that a liquid mixture is dispensed through a packed column that removes analytes and impurities. The procedure can be accomplished on a large scale using manifolds to hold multiple columns so that several samples can be processed simultaneously.

Generally, the major challenge in developing a simultaneous method for the quantitative analysis of target compound and its metabolites is that one of the analytes is highly water-soluble whereas the other one is highly hydrophobic. Therefore, if we use the standard two-phase LLE methods, there is often a loss of one or the other analyte and this could compromise the recovery and the method sensitivity for either of them. The intracellular concentrations of nucleoside analogues and its phosphorylated metabolites, such as entecavir and its mono-, di-, and tri-phosphates [46], are extremely low. In this case, conventional reversed-phase liquid chromatography can't meet the demands for detection limits and sensitivity, and then an LC-MS (MS/MS) method is needed. For MS detection, SPE is prior to protein precipitation because of its great capability of impurity cleaning. Emotte C., et al. [47] found that for blood samples, protein precipitation were not always compatible with LC-MS/MS analysis as they observed some sensitivity loss and MS clogging during the sample analysis. Consequently, they found that C18 SPE was suitable for fingolimod and fingolimod-phosphate extraction from biological matrices due to its automation and on-line operation with LC-MS/MS detection and the SPE method offered high- speed, high sensitivity for the sample pre-concentration. Lane R. Bushman, et al. [48] developed a LC-MS/MS method with solid phase extraction to determine nucleoside analogue mono-, di-, and tri-phosphates in cellular matrix. Firstly, the SPE cartridges were activated by wash with 2mL ultrapure water, 1.5mL 1 M KCL, and 2mL 5mM KCL. After the activation, samples were loaded onto SPE cartridges. The MP elution was accomplished with 5 mL of 75 mM KCL; elution of DP was accomplished with 7 mL of 90 mM KCL; and the final TP elution was accomplished with a single 2 mL wash with 1 M KCL. Then the isolated MP, DP, and TP NA fractions were then treated with 100 μL of acid phosphatase in 1 M sodium acetate, pH 5, to dephosphorylate to their corresponding parent nucleoside analogue. The mixture was vortexed and incubated for 60 min at 37 in water bath. Then the Phenomenex Strata-X SPE column was used to de-salt and concentrate the samples. Through all these procedures, the samples can be injected into the LC-MS/MS system.

Liquid Chromatography

HPLC-UV

High performance liquid chromatography (HPLC), as a powerful

tool in analysis, is basically a highly improved form of liquid chromatography. Instead of the natural gravity which allows the solvent dripping through the column, the HPLC system has a pump which provides high pressure required to move the mobile phase and sample components through the densely packed column. That makes the separation much faster. HPLC also has some characteristics such as high efficiency, high sensitivity and wide application compared with the ordinary column chromatography. These advantages make it become a good choice for the determination of nucleoside analogues and its metabolites.

Homma, M.et al [49] developed a high-performance liquid chromatographic (HPLC) assay for ribavirin in whole blood to estimate concentrations of free ribavirin and phosphorylated anabolites in erythrocytes. Reversed-phase HPCL with UV detector was used for this determination, and the wavelength was fixed at 235 nm. As the mobile-phase solvent, 10 mM ammonium phosphate buffer (pH 6.5) was used at a flow rate of 0.7 ml/min for analysis of enzyme-treated samples. For nontreated samples, the pH of the mobile phase was adjusted to 2.5. This reversed-phase HPLC method was optimized for determination of ribavirin in whole blood or erythrocytes.

HPLC-UV methods are the most simple and cost-saving among all the methods. But, mostly HPLC-UV methods are even not sensitive enough to monitoring the concentrations of nucleoside analogues, especially for the samples from human volunteers. As to their phosphorylated metabolites, there are only few articles published on this topic. And no HPLC-UV methods are reported to be applied to the clinical pharmacokinetic studies.

LC-MS/MS

Liquid chromatography (LC) coupled to mass spectrometry (MS) or tandem mass spectrometry (MS/MS) has become increasingly important in drug and biomedical analysis. As an experimental technique developed in the early 1990s, it has now become a reliable and robust analytical technique for routine analysis. Compared with other commonly employed techniques, LC–MS/MS techniques can provide specific, selective and sensitive quantitative results with relatively reduced sample preparation and analysis time.

A sensitive and specific LC–MS/MS method was developed for determination of the nucleoside analog mono-, di-, and tri-phosphates in cellular matrix [48]. Red blood cells were firstly isolated by flow cytometry. Once cell samples were isolated, purified and counted, the cells were lysed with 0.5 mL cold methanol-ultrapure water (70:30, *v/v*) and stored at −80°C. Then lysed cellular matrix was made. Mono-, di-, and tri-phosphates of lamivudine (3TC) and lamivudine-isotopic internal standard ($^{15}N_2$, $^{13}C_1$, 3TC-iso) were extracted from lysed cellular matrix using Solid Phase Extraction (SPE), then the samples were injected into the LC-MS/MS system. The mobile phase had two conditions: one contained 2% acetonitrile and 0.1% formic acid in ultrapure water at an isocratic flow of 250 µl/min, and another contained 6% 2-propanol and 0.1% acetic acid in ultrapure water at an isocratic flow of 200 µl/min. A Thermo Scientific TSQ Vantage triple quadrupole mass spectrometer coupled with a HESI II probe was used as detector and was operated in the positive ionization mode. Selected reaction monitoring (SRM) was used to quantify mono-, di-, and tri-phosphates of lamivudine (3TC) and lamivudine-isotopic internal standard ($^{15}N_2$, $^{13}C_1$, 3TC-iso), respectively. In both conditions, standard curves were linear between 0.1 pmol/sample and 200 pmol/sample for 3TC. The method was successfully used to analyze hPBMC and RBC samples containing nucleoside analogue-phosphates in either HIV-negative or HIV infected subjects, as well as for clinical investigation of drug pharmacokinetics.

LC-MS/MS methods are much more sensitive than HPLC-UV methods for most small molecules. So, it is reasonable for the researchers to choose LC-MS/MS methods while determining the nucleoside analogues and their phosphorylated metabolites. Unfortunately, for the remarkable strong polarity of the phosphorylated metabolites LC-MS/MS is not always a sensitive method, especially it is used with a mobile containing high proportion of water solution. The normal way to resolve this problem is usually to hydrolyze the phosphorylated metabolites to the original moieties, which steps usually sharply increase the cost for both time and money.

Radioimmunoassay

Radioimmunoassay (RIA) is a method based on the antigen-antibody binding reaction. Berson and Yallow of Albert Einstein School of Medicine initially developed the method to determine insulin levels in human plasma in 1960 [50], which was considered to be a revolution in medical investigations at that time. Due to its specificity, high sensitivity, simple operation, and does not require sample pre-treatment,it is widely used in the clinical diagnosis of disease.

A Cartridge Radioimmunoassay method for determination of intracellular levels of lamivudine triphosphate (3TC-TP) in the peripheral blood mononuclear cells of human immunodeficiency virus-infected patients has been developed [51]. The procedure involves rapid separation of 3TC-TP by using Sep-Pak cartridges, dephosphorylation to 3TC by using acid phosphatase, and measurement by radioimmunoassay using a newly developed anti-3TC serum. Peripheral Blood Mononuclear Cells (PMBC) was firstly isolated by centrifugation over Ficoll-Hypaque. Nucleotides which were extracted from PBMCs were kept at -20°C until analysis. Then Sep-Pak cartridges were used for separation of 3TC-TP. The cell lysates (cultured or HIV-infected PBMCs) were loaded onto the cartridges, and sample fractions were collected in polypropylene tubes. 3TC and its mono-, and diphosphate derivatives were eluted from the cartridge with 8 ml of 95 mM KCl, and 3TC-TP was eluted with 5 ml of 300 mM KCl. Then phosphate groups were cleaved by the addition of acid phosphatase (sweet potato type XA). After that, the dephosphorylated cell or plasma samples with anti-3TC antiserum and [^{3}H] 3TC label were combined, and the mixture was cultured for 2 h at room temperature, and goat anti-rabbit secondary antibody was added to each tube. After a stand for 30 min, the samples were centrifuged and then suspended with the pellets in 0.1 N HCl. Then the radioactivity in 500 µl samples was determined, and the drug concentrations were calculated from the standard curve. In seven subjects, intracellular 3TC-TP levels ranged from 2.21 to 7.29 pmol/106 cells, and concentrations of 3TC in plasma determined in these subjects ranged from 0.34 to 9.40 mM. This method permitted the analysis of a large number of patient samples, which proved to be very useful for in vivo pharmacodynamic studies.

Radioimmunoassay and other immunoassay methods like it can be good choices for determination of nucleoside analogues and their phosphorylated metabolites only if we can find out the appropriate antibodies. Actually, as be well known, it is quite difficult and time-consuming to develop that. If there is no certain necessity, this way is often avoided for its application values are usually below the developing costs.

Capillary Electrophoresis

Capillary electrophoresis (CE) (or high-performance CE, HPCE)

is an instrumental evolution of traditional slab gel electrophoretic techniques [52].This kind of technique can separates ions based on their electrophoretic mobility with the applying of an high voltage. The electrophoretic mobility is dependent upon the charge of the molecule, the viscosity, and the molecular size. The particle moving rates are directly proportional to the applied electric field: the greater the field strength, the fast the mobility. If two ions are at the same size, the one with greater charge will move faster. For ions with the same charge, the smaller particle has less friction and overall the faster migration rate. Capillary electrophoresis is used most predominately because it gives faster results and provides high resolution separation. The main advantages of capillary electrophoretic techniques include high separation efficiencies, low sample consumption and short analysis time, and it is easily automated and consumes limited amounts of reagents, generating low volumes of waste [53,54].

Breadmore MC, et al [55] demonstrated a simple and efficient method for separation of ribavirin and 5-methylcytidine (internal standard) by capillary electrophoresis. The detection and quantitation limits were determined to be 0.05 and 0.10 μg/mL, respectively, which is suitable for therapeutic drug monitoring of ribavirin in human plasma and serum samples. Puli, Nantou, et al, developed a simple and rapid capillary electrophoretic method for the simultaneous determination of thymidylate (TMP) and thymidine 5'-diphosphate (TDP) in enzyme assays without using radioactive-labeled substrates. Using deoxyadenylate as an internal standard, this method had a linear range of 5-200 mM, and the concentration limits of detection of TMP and TDP were 2.6 and 3.8 mM, respectively.

For the multi-charged nucleoside analogues' phosphorylated metabolites, capillary electrophoresis provides the powerful separation capacity, which gives dozens of times theoretical plates more than HPLC methods. When it comes to the ability of quantification, the accuracy and precision of capillary electrophoresis methods are often not able to match the basic requirements particularly for analyzing the biological samples. Capillary electrophoresis need to be improved significantly before it could be widely used in practical clinical studies rather than only in separation theoretical researches.

Conclusion

The growing importance of nucleoside analogues has stemmed both from the development of newer compounds with broad applicability to common cancers and from an understanding of their mechanisms of action, enabling pharmacological intervention to potentiate the anti-tumor effects of these compounds.

In this paper we review anti-viral and some anti-tumor nucleoside analogs commonly used in the clinic and methods using RP-HPLC, LC-MS/MS, radioimmunoassay and capillary electrophoresis (CE) for analysis of these anti-viral and anti-tumor nucleoside analogues and their phosphorylated metabolites (summarized in Table 1). Further investment and research can pay close attention to the development of more compatible method with high-speed and high sensitivity. Meanwhile, sample preparation also plays an indispensable role in the selectivity and sensitivity of the methods and more effective sample extraction procedures with high recovery is demanded as well.

Methods	Advantage	Disadvantage
HPLC-UV	Simple Cost-saving	Not sensitive enough
LC-MS/MS	Sensitive Most used	Needing hydrolysis Costly Complicated and time-consuming
Immunoassay	Simple and easy Practicable Sensitive	Hard to establish antibodies
Capillary electrophoresis	Strong separation ability Well matched with high polarity solution	Absence of sensitive detector Unstable migration property

Table 1: Comparison of the analytical methods for anti-viral and some anti-tumor nucleoside analogs.

Acknowledgement

This work was supported by the National Natural Science Foundation of China (No.81102499), the Fundamental Research Funds for the Central Universities of Central South University and Hunan Science and Technology Project (No. 2011SK3261).

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