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The determination of acyclovir in sheep serum, human serum, saliva and urine by HPLC

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Objective The aim of this study is to determine acyclovir concentration in sera of sheep and human as well as human urine and saliva.

Methods Acyclovir determination is achieved on C8 (150x4.6 mm) column by high performance liquid chromatography (HPLC) with fluorescence detector. Extraction of samples with HClO4 helped to precipitate proteins whilw shortening clean-up steps.

Results Compare to prior studies, utilization of 0.02 M HClO4 organic solvent as a mobile phase reduced the separation expenses. Besides mobile phase was delivered at isocratic condition. Each sample has been reproducible within 10-30 minutes depending on kind of sample. Recovery of acyclovir from sheep sera was 81.6%. After oral administration of 200mg dosage to healthy volunteers, saliva acyclovir concentration reached a level of 1.25 μg/ml±3.4 SE within one hour. Serum acyclovir concentrations seem to be negatively correlated with saliva concentration (r:-0.99, p<0.009).

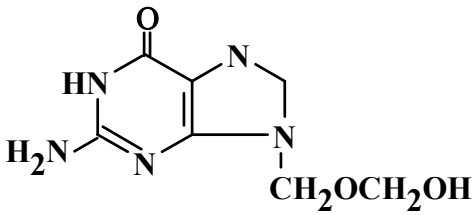
Conclusion Acyclovir concentration in sera of sheep and human as well as human urine and saliva can be easily determined on C8 column by high performance HPLC. The results indicated that saliva may be the one of the major excretion route for acyclovir in healthy individuals.

Key words Acyclovir, human, sheep, serum, urine, saliva, HPLC

Introduction

Acyclovir (9-[(2-hydroxyethoxy)methyl] guanine) is a nucleoside analog with potent anti viral activity for herpes simplex viruses (HSV), varicella zoster virus (VZV), epstein-Barr virus (EBV), cytomegalovirus (CMV) and human herpes virus 6 (HHV-6) (1,2,3,4) . Although acyclovir was first synthesized for the anti cancer producing program, a recent review on anti viral potency of acyclovir for infectious mononucleosis, chronic hepatitis, papillomatosis and AIDS indicates the importance of this drug (1,4,5) .

Acyclovir exerts its antiviral activity by acting as substrate that inhibits viral DNA polymerase. Phosphorilation of Acyclovir to acyclovir monophosphate occurs via viral or cellular thymidine kinase (4,5,6,7).



Acyclovir (9-[(2-hydroxyethoxy)methyl] guanine)

Acyclovir and its sodium salt have molecular weight of 225-247 daltons and their water solubility is above 1.3 mg/ml and 100 mg/ml, respectively. Pharmocokinetic profiles of acyclovir in serum , plasma, urine, cerebrospinal fluids, milk, lung, liver, heart, brain , spinal cord, saliva, tears have been recently reviewed (4,7,8) .

Determination of acyclovir has been achieved by radioimmunoassay (9,10), HPLC (11, 12, 13, 14).

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This paper describes a somewhat enhanced HPLC method for the determination of guanine nucleoside derivative acyclovir in serum, saliva and urine compare to prior publications (13).

Material and Method

Materials: Acyclovir powder was kindly supplied by Dr. H. Mascher (Pharm analyt labor Gmbh, Austria). All solvents and other chemicals were purchased from Sigma ACS grade.

Mobile phase: The mobile phase was 0.02 M $HClO_4$ with pH 2.0 prepared by double distilled water. The mobile phase was filtered (Millipore, 0.45 mm) and degassed under vacuum prior use.

Chromatography: A Model LC-10AD HPLC pump (Shimadzu, Japan) was used to deliver the mobile phase isocratically at a flow rate of 1 ml/min. Samples were injected through Rheodyne 7124 injection valve (fitted with 20-ml loop). Shimadzu RF-10A model spectrofluoremetric detector was operated at excitation 260 nm. and emission 376 nm

Samples were separated by C_8 colon (150x4.6 mm, Shimadzu, Japan) at ambient temperature. Results were calculated by C-R6A model chromatopac integrator (Shimadzu).

Preparation of standard solutions: Stock acyclovir standard was prepared by dissolving 20 mg powder in mobile phase, dilution was made by mobile phase to reach 300 ng/ml.

Preparation of samples: To measure the recovery of acyclovir, 500 ml sheep serum samples collected from slaughter house in Van and were divided into 2 groups. 200 ml and 400 mL of acyclovir standards were added on one group sera to reach 60 ng/ml and 120 ng/ml. Control sera received same volume of dd.

water instead of acyclovir. All sera were extended to 1 ml with addition of 30% $HClO_4$ to precipitate proteins. Clear supernatants were obtained by centrifuging at 4000 rpm for 15 minute. After filtration through 0.45 mm cellulose membrane, all samples were injected to C_8 colon directly. Standards and recovery were followed as previously reported (11).

Six volunteer patient's serum, saliva and urine were collected prior and after 200 mg oral acyclovir tablet administration. All samples were treated as above and statistical evaluatin (ANOVA; correlation) was made on SPSS software package.

Results and Discussion

Minimum concentration of acyclovir that could be determined by UV detector was 10-20 ng/ml. This sensitivity was increased up to 50 pg./ml with fluorescence detection methods. Measuring the nanogram quantities with fluorescence detector has been reported (11, 13, 14). Previous papers reported to use C18 column to separate acyclovir (14). However this study has utilized C8 column which gave a better peak shape.

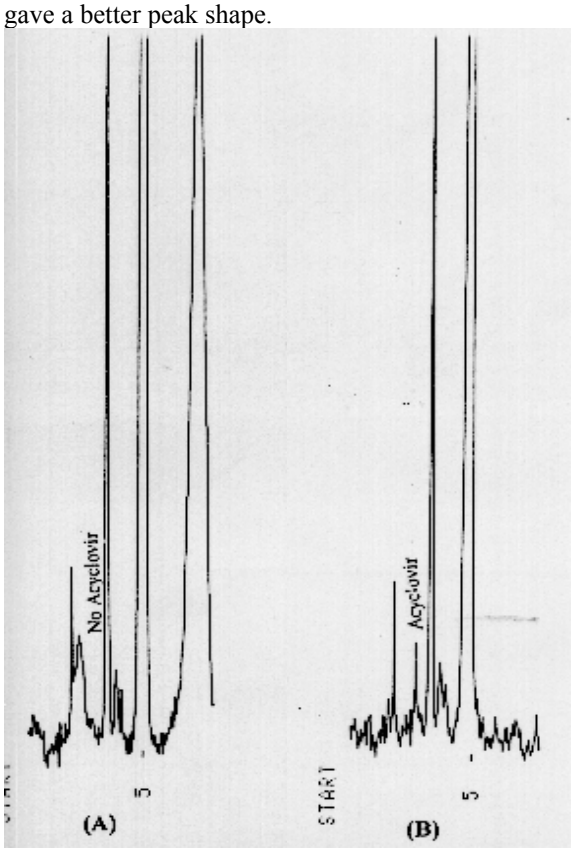

Figure 1. Detection of acyclovir in sheep sera. (A) Sheep sera prior acyclovir addition (B) Sheep sera after acyclovir addition. Separation of acyclovir has accomplished on C8 column. Detection was made at excitation 260 nm and emission 375 nm. Flow rate was 1.5 ml /min using 20 mM HClO4 as a mobile phase (pH 2.0).

A previous paper (14) reported mobile phase as 0.02 M perchloric acid-acetonitrile (55:45, v/v). However utilization of plain 0.02 M perchloric acid with C8 column gave quite reasonable results.

These reduce expensive mobile phase utilization. Besides, no further cleanup and reproducing time periods were needed. Retention time for acyclovir was 2.7 minutes. Full chromatograms of sheep sera with and without acyclovir addition have been shown in figure 1.

No reports has been found on determination of acyclovir measurement on sheep sera. This chromatogram indicated that no interfering peak exists in sheep sera.

Determination of acyclovir in human urine sera depends on patient's diet. In general we could determine acyclovir in urine without further clean-up and interference (Figures 2A,2B).

Sometime acyclovir saliva concentrations seem to interfere with naturally found nucleotides in saliva (Figures 3A,3B). Acyclovir levels of sera, saliva and urine of human after receiving 200 mg dose have been shown in figure 4.

This paper has only reported acyclovir in urine rather than these two metabolites. Two hours after acyclovir administration, mean urinary acyclovir concentration was 7.6 µg/ml ±3.4 SE.

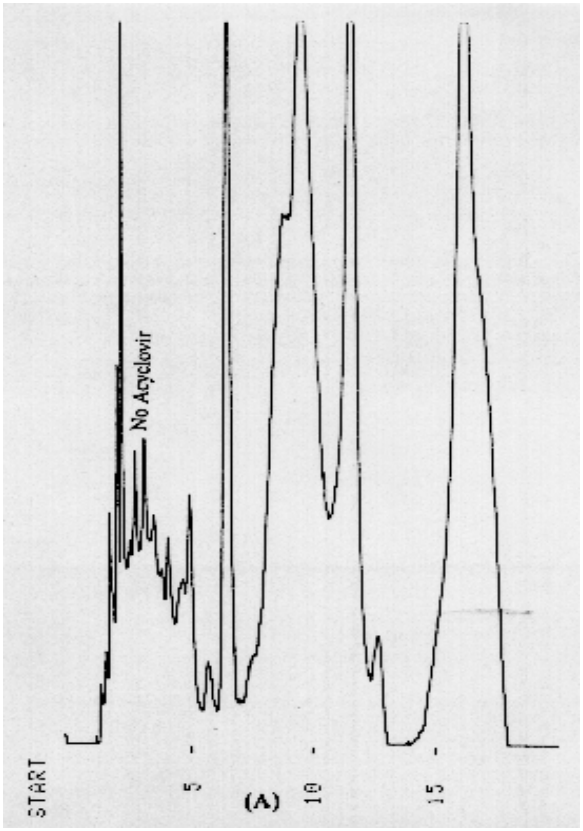

Figure 2A. Detection of acyclovir in human urine. Human urine prior acyclovir addition.

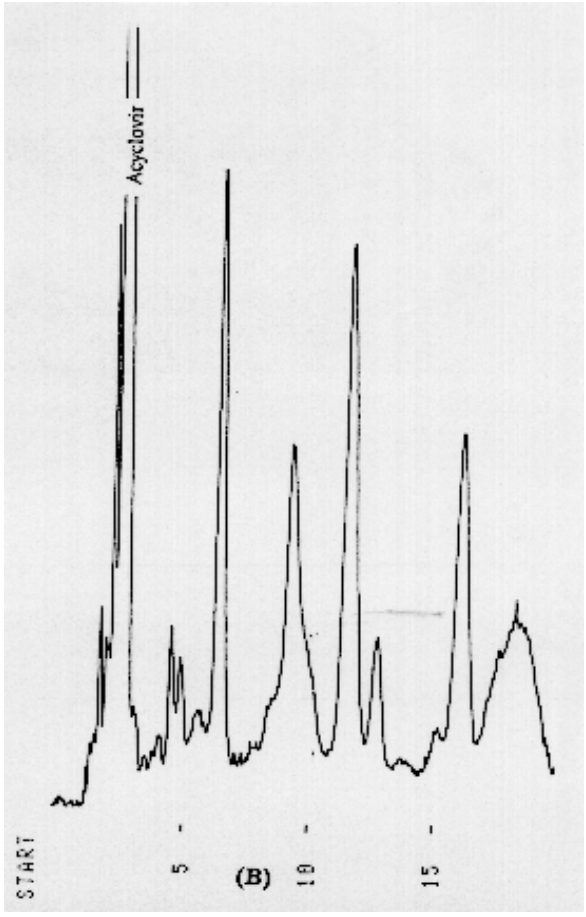

Figure 2B. Detection of acyclovir in human urine. Human urine after acyclovir administration. Separation of acyclovir has accomplished on C8 column. Detection was made at excitation 260 nm and emission 375 nm. Flow rate was 1.5 ml /min using 20 mM $HClO_4$ as a mobile phase (pH 2.0).

Urine is also the major excretory way of acyclovir. Excretion of acyclovir from urine is apparently 6 times higher than saliva within first hour (figure 4).

Mean plasma concentrations for studies of pharmacokinetics in human indicated that infusing 0.5-5.0 mg/kg acyclovir i.v. had given 28.16-148.28 µg/ml in plasma (6).

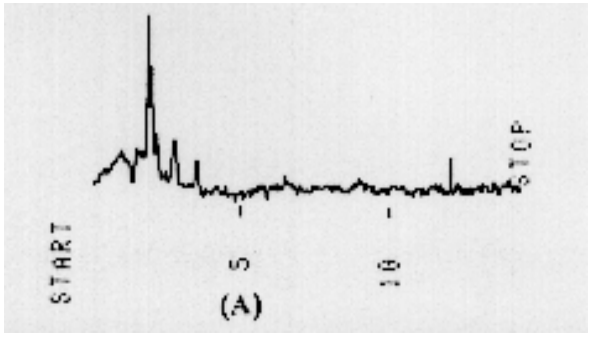

Figure 3A. Detection of acyclovir in human saliva. Human saliva prior acyclovir administration.

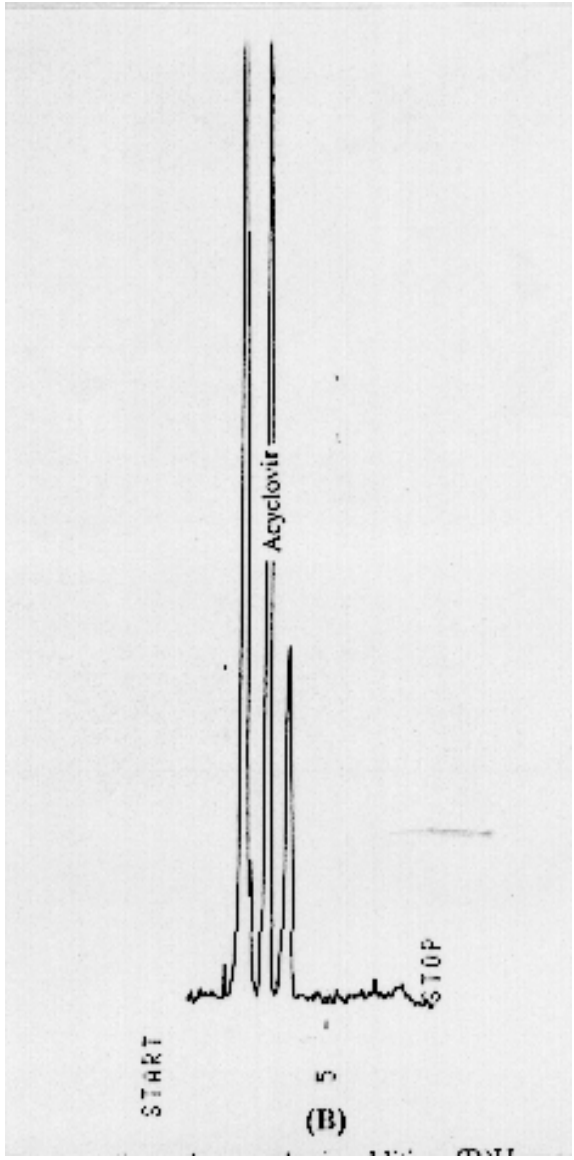

Figure 3B. Detection of acyclovir in human saliva. Human saliva after acyclovir administration. Separation of acyclovir has accomplished on C8 column. Detection was made at excitation 260 nm and emission 375 nm. Flow rate was 1.5 ml /min using 20 mM HClO4 as a mobile phase (pH 2.0).

Serum results were consisting plasma concentration that were obtained one hour after acyclovir administration.

Rabbit studies indicated that animals receiving 200 mg per animal, maximum acyclovir concentrations reached within 45 minutes in plasma was 29.3 µg/ml (8).

Examining figure 4 indicated that mean serum levels were 56.47 ng/ml±4.6 SE at 1st hour and 97.62 ng/ml ± 43.0 SE at 2nd hour.

Figure 4. Acyclovir concentration of human sera, saliva and urine prior and after oral acyclovir administration

Conclusion

Under the isocratic separation condition, determination of acyclovir in sheep and human sera as well as human urine have been accomplished within minutes by means of HPLC.

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