DNA-HARMALOL INTERACTION, THE EFFECTS OF HARMALOL ON THE SOLUTION STRUCTURE OF CALF-THYMUS DNA STUDIED BY FTIR SPECTROSCOPY

ABBAS HADJIAKHOONDI*, SHOHRE NAFISI ** and HAVA DIARI *

*Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

** Department of Chemistry , Islamic Azad University, Tehran Center Branch, Tehran, Iran.

ABSTRACT

The interaction of hamalol with calf-thymus DNA was investigated at physiological pH with drug/DNA (phosphate) molar ratio(r) of 1/40. Fourier transform infrared difference spectroscopy were used to establish correlations between spectral changes and drug binding mode, sequence selectivity, DNA conformation and structural properties of harmalol-DNA complexes in aqueous solution. Spectroscopic results indicated that harmalol is a weak intercalator with affinity for A-T rich regions. At low drug concentration(r=1/40), the A-T region is the main target of drug intercalation.

Key words: Calf-thymus DNA, *Harmalol*, FTIR

INTRODUCTION

Harmalol which is isolated from *peganum harmala L.* as well as *Banisteria Caapi* spruce (1-2) has been shown to play an important role in human cancer (3). The exact mechanism by which Harmalol exerts its anticancer activity is not clear and investigation of its interaction with cell particles such as nucleic acids, DNA polymerases and proteins is of great biological importance. Vibrational spectroscopy (infra-red) is often used to characterize the nature of drug/DNA interaction and to monitor the effects of various drugs on DNA structure (4-6). Recently, vibrational spectroscopy has been applied to analyze the nature of vitamin C, aspirin, chlorophyllin, diethylstillbestrol (DES) complexes with DNA (7-10). Our interest on biochemical activities of harmolol prompted us to investigate the drug binding sites, sequence specificity, helical stability and DNA secondary structure of DNA-harmol interactions in aqeous solution by FTIR spectroscopy. In this communication, the interaction of harmolol with calf thymus DNA with molar ratio of r=1/40 in water/ethanol (75/25 v/v) solution of pH=6-7 by FTIR spectroscopic technique is determined.

MATERIALS AND METHODS

Chemicals: Highly polymerized calf-thymus DNA sodium salt (7% sodium content) was from Sigma Chemical Co. and used as supplied. Harmalol was purchased from Fluka Chemical Co. and used without further purification.

Preparation of stock solution: sodium DNA solution was prepared in 4% w/w (0.1M phosphate) in 0.1M NaCl solution at 5°C for 24h with occasional stirring to ensure formation of a homogeneous solution. A solution of harmalol was also prepared in water/ethanol solution (75/25 v/v). Mixtures of drug and DNA were prepared by adding harmalol solution dropwise to DNA solution with constant stirring to give the desired drug/DNA molar ratios of 1/40. Solution pH was kept near 7.5 to 6.5 by NaOH solution (0.1 M). The IR spectra were recorded 4 h after initial mixing of drug and DNA solution on a BOMEM MB series Fourier transform infrared spectrometer with a nitrogen-cooled HgCdTe detector and a KBr beam splitter. Solution spectra were taken using CaF_2 windows with a resolution of 2 cm^{-1} and 100-500 scans. Water subtraction was carried out as reported (11). A good subtraction was achieved as shown by a flat baseline around 2200 cm^{-1}, where the water combination mode is located.

RESULTS AND DISCUSSION

The FTIR spectra related to harmalol interaction with DNA are tabulated in Table 1. The IR spectrum of the free harmalol in aqueous solution contains several important bands with strong and medium intensities at 1723 and 1659

Correspondence: Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

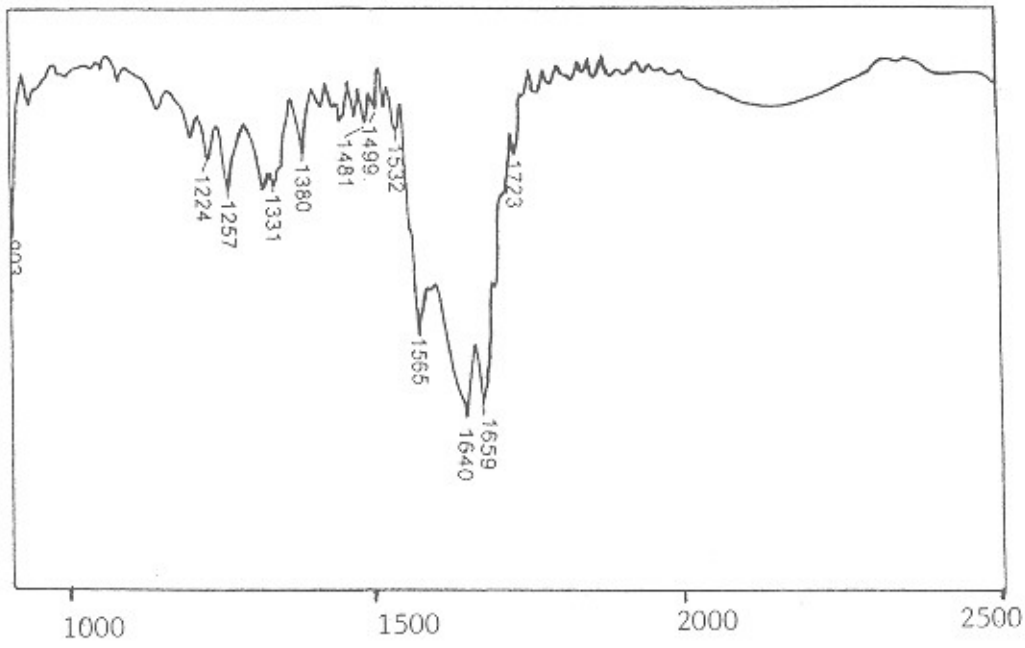

Figure 1. FTIR spectrum of Harmalol

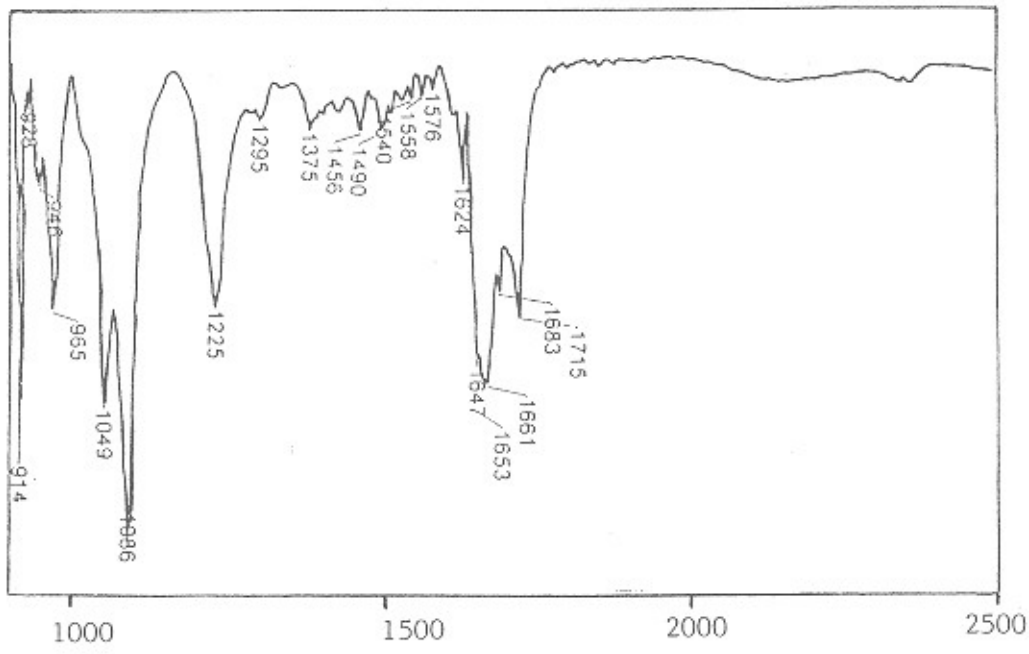

Figure 2. FTIR spectrum of DNA

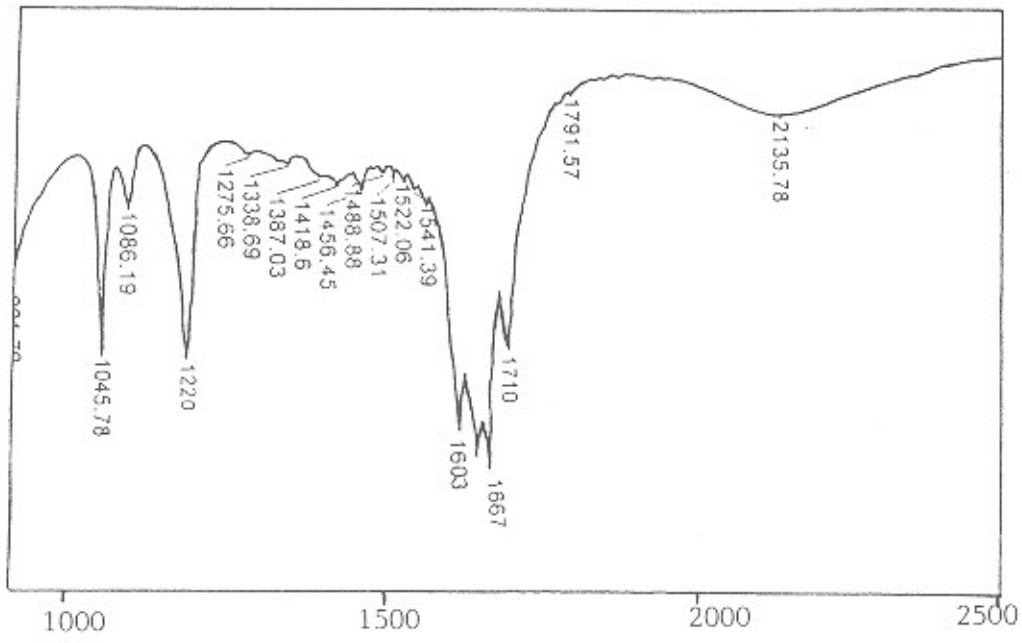

Figure 3. FTIR spectrum of DNA-Harmalol complex

cm^{-1} (C=O, C-O and C-N stretching) and 1640 cm^{-1} (assigned to the N-H and H_2O bands) (12). The absorption bands at about 1450-1550 cm^{-1} in the harmalol spectrum corresponds to C=N vibrations. The medium and weak absorption bands at 1040, 1142, 1257, 1331, 1380 and 1450 cm^{-1} are assigned to O-H and C-H deformations, C-C and C-O stretching.

Drug-DNA complexation: At low harmalol concentration (r=1/40), the band at 1715 cm^{-1} (G,T) in the DNA spectrum shifted towards a lower frequency at 1710cm^{-1} in the DNA harmalol spectrum. No intensity variation is observed for this frequency upon drug interaction. This is indicative of no major harmalol interaction *via* G-C base pairs at this stage. However major intensity increase was observed in the infrared bands of 1661 cm^{-1} (mainly thymine) 1609 cm^{-1} (mainly adenine) and at 1225cm^{-1} (PO_2 antisymmetric stretching (Table I). The intensity increase of these vibrations is also associated with the shift of the bands of 1661 to 1667 cm^{-1}, 1609 to 1603 cm^{-1} and 1225 to 1220 cm^{-1}. The observed spectral changes are due to the drug - DNA interaction *via* backbone phosphate group and thymine O-2 and adenine N-7 atoms (with N-H and O-H groups of harmalol) that are not normally involved in Watson-Crick hydrogen bonding network. Such interacion does not bring about helix destabiliztion (Figures 1, 2, 3) (8-10). In conclusion, at low drug concentration (r=1/40),.

Table 1. Assignment of FTIR frequencies (cm^{-1}) of calf-thymus DNA in aqueous solution in the presence of harmalol at pH=7-6 with molar ratio r=1/40 in the region 2500-900 cm^{-1} .

	Harmalol / DNA	Assignments
DNA	1/40	R
1715 s	1710 s	G, T
1661 w	1667 s	T, G, A, C
-	1659 s	Harmalol
1653 s	1643 s	T
1647 w	1645 s	H_2O
1609 w	1603 s	A
1490 m	1488 w	A,G
1375 w	1370 w	T, G, A, C
1295 w	1275 w	A, C
1225 s	1220 s	PO_2
1086 vs	1086 m	PO_2
1049 s	1045 s	T, G, A, C

R: drug/DNA, (p): molar, A: adenine, C: cytosine, T: thymine, G: guanine, s: strong, w: weak, m: medium, vs: very strong.

the interaction of harmolol with calf thymus DNA results minor perturbations of the backbone phosphate group and the A-T sites. Similar changes has been reported in aspirin-DNA complexation at the same concentration

ACKNOWLEDGEMENT

The authors appreciate Dr. H. Zamanizadeh and F. Zamani for instrument service and technology cooperation office (T.C.O) of presidency for supporting this project.

REFERENCES

1. Cordell G. A. (1989) The Dictionary of Alkaloids , Chapman and Hall, London: p: 487.
2. Cordell G. A. Dictionary of Natural Products (1994) Chapman and Hall, London, vol: III: p: 2677
3. Al-Allaf, T. A; Ayoub, M. T, Rashan, L. J. (1990) Synthesis and characterization of novel biologically active platinum (II) and palladium (II) complexes of some β-carboline alkaloids. J. Inorg, Biochem. 38: 47-56.
4. Morjani, H; Riou, J. F, Nabiey, I; Lovalle, F., Manfait, M. (1993) Molecular and cellular interactions between intopolicine DNA and topoisomerase II studied by surface-enhanced raman scattering spectroscopy. Cancer Res. 53: 4784-4.
5. Le Gal, J. M, Marjani, H and Manfait, M. (1993) Ultrastructural appraisal of the multi-drug resistance in K562 and IR73 cell lines from fourier transform infrared spectroscopy Cancer Res. 53: 3681-3686.
6. Sequais, J. M, Koglin, E, and Malfoy, B. (1984) Comparison of different spectroscopic methods for the interaction of antitumor compounds with deoxyribonucleic acid, FEBS Lett, 173: 95-98.
7. Neault, J. F, Naoui, M. and Tajmir-Riahi, H. A. (1995) DNA-Drug interaction, the effect of vitamin C on the solution spectra of calf-thymus DNA studied by FTIR and laser raman. J. of Biomolecular Structure and Dynamics. 13: 3.
8. Tajmir-Riahi, H. A; Neault, J. F; Naoui, M; Manfait, M. (1996) Aspirin-DNA interaction studied by FTIR and lase raman difference spectroscopy. FEBS Lett, 382: 26-30.
9. Tajmir-Riahi, H. A; Neault, J.F. (1996) DNA-Chlorophyllin interaction. J. Physical Chem. B. 103: 1610-1611.
10. Tajmir-Riahi, H.A; Neault, J. F. (1996) Diethylstilbestrol-DNA interaction studied by fourier transform infrared and raman spectroscopy. J. Biol. Chem. 271: 8140-3.
11. Alen, A; Dupuis P. (1996) FTIR and raman investigation of cadmium binding by DNA. Inorganica. Chimica. Acta, 157: 171-181.
12. Spenser, I.D. (1959) A synthesis of harmaline. Can J. Chem, 37: 1851.