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Short Communication

Ibuprofen binds to DNA

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Abstract

Molecular modelling and UV spectrophotometric studies suggest that Ibuprofen binds to DNA with greater preference to GC than AT. The study raises the question whether ibuprofen could be a 'lead-molecule' in the search for better anti-cancer drugs.

Key words: Anti-cancer drugs, DNA, molecular modelling, Ibuprofen, anti-inflammatory drugs, binding.

1. Introduction

Ibuprofen is widely used as an analgesic and anti-inflammatory drug[1,2]. The molecular mechanism of its action, like other anti-inflammatory drugs, is believed to be in the inhibition of prostaglandin biosynthesis in the body[3,4]. However, other mechanisms may be there, but are as yet not well established[4]. We pose the question whether anti-inflammatory action of these drugs can arise from binding to DNA or more simply how do these agents interact with DNA as there is an urgent need to look for novel leads to cancer therapy.

In two recent communications from one of us[5,6], a new molecular modelling approach was outlined which can enable one to arrive at the geometry of the active site of a receptor. As part of an ongoing research in the application of this new procedure, here we report of an experimental work carried out after a suggestion, from theoretical modelling studies, that ibuprofen can weakly bind to DNA in a pseudo-intercalative manner. The results are presented in two stages. First, the theoretical calculations performed on ibuprofen interacting with nucleic-acid components, followed by UV spectroscopic studies on the binding of ibuprofen with calf-thymus DNA, poly(dA-dT) and poly(dG-dC).

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2. Theoretical studies

The binding energy of a drug-(model) receptor complex comprises electrostatic, induction, dispersion (long-range forces) and non-bonded repulsion (exchange repulsion), and charge-transfer energy terms (short-range forces). As we are primarily interested in the pharmacodynamic phase of this drug's action, we exclude the discussion of the 'ill-defined' term of hydrophobic interactions. Of the long-range terms, only the electrostatic term can either be attractive or repulsive. If this term is attractive, then it is likely that the drug may bind at the receptor site. On this premise, we computed the electrostatic interaction energy for interaction between ibuprofen with guanine, thymine, cytosine and adenine, in the sandwich conformation of the complex at inter-planar distances of 3 to 5 Å (Fig. 1).

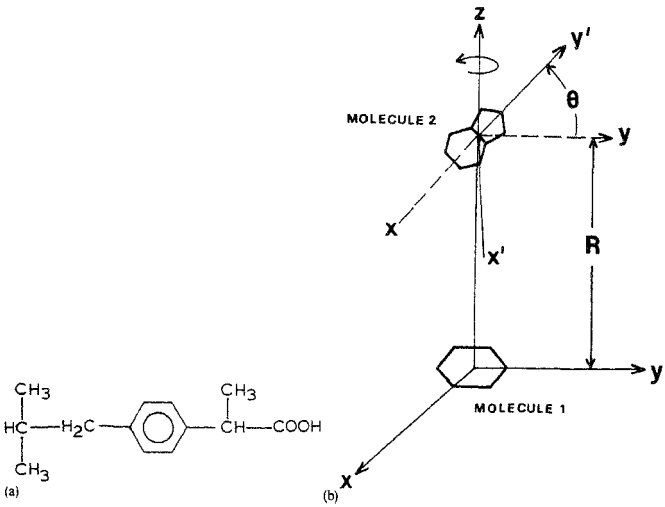

FIG. 1. Structure of the molecular complex considered in the present work.

The geometries of the molecules and the net charges on all the atoms were taken from the data bank drug design and receptor identification. It is noted (Table I) that interaction is favouring the formation of the complex with more binding to GC than AT. Based on this, we carried out UV spectroscopic studies to check whether ibuprofen binds with DNA.

Table I
Electrostatic interaction energy between ibuprofen and nucleic acid bases (fig. 1 for geometry)

Base	R(Å)	O°(deg.)	Energy (kcal/mole)
Guanine	3.5	210	−0.98
Thymine	3.5	60	−1.00
Adenine	3.5	90	−0.35
Thymine	3.5	300	−0.51

3. Material and methods

Poly(dA-dT) and poly(dG-dC) (Pharmacia P. L.) were used without further purification. Ibuprofen was a gift from Dr. S. Chandrasekhar (IISc) and c.t. DNA (Sigma) was deproteinated by phenol extraction and extensively dialysed against 20 mM NaCl, pH 7.1, before use. The concentrations of the drug and polynucleotides are expressed in terms of moles per litre, and measured using molar extinction coefficients ($nm^{-1} cm^{-1}$) poly(dA-dT) $\varepsilon_{260} = 6.7$, poly(dG-dC) ε_{254} 8.4, c.t. DNA ε_{260} 6.6 and Ibuprofen ε_{263} 0.38.

Ibuprofen stock solution was made in 60% methanolic buffer solution and dilution to required concentration with 20 mM NaCl solution, pH = 7.1. All the experiments were carried out at room temperature of 20°C employing a Beckman DU8B spectrophotometer using 1-cm quartz cuvettes.

Figure 2 shows the changes in the UV spectrum of the drug on addition of c.t. DNA. The appearance of a new peak between 290 and 295 nm clearly indicates the binding of the drug with DNA. Further, the interaction of ibuprofen with polynucleotides and c.t. DNA are characterised (fig. 3) by an increase in absorption of DNA with a small blue shift in the peak position of DNAs on addition of the drug.

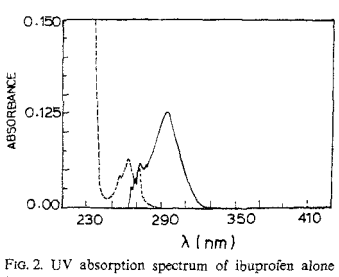

FIG. 2. UV absorption spectrum of ibuprofen alone (----) (130 μm) and in the presence of c.t. DNA (448 μm) (——). Note the appearance of a peak at 295 nm on drug-DNA complexation.

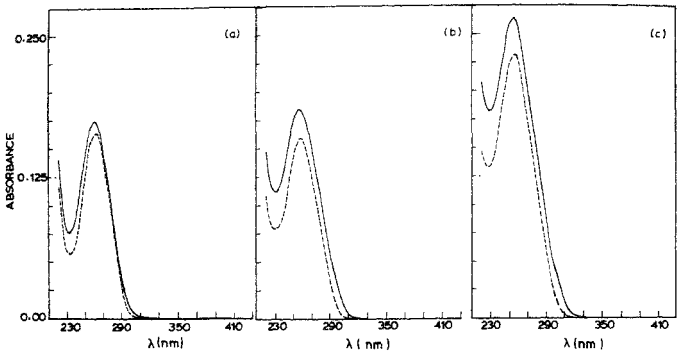

FIG. 3. UV absorption spectra of DNA alone (25 μm) (----) and in the presence of ibuprofen (5 μm) (——) a) Poly d(dA-dT), b) Calf-thymus c.t. DNA, c) Poly d(dG-dC).

These results indicate the binding of the drug with DNA, but not of any base specificity. We tried to monitor the reaction with CD spectroscopy, but the drug-DNA complex did not show any induced band in the DNA non-absorption region, *i.e.*, above 300 nm. However, the difference spectra of the drug-DNA complex and DNA showed weak positive band in 300–305 nm region (figure not shown).

This result raises several questions of fundamental importance to the understanding of the basis of all drug action, principally among them: If binding is the criteria for activity, then how do we distinguish binding to an active from those with inactive or antagonist sites. What is the optimal geometric nature of the bound complex? Electrostatic energy term being a long-range force, normally one expects it to be a facilitator for an 'early recognition' of binding site and in this perspective will an analog of ibuprofen form a better anti-cancer agent? or can we think of utilising ibuprofen as a model 'lead' molecule in the quest for less toxic and more effective anti-cancer drugs? Perhaps, detailed calculations of all the terms, 2D-nmr and crystallographic studies are likely to provide answers to many of these queries.

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