RESEARCH ARTICLE

Hemolytic and DNA Nicking Studies of Anti-Cancer Peptide

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ABSTRACT

Background: In recent years studying of in vitro toxicity encompass their possess significance to proceed any compound to further pharmaceutical procedure. In this we taken peptide which is supposed to be bind with DNA to produce anti-cancer possessions so it requires studying for its genotoxic possessions.

Aims & Objective: To study Hemolytic and DNA nicking of anti-cancer peptide.

Materials and Methods: Simple agarose gel was used to electrophoresis to determine the DNA nicking property and hemolytic assay also performed to ensure the safety of the peptide in biological system.

Results: It was found that peptide is being safe even at elevated concentration 40 ng which cannot be achieved in biological distribution and the hemolytic assay shows the IC50 value 25.24 µg/ml which is also an awfully higher concentration in in vivo terms.

Conclusion: So it is concluded the peptide have safe use in biological system.

Key Words: Peptide; Electrophoresis; DNA Nicking; Haemolytic Assay

INTRODUCTION

The studies of new DNA cleavage agents are of considerable importance in molecular biology and organic chemistry. The interaction of peptides with DNA is central to the control of many cellular processes including DNA replication, recombination and repair, transcription, and viral assembly. Peptides have several advantages over currently used onco therapeutics, such as selective cytotoxicity for tumor cells, bypass of the multidrug-resistance mechanism, and additive effects in combination therapy. The hydrophilic, cationic part is proposed to initiate electrostatic interaction with the negatively charged components (e.g., 3–9% phosphatidylserine) of the membrane of tumor cells.[1] The peptide p-ACC1 designed for tumor suppressor by interaction with BRCT domains[2] and it was checked for its genotoxicity and hemo interactions.

MATERIALS AND METHODS

Genotoxicity Test

Plasmid nicking assay was carried out by the method described by kitts et al., 2000 tris Hcl Buffer (10 mM) was adjusted to the pH 7.5. pUC19 (0.5 µg) was taken in a microfuge tube. 10 µl (20, 40, 80 ng) of peptide (Bio concept) was added to the microfuge tube 0.5 µg of pUC 19 were mixed with 215 µM of H_2o_2 were served as negative control[3] incubated for 2 hours. 10 µl of the tracking dye was added to the tube (40 mM EDTA, 0.05% bromophenol blue and 50% (V/V) glycerol). After incubation the mixture was subjected to electrophoresis. The reaction mixture was run on 0.8% agarose gel for 40mins.Ethidium bromide stained gel was viewed with UV illuminator.[4] Finally the figure was treated with Phoretix 1D, Image J softwares for construal.

Hemolytic Assay

Hemolytic activity was evaluated as described previously by Andra et al., 2008 with a slight modification. Human erythrocytes washed with Phosphate Buffer Saline (pH 7.4) and centrifuged at 8000 G for 10 min. After washing with PBS (until the supernatant was colourless), then the erythrocytes were Re-suspended and diluted to 10 times of the original volume with PBS kept as stock. Then, 150 µl of peptide (2-16 µg/ml) in PBS was incubated with 150 µl of stock erythrocyte suspension (4% v/v) for 60 min at 37°C. After the incubation period, the reaction mixtures were centrifuged at 1,000 x g for 10 min to remove intact erythrocytes. The 10 fold dilution of the supernatant of released hemoglobin was measured at 540 nm using a micro plate reader.[5] The triplicates experiment was done. Finally the haemolytic activity was expressed as a percentage hemolysis using the following equation.

% of Haemolysis = [(Abs sample-Abs buffer) / (Abs maximum-Abs buffer)] × 100

Abs sample is RBC with peptide solution in PBS. Abs buffer's Red blood cells in PBS 'Absmax' is red blood cells with 1% (v/v), Tritonx -100 in PBS .No hemolysis (0%) and full hemolysis (100%) were observed in the presence of PBS and 1%(v/v)Tritonx-100 respectively. Finally the IC 50 Value was calculated by using PRISM version 5 software.

RESULTS

DNA Nicking Assay

The results shows in the DNA nicking assay describes clearly there is no significant harm in the DNA even in higher concentration (80 ng) the super coiled DNA does not get much affected by the peptide shown in figure 1 further the reports characterizes the experimental system and presents evidence that DNA strand interruptions indicated by binding to circular DNA from the same preparation was significantly high (lanes 3 - 4), indicating that binding to linear DNA may be due to non-specific aggregation shown in figure 3. Further the length of lane 3, 4 & 5 shows gentle significance.

Hemolytic Assay

The peptide was checked for the hemolytic

properties at varying concentrations (2-16 µg/ml) it was shown the peptide does not turn out any higher changes in the hemolysis even in higher concentration which may not be possible in in vivo distribution 4 µg/ml shown in figure 4.

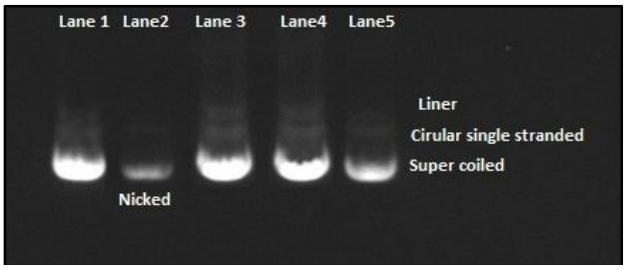

Figure-1: DNA Nicking Assay of Peptide (Lane 1: Control pUC19 plasmid; Lane 2: H_2O_2 + Control pUC19 plasmid; Lane 3: 20 ng peptide + Control pUC19 plasmid; Lane 4: 40 ng peptide + Control pUC19 plasmid; Lane 5: 80 ng peptide + Control pUC19 plasmid)

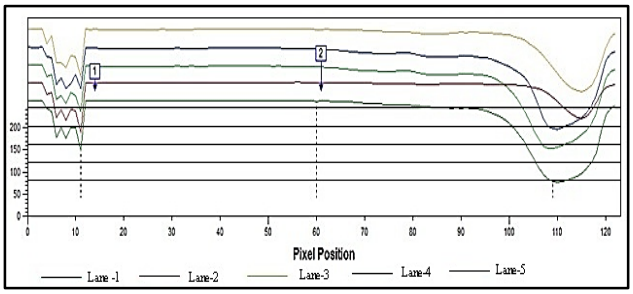

Figure-2: Multiple lanes overlayed with reference lane

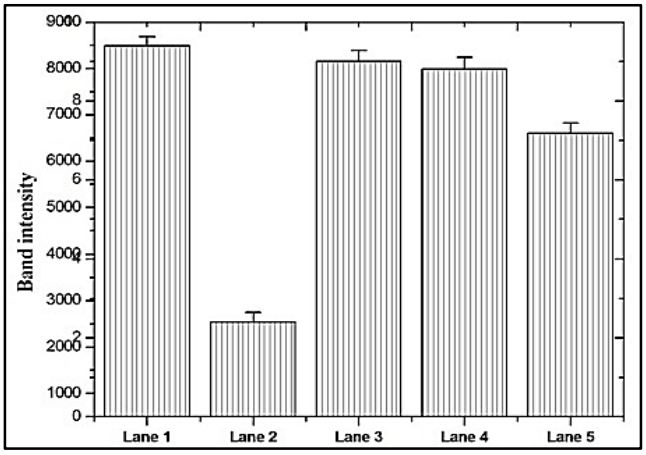

Figure-3: Densitometry values of agarose gel

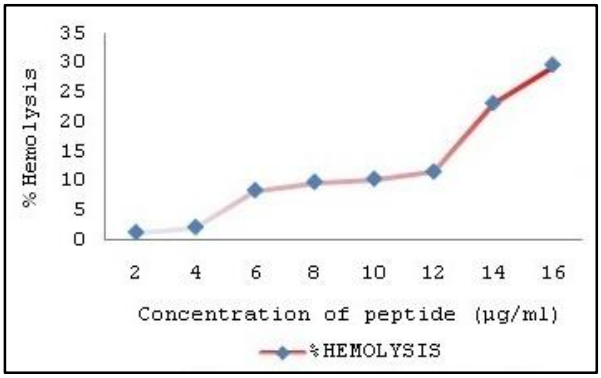

Figure-4: Peptide-Heam interaction

DISCUSSION

To assay the molecules for their genotoxic property studied for various features like single and double strand break ,loss of excision repair, cross-linking, alkali-labile sites, point mutations and structural and numerical chromosomal aberrations.[6] DNA nicking assays provides a simple, rapid and extremely sensitive method for detecting toxicity of sequence-specific DNA-binding proteins. Proteins which bind specifically to a labeled DNA fragment retard the mobility of the fragment during electrophoresis. This assay in another hand provides uncooked suggestion about affinity, abundance, association rate constants, dissociation constants and binding specificity of DNA-Binding proteins. Since the peptide encompass the possessions of binding to DNA it's obligatory to study the DNA negative properties before proceed further. In this the gentle significance in mobility shift in lane 2, 3, 4 perhaps by reason of speed variation in moving through gel it gives a raw thought that the size or charge variation might happened with the exacting lanes in the lesser extent their shape variation also been consider for this however, it can be assumed that the peptide is capable of binding to the circular DNA, and it as occurred shift up on the gel. Hence the pUC19 does not have specific binding domain.[7] Hence the increment in the biding may be due to non-specific phosphorylation by the peptide.

The US FDA recommends that for even the excipients intended to biological administration, an in vitro hemolysis study be supposed to performed at the higher than intended concentration for biological administration to test for hemolytic potential.[8] In the case of peptides the amphipathicity can be directly correlated with hemolytic activity and inversely correlated with anti-proliferative activity. Since the pACC1 encompass the soft amphipathicity. It is intended to encompass hemolytic potential. Taking the above fact in to consideration the heam interface of peptide was checked with extremely elevated concentration (2-16 µg/ml). The consequences shows companionable even compared with the well-known haemolytic

peptide mellitin has been reported to cause 50% haemolysis at 7.5 µg/ml. significantly our peptide shows 25.24 µg/ml. so this imply that the application of this peptide might be possible for safe use treatment.

CONCLUSION

The Peptide has no impact on genotoxicity as well as haemolytic property even in higher concentration so it can be further studied for their therapeutic applicability.

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