Research Article

In-vivo anticancer activity of Curcumin-Hyaluronic acid conjugate

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Abstract

Objective: Polymer-drug conjugates have gained much attention largely to curcumin lower drug solubility and to enhance drug stability. Curcumin is widely known for its medicinal properties including its anticancer efficacy. One of the serious drawbacks of curcumin is its poor water solubility which leads to reduced bioavailability. **Materials and methods:** Curcumin – HA conjugate was assessed for anticancer activity in EAC induced mice. And haematological parameters, differential count, body weight changes and mean survival time was assessed. **Results:** The results showed Conjugate (HA–Cur) produced extremely significant as anticancer activity by change in haematological parameters, differential count, body weight changes and mean survival time. **Conclusion:** Conjugated curcumin also showed potential anticancer activity.

***Keywords*:** Curcumin, conjugate, anticancer, hyaluronic acid (HA)

Introduction

Cancer is a fatal disease characterized by uncontrolled growth of abnormal cell and standing next to the cardiovascular disease in terms of morbidity and mortality (Sashidhara et al., 2010). Globally, cancer is the foremost cause of death accounting for 7.6 million deaths (around 13% of all deaths) in 2008 (Ferlay et al., 2008). International Agency for Research on Cancer reckons by 2030, it is predicted that every year there will be 26 million new cases of cancer and 17 million cancer deaths (Kumari et al., 2018).

Curcumin (1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, Cm) is a hydrophobic polyphenol secluded from the root of the Curcuma longa, a spice that has been extensively premeditated and its immense therapeutic potential is entrenched (Simion et al., 2016). Curcumin being an ingredient of turmeric possess a range of pharmacological activities such as anti-oxidant, anti-inflammatory, anti-proliferative, anti-metastatic, anti-cancer and anti-atherosclerotic. It is eminently pleiotropic molecule that inhibits cell proliferation and induces apoptosis in cancer cells. Even though its vital chemical instability, biological activities, photo-instability and poor bioavailability limits its utilization as an effective therapeutic agent. Therefore, enhancing the bioavailability of curcumin may improve its therapeutic index for clinical setting (Waghela et al., 2015).

A extensive variety of pharmacological activities has been endorsed to curcumin; however due to the low bioavailability of this pigment when administered orally, the translation of its experimental biological benefits into clinical trials is observed difficult in both rodents and humans (Vareed et al., 2008), which is explicated by its poor absorption due to the low solubility in water, limited tissue distribution, and rapid rate of metabolism in liver and intestine followed by the rapid excretion from the body (Anand et al., 2008). So the low bioavailability of curcumin emerges as a foremost barrier to reach its adequate circulating levels related to desirable pharmacodynamic actions, hampering its clinical approval as a therapeutic agent for numerous diseases (Gutierres et al., 2015).

The present study was undertaken to evaluate the anti-cancer activity of Curcumin-Hyaluronic acid conjugate

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using Ehrlich Ascites Carcinoma (EAC) cells in mice.

Materials and methods

Materials

Hyaluronic acid sodium salt from streptococcus equi sp. (HA) was purchased from Fluka, Bangalore, India. Curcumin 95% total curcuminoid content, from turmeric Sami Labs Pvt. Ltd, 1, 3-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) will be obtained from Sigma–Aldrich, Bangalore, India. All other chemicals used for this study will be of analytical grade.

Cell Lines and cell culture

The Ehrlich Ascites Carcinoma (EAC) cell lines (National Centre for Cell Science, Pune, India) were used for the assay of Ehrlich Ascites Carcinoma (EAC) and cancer cells were obtained from the Amala Cancer Research Centre, Thrissur, Kerala.

Experimental Animals

Healthy Swiss albino mice of body weight 25±3 g were procured from Central Animal Facilities, Acharya & BM Reddy College of Pharmacy, Bengaluru. Animals were housed in polypropylene cages and maintained under standard conditions. They were fed with standard diet and water. The animals were maintained as per CPCSEA (Committee for the Purpose of Control and Supervision of Experimental Animals) guideline for the care and use of laboratory animals. The study protocol (IAEC/ABMRCP/2016-2017/1) was approved by Institutional Animal Ethics Committee (IAEC), Acharya & BM Reddy College of Pharmacy, Bengaluru.

Treatment protocol

Swiss albino mice were randomly divided into 4 groups of 8 mice each, group I, II, III and IV were induced with Ehrlich Ascites Carcinoma where group I served as control, group II, treated with Curcumin (60 mg/kg), group III treated with Curcumin-HA (60mg/kg) and group IV treated with 5 FU (20 mg/kg) (Bose et al., 2008).

Cancer cell count and induction

0.1ml of normal saline (0.9%) was injected intraperitoneally into the donor mouse. Immediately after injecting saline, 1 ml of ascites fluid was collected from the peritoneal cavity and diluted with normal saline up to 10 ml. 10 µl ascites fluid from this was taken and placed on Neubauer's chamber and the number of cells appeared on chamber were calculated and concentration of 1×10^6 cells were injected to each mouse i.p.

Hematological parameters

To study the effect of the Curcumin-HA (60mg/kg) conjugate on haematological parameters of EAC cells bearing mice, blood was withdrawn from each mouse by retro-orbital plexus method and was collected in 12 µl of EDTA tube, and haematological parameters were performed for RBC, WBC, haemoglobin content and differential count (Hu et al., 2005).

Measurement of mean survival time (MST) and Percentage increase in life span (% ILS)

The effect of Curcumin-HA (60mg/kg) conjugate on tumour growth was monitored by recording the mortality rate daily until all the animals were dead and % ILS was calculated by using the formula (Hu et al., 2005).

% ILS

= [MST of treated group/ MST of control group -1] × 100

Statistical analysis

All the data are expressed as Mean ± SEM (n=6) and SD. Data analyzed by software GraphPad Prism 7 and the parameters were analyzed by one way ANOVA followed by Dunnett's t-test for multiple comparisons.

Results

Haematological parameters

The result showed decrease in Hb level in the cancer control, i.e. (8.76±0.17 g/dL). Curcumin and Curcumin – HA conjugate also showed raise in Hb towards normal i.e. 10.36±0.28 gm/dL and 12.36±0.28 gm/dL respectively. With the standard 5 FU, the level of Hb was found to be increased significantly (12.1±0.11 gm/dL).

Treatment with Curcumin – HA conjugate (60 mg/kg) showed an extremely significant increase (10.4±0.05) in RBC count compared to EAC cancer control group (7.01±0.42), while significant increase in RBC count on administration of 5 FU (20 mg/kg) (9.5±0.11).

The result shown that the level of WBC was increased in EAC cancer control mice ($25.23\pm1.19\times10^3$/ul) when compared with Curcumin – HA conjugate (60 mg/kg) showed an extremely significant decrease (12.6±0.05) of WBC count compared to EAC cancer control group, while significant decrease (14.97±0.4) of WBC count on administration of 5 FU (20 mg/kg).

Differential count

The result showed decrease in Lymphocytes in the cancer control, i.e. 14.9±7.45%. Curcumin and Curcumin – HA conjugate also showed raise in lymphocytes towards normal i.e. 28.8±1.13% and 21.33±1.34% respectively. With the standard 5 FU, the level of lymphocytes was found to be increased significantly (77.07±5.24%).

Table 1. Effect of Curcumin-HA conjugate on Haematological parameters

Groups	Hb (g/dl)	RBC ($1x10^6$ /mm^3)	WBC $1x10^3$/mm^3
Control 10% DMSO (4ml/kg)	8.76±0.17	7.01±0.42	25.23±1.19
Curcumin (60mg/kg)	10.36±0.28*	9.33±0.26**	17.5±0.34**
Curcumin -HA (60mg/kg)	12.36±0.28***	10.4±0.05***	12.6±0.05***
5-FU (20mg/kg)	12.1±0.11**	9.5±0.11**	14.97±0.40**

n=6, values were expressed as Mean ± SEM Data analyzed by one-way ANOVA followed Dunnett's t-test for multiple comparisons. *P < 0.05 (*), P < 0.01 (**)* and *P< 0.001(***)* were taken as significant.

Table 2. Effect of Curcumin-HA conjugate on Differential count

Groups	Differential count %		
	Lymphocytes	Neutrophils	Monocytes
Control 10% DMSO (4ml/kg)	14.9±7.45	63.60±4.49	14.20±3.30
Curcumin (60mg/kg)	28.8±1.13	55.40±1.90	15.80±0.81
Curcumin -HA (60mg/kg)	21.33±1.34 *	57.87±1.73	20.80±0.49
5-FU (20mg/kg)	77.07±5.24 ***	21.00±4.98 **	1.93±0.27 ***

n=6, values were expressed as Mean ± SEM Data analyzed by one-way ANOVA followed Dunnett's t-test for multiple comparisons. *P < 0.05 (*), P < 0.01 (**)* and *P< 0.001(***)* were taken as significant.

Table 3. Effect of Curcumin-HA conjugate on Mean survival time (MST) and % increase in life span (% ILS)

Groups	MST (Days)	% ILS
Control 10% DMSO (4ml/kg)	14.44 ± 2.24	-
Curcumin (60mg/kg)	21.13 ±4.18*	46.32
Curcumin -HA (60mg/kg)	28.89 ±7.13***	100.06
5-Fu (20mg/kg)	23.33±1.58**	61.55

n=6 and values were expressed as Mean ± SEM Data analyzed by one-way ANOVA followed Dunnett's t-test for multiple comparisons. *P < 0.05 (*), P < 0.01 (**)* and *P< 0.001(***)* was taken as significant.

Treatment with Curcumin – HA conjugate (60 mg/kg) showed an decrease (57.87±1.73%) in neutrophils compared to EAC cancer control group (63.60±4.49), while significant decrease in neutrophils count on administration of 5 FU (20 mg/kg) (21.00±4.98).

The result shown that the monocytes were increased in EAC cancer control mice (14.20±3.30) when compared with Curcumin – HA conjugate (60 mg/kg) showed an increase (20.80±0.49) of monocytes count compared to EAC cancer control group, while significant decrease (1.93±0.27) of monocytes count on administration of 5 FU (20 mg/kg).

Mean Survival Time and % ILS

The result shown that In EAC cancer control mice the mean survival time was 14.44±2.24 days. Whereas, it was significantly increased on treatment with Curcumin – HA conjugate (60 mg/kg) by (100.06%) 28.89±7.13 days. Where, comparison on treatment with standard drug 5 FU (20mg/kg) increased the life span by 61.55% and increased the mean survival time significantly 23.33±1.58.

Change in body weight

The result showed that In EAC cancer control mice the body weight was 20.6±1.38 gm. whereas, it was significantly

Table 4. Effect of Curcumin-HA conjugate on body weight

Groups	Gain in body weight (Mean ± SEM)	% Decrease in body weight
Control 10% DMSO (4ml/kg)	20.6±1.38	-
Curcumin (60mg/kg)	16.3±1.41**	20.873
Curcumin -HA (60mg/kg)	13.32±0.82***	35.436
5-Fu (20mg/kg)	18.59±0.85*	10.194

n=6 and values were expressed as Mean ± SEM Data analyzed by one-way ANOVA followed Dunnett's t-test for multiple comparisons. *P < 0.05 (*), P < 0.01 (**)* and *P< 0.001(***)* were taken as significant.

decreased in body weight on treatment with Curcumin – HA conjugate (60 mg/kg) by 35.436 % i.e. ***(P<0.01)*** Whereas, comparison on treatment with the standard drug 5 FU (20mg/kg) decreased the body weight by 10.194 %.

Discussion

Although curcumin has shown remarkable potential as an anticancer drug, its poor solubility in water leads to poor bioavailability. Previous studies to address this issue of reduced bioavailability include formulating curcumin in various carriers such as polymers, liposomes, polymeric micelles, emulsion and nanospheres. Polymer drug conjugates have distinctive advantages over conventional polymeric nanosized carriers due to its high drug content, good water solubility and increased drug half life in the body. We reasoned that HA–Cur conjugate is a potential curcumin delivery vehicle considering the several advantages of HA. The extreme hydrophilicity of HA and its poor solubility in most organic solvents, however, restricted the direct conjugation of HA with curcumin which is highly hydrophobic. We found that Water/DMSO mixture (1:1 V/V) dissolves both HA and curcumin enabling the conjugation to form the drug conjugate. The results are a reflection of the stability of the conjugate at physiological pH. However the degradation profile of pure curcumin shows about 60% degradation with in 25 min. After that there is no considerable absorption in the 420 region, showing almost complete degradation of curcumin. In one of the study (Wang et al., 1997) also demonstrated 90% decomposition of curcumin within 30 min. The high stability of the conjugate comparing to curcumin can be traced to the formation of micelles. In the micelle, we presumed that conjugated curcumin exist in the inner core due to its hydrophobicity. The hydrophilic HA molecules, on the other hand, protrude outwardly. The micelle formation thus protects curcumin from the deprotonation and subsequent fragmentation in the alkaline media. The conjugation of curcumin to HA advantageously stabilizes curcumin against hydrolysis and hence enhance its aqueous stability.

Curcumin has two –OH (phenolic) groups and one active methylene group. These points are potential sites for conjugation. Blocking of –OH groups may reduce medicinal features of curcumin. To get an insight whether the conjugation affected inadvertently the ability of curcumin to kill cells, cytotoxicity of the conjugates was evaluated for *in vivo* anticancer activity.

The data indicate that only low quantity of conjugate is required to impart cytotoxicity which we reasoned is due to the high solubility of the conjugate. It is expected that the improvement in the conjugates Cytotoxicity was due to the water solubility and cell internalization ability of HA–curcumin conjugate.

Conclusion

The potential of curcumin as a drug have been investigated widely. One of the serious drawbacks of this molecule is its reduced water solubility and instability beyond neutral pH. Conjugated curcumin showed potential anticancer activity.

Conflict of interest

The authors declare that no potential conflict of interest.

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