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Effect of embelin on carbohydrate moieties of glycoprotein in tumour-bearing rats

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Abstract

Objective: To investigate the changes taking place in the carbohydrate moieties of the glycoprotein in plasma, liver and kidney of the tumour-bearing rats on embelin treatment. Materials and methods: Fibrosarcoma was induced to three groups of animals by injecting the tumour cell line. To two groups, embelin was administered after the tumour became palpable, in two doses (50 and 100 mg/kg) continuously, for 20 days. On 21st day, the animals were sacrificed, blood collected and liver and kidney separated. Total hexose, hexosamine and sialic acid in plasma, liver and kidney tissues were estimated. Results: In tumour-bearing control animals, the above parameters in plasma, liver and kidney tissues were elevated. Continuous administration of embelin lowered these values to near normal. Conclusion: Embelin significantly reversed the elevated levels of carbohydrate moieties of glycoprotein in tumour-bearing rats. This further supports the anti-tumour property of embelin.

Key words: Embelin, fibrosarcoma, carbohydrate moieties of glycoprotein.

1. Introduction

Embelin, a naturally occurring benzoquinone, isolated from the berries of *Embelia ribes* Burm. (Family: Myrsinaceae) showed significant anti-tumour activity in rats. Embelin in doses of 50 and 100 mg/kg body weight produced 52.87% and 68.86% tumour regression respectively when compared with tumour-bearing controls besides enhancing the survival time of the animals. There were no deaths due to toxicity observed during the experiment [1]. In this study, the effect of the drug on carbohydrate moieties of glycoprotein such as total hexose, hexosamine and sialic acid of the plasma, liver and kidney of the animals was investigated in sequel.

Glycoproteins are a group of complex proteins having oligosaccharides bound to them. The monosaccharide portion of the oligosaccharides contains sugars, hexosamines and derivatives of N-acetylneuramic acid, viz. sialic acid. These molecular constituents are necessary for

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maintaining the integrity of the extra-cellular matrix and cellular contents [2] and within the cell they have varied biological role such as transport of hormones, haemoglobin binding and blood coagulation [3]. Impairment occurring in the cellular macromolecules result in the degeneration of the matrix and loss of tissue function [4].

2. Materials and methods

2.1 Isolation of embelin

Coarsely powdered berries of *E.ribes* (1.5 kg) were exhaustively extracted with *n*-hexane by cold extraction method (3x2L). After 72h, the solvent was decanted and distilled off over boiling water-bath. The extract so obtained was concentrated *in vacuo* and subjected to column chromato-graphy over silica gel (100-200 mesh). Elution of the column with benzene yielded an orange powder which on crystallisation with ether afforded orange plates of embelin (Yield: 4.5g).

2.2 Animals

Male albino rats of Wistar strain, weighing 125-150 g obtained from Fredrick Institute of Plant Protection and Toxicology, Padappai (Chengalpattu district) were maintained on rat chow (M/s. Lipton India Ltd., Mumbai) and water *ad libitum*. They were housed under standard environmental conditions.

2.3 Animal studies

Rats were divided into four groups each containing six animals. The first group consisted of normal rats, which had only feed and water. Methylcholanthrene-induced fibrosarcoma was transplanted in the remaining animals by injecting 0.2 ml of 10% suspension of minced tumour cell line in saline in the axillary region through a puncture [5]. Treatment was started after the tumour has developed into a palpable stage. The second group contained tumour-bearing control rats, which received only the vehicle. The third and fourth groups received embelin in two doses (50 and 100 mg/kg body weight respectively) for 20 consecutive days. The dosages were chosen based on our earlier study. Embelin was administered by oral route in olive oil suspension.

The animals were sacrificed on the 21st day, 24 h from the administration of the last dose of the drug, blood was collected by cardiac puncture and the plasma was separated. The liver and kidney were immediately removed and washed with chilled physiological saline. The tissues were homogenised in 0.1M Tris-HCl buffer at 4°C in a Potter-Elevejem homogeniser and used for biochemical estimations.

2.4 Biochemical estimations

The plasma and tissues were defatted in chloroform-methanol and after hydrolysis the estimation of total hexose, hexosamine and sialic acid were carried out as per the standard procedures [6-8].

2.5 Statistical analysis

The results obtained were analysed using Student's *t* -test and the values with $p<0.05$ were considered to be statistically significant.

3. Results

Table 1 summarises the levels of total hexose, hexosamine and sialic acid in the plasma, liver and kidney of the experimental animals.

In plasma, the tumour-bearing control animals had high levels of above components compared to normal ones. Treatment with 50 mg and 100 mg of embelin showed appreciable decrease in their levels and the decrease was significant with 100 mg dose.

In liver tissues, they showed an increase in the tumour-control group, which reverted to normal values with 100 mg dose of embelin. Likewise, in kidney tissue too, the elevated levels of these

Table 1
Effect of embelin on carbohydrate moieties of glycoprotein in plasma, liver and kidney of rats

Treatment	Plasma			Liver			Kidney		
	Total hexose mg/dl	Hexosamine mg/dl	Sialic acid mg/dl	Total hexose mg/dl	Hexosamine mg/dl	Sialic acid mg/dl	Total hexose mg/dl	Hexosamine mg/dl	Sialic acid mg/dl
Normal rats	212.34 ±7.74	45.37 ±1.25	93.78 ±3.53	31.82 ±1.07	10.81 ±0.51	6.13 ±0.21	25.98 ±0.86	21.22 ±0.77	8.54 ±0.42
Tumour-bearing control	273.41 ±9.85**	59.88 ±1.97***	125.61 ±5.41**	38.31 ±1.20**	12.43 ±0.47*	8.47 ±0.31***	31.15 ±1.18**	27.15 ±1.07**	13.97 ±0.54
Embelin									
50 mg	242.38 ±9.53#	51.25 ±2.21#	100.74 ±5.22#	34.16 ±1.32	10.80 ±0.45#	7.97 ±0.38	29.64 ±1.01	25.48 ±0.97	13.08 ±0.44
100mg	225.52 ±8.68##	47.13 ± 1.89##	90.56 ±4.22##	29.67 ±1.05#	10.77 ±0.51#	5.85 ±0.25###	27.21 ±1.11#	22.37 ±0.89#	10.36 ±0.46##

Each value represents the mean + SEM of 6 observations.
* p<0.05; ** p<0.01; *** p<0.001 when compared to normal rats; # p< 0.05; ## p<0.01; ### p< 0.001 when compared to tumour-bearing rats (Student's *t* - test)

parameters were brought back to almost normal levels with 100 mg dose.

4. Discussion

In cancer chemotherapy, additional markers, which are present in appreciable quantities in the tissue or fluid give clinically useful information that can be related to the underlying tumour burden. The carbohydrate moieties of glycoprotein, which are integral part of the surface structure of cells, show alteration in malignant or transformed conditions. These changes might be correlated to the abnormal behaviour displayed by the tumour cells [9]. Further it has been reported that the levels of circulating glyco-components like glycoproteins are often increased in patients with cancer [10].

In the present study, the increased levels of total hexose, hexosamine and sialic acid are observed in the tumour-bearing control animals, which is in line with the earlier findings. Hexose and fucose are the neutral sugars of glyco-components that help in increasing the solubility of proteins. Hexosamine provides positive charges while uronic acid and sialic acid offer negative charges to the cell surface [11].

Any change in the glyco-component content may subsequently increase their polarity and alter the permeability of hydrophilic and hydrophobic substances, which are pronounced in tumour condition [12].

The relation between tumour burden and the circulating sialic acid has been reported earlier as a method for monitoring response to therapy [13]. Sialic acid is a family of acylated derivatives of neuraminic acid, which usually occurs as a terminal component of carbohydrate chains of glycoproteins and glycolipids. Neoplasms often have an increased concentration of sialic acid on tumour cell surface. Hence, total sialic acid levels have been recognised as a valuable non-specific marker of tumour-burdon [14].

In this study, it has been observed that the sialic acid levels in the plasma, liver and kidney tissues were elevated in tumour-bearing animals. Continuous administration of embelin significantly attenuated the elevation of sialic acid levels in plasma and the tissues which provides further support for the drug's antitumour potential.

The changes seen in apparently non-involved tissues such as liver and kidney of the host, is possible due to the fact that a few tumour cells do reach the other tissues, remain viable therein and release substances *in situ,* which alter the rate of synthesis or degradation of certain specific proteins.

Therefore, the changes produced by embelin in the carbohydrate moieties of the glycoprotein apparently exhibit a positive step towards the rejuvenation of altered cellular constituents.

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