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Serum [^{3}H]-fucose labelled glycoproteins in *Fasciola hepatica* infected and ascite Zajdela hepatoma bearing rats

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Summary

The status of soluble [^{3}H]-fucose labelled glycoproteins from *Fasciola hepatica* infected and ascite Zajdela hepatoma bearing rats was investigated and compared. Isolated blood sera were fractionated by preparative isoelectrofocusing (pH 3 – 10) and radioactivity of the fractions was measured. Different numbers of fractions with common or specific isoelectric points (pI) were separated in the sera from: healthy rats – 4.60, 5.00, 6.05, 6.95, 7.30, 7.70, 9.30; ascite Zajdela hepatoma bearing rats – 4.60; 5.00; 5.40; 6.25; 6.90; 7.50, 8.30; rats with acute fasciolosis – 4.55, 4.95, 5.20, 6.05, 6.35, 7.10, 7.35, 8.00, 8.65, 9.20; rats with chronic fasciolosis – 4.25, 4.65, 5.05, 5.70, 6.00, 6.45, 7.05, 7.45, 8.65, 9.05, 9.30; tumour bearing rats with acute fasciolosis – 4.55, 4.75, 5.20, 5.75, 6.45, 6.90, 8.30, 8.90; tumour bearing rats with chronic fasciolosis – 4.25, 4.55, 5.00, 5.55, 5.75, 6.45, 6.90, 7.40, 8.00, 8.35, 8.90. The highest radioactivity was measured in acid zone for all groups but at different and specific pI.

Key words: sera glycoproteins; fasciolosis; ascite Zajdela hepatoma; rats

Introduction

Inhibition on the experimental tumour development during the chronic stage of fasciolosis and its stimulation at the background of acute fasciolosis has been established in our previous investigations (Tsocheva, 1986). Later, different biologically active substances, inhibitors of cell proliferation, glycoproteins, have been isolated from *Fasciola hepatica* tissues and *F. hepatica* infected host liver and spleen tissues (Tsocheva & Topashka-Ancheva, 1992; Tsocheva *et al.,* 1992a, 1995a, 1995b, Tsocheva-Gaitandjieva *et al*., 1999). We suppose that probably they take place in some mechanisms of the inhibiting action of fasciolosis on experimental carcinogenesis.

Malignant cell transformation and differences in the oncogenic capacity of certain tumours are closely related to the appearance of newly formed tumour specific antigens in the serum which are glycoproteins and which possess differences in the structure of their oligosaccharide chains compared to normal cells (Ivanov *et al*., 1998).

The helminth *F. hepatica* interacts with the host body mainly through the tegument. It is established that *F. hepatica* tegument consists of membrane glycoproteins (Threadgold, 1976). The character of the tegument secretion is changed during the different stages of *F. hepatica* development and maturation (Dalton & Joyce, 1987; Dalton *et al.*, 1985).

The present study aims to find out and to compare the status of soluble [^{3}H]-fucose labelled glycoprotein fractions in sera of healthy rats, ascite Zajdela hepatoma bearing rats, *F. hepatica* infected rats and animals treated with the combination of the both pathogenic factors, investigated in acute and chronic stage of fasciolosis.

Materials and Methods

The experiments were carried out on 55 male albino Wistar rats, aged 30 days, divided into the following groups: Group I – controls (healthy rats) – 12; Group II – ascite Zajdela hepatoma transplanted rats – 12; Group IIIA – experimentally infected with *F. hepatica* rats, investigated in acute stage of fasciolosis – 8; Group IIIB – *F. hepatica* infected rats, investigated in chronic stage of fasciolosis – 7; Group IVA – rats infected with *F. hepatica* and transplanted with ascite Zajdela hepatoma during the acute stage of fasciolosis – 8; Group IVB – *F. hepatica* infected rats transplanted with ascite Zajdela hepatoma at the background of chronic stage of fasciolosis – 8.

The rats were orally infected with 15 metacercariae of *F. hepatica* on day 1st of the experiment.

Transplantation of ascite Zajdela hepatoma was done on the day 7th or on the 17th week of the experiment and 7 days were necessary for the tumour development.

Animals from groups A were sacrificed on the 14th day from the beginning of the experiment (acute stage of fasciolosis) and rats from groups B – on the 18th week p.i. (chronic stage of fasciolosis).

Healthy animals from the control group and tumour bearing rats breaded in the same living conditions were sacrificed on the 14th day or on the 18th week too.

Three rats from each group were injected i.v. with [6-^{3}H]-fucose (Specific activity = 15.3 TBq/mol, Amersham, England) in a dose of 10 μCi per 100 g body weight, one hour before the keeling of the animals.

Blood sera isolated from the animals were fractionated by 110 ml ampholyne column LKB for preparative isoelectrofocusing after sacrifice (Vesterberg, 1981). The procedure was performed with 20 mg serum protein. The protein content was determined by the method of Bradford, 1976. The pH measuring of the collected fractions was done with micro-pHmeter Radiometer. Fractions with pH between 3.0 and 10.0 were kept and their radioactivity was measured in scintillation mixture by scintillation spectrometer LKB – 1110. Relative radioactivity (%) of the peaks was estimated.

Results

Seven peaks of radioactivity of [^{3}H]-fucose labelled soluble glycoproteins are established in sera of healthy rats (Group I). Their isoelectric points (pI) are – 4.60, 5.00, 6.05, 6.95, 7.30, 7.70 and 9.30. The measured radioactivity is the highest in one peak in the acid zone with pI 4.60, slighter – in peaks 5.00, 6.95, 9.30 and the lowest – in peaks with pI 6.05, 7.30 and 7.70 (Table 1 and Fig. 1).

Seven peaks of radioactivity of [^{3}H]-fucose labelled soluble glycoproteins are established in sera of rats bearing ascite Zajdela hepatoma (Group II) – respectively at pI 4.60, 5.00, 5.40, 6.25, 6.90, 7.50 and 8.30. The radioactivity is the highest in the peaks of acid zone with pI 4.60, 5.00 and slighter – at pI 5.40, 6.25, 6.90, 7.50, 8.30 (Tab. 1, Fig. 2).

Glycoproteins from the sera of rats with acute fasciolosis (Group IIIA) possess 10 peaks of radioactivity of [^{3}H]-fucose incorporation at pI 4.55, 4.95, 5.20, 6.05, 6.35, 7.10, 7.35, 8.00, 8.65 and 9.20. The highest radioactivity is established again in acid zone at isoelectric points 4.95 and 5.20. Slighter is the radioactivity at pI 6.05, 6.35, 7.35 and 8.00 and the lowest – at pI 4.55, 7.10, 8.65 and 9.20 (Tab. 1 and Fig. 3).

The peaks of radioactivity of [^{3}H]-fucose labelled soluble glycoproteins established in sera of rats with chronic fasciolosis (Group IIIB) are 11 in number – with pI 4.25, 4.65, 5.05, 5.70, 6.00, 6.45, 7.05, 7.45, 8.65, 9.05 and 9.30. The highest activity is established in acid zone at pI 4.65 and 5.05, slighter – at pI 5.70, 6.00 and 6.45 and the lowest – at pI 4.25, 7.05, 7.45, 8.65, 9.05 and 9.3 (Tab. 1, Fig. 4).

Eight peaks of radioactivity of [^{3}H]-fucose labelled soluble glycoproteins in sera of ascite Zajdela hepatoma bearing rats at the background of acute stage of fasciolosis (Group IVA) are established at pI 4.55, 4.75, 5.20, 5.75, 6.45, 6.90, 8.30 and 8.90. The highest activity is presented in acid zone at pI 4.55, 4.75, 5.20, slighter – at pI 5.75, 6.45 and the lowest – at pI 6.90, 8.30, 8.90 (Tab. 1 and Fig. 5).

Eleven peaks of radioactivity of [^{3}H]-fucose labelled soluble glycoproteins are established in sera of rats with ascite Zajdela hepatoma at the background of chronic stage of fasciolosis (Group IVB) with pI 4.25, 4.55, 5.00, 5.55, 5.75, 6.45, 6.90, 7.40, 8.00, 8.35 and 8.90. The highest activity is present again in the acid zone at pI 4.25 and 4.55, slighter – at pI 5.55 and 5.75 and the lowest – at pI 5.00, 6.45, 6.90, 7.40, 8.00, 8.35 and 8.90 (Tab. 1 and Fig. 6).

Table 1. Isoelectric points (pI) of [^{3}H]-Fucose labelled soluble glycoproteins isolated from sera of healthy rats (I), ascite Zajdela hepatoma bearing animals (II), *Fasciola hepatica* infected rats (IIIA,B), and rats treated with the both pathogenic factors (IVA,B) after separation by preparative isoelectrofocusing

pI					
Groups					
I	II.	IIIA	IIIB	IVA	IVB
Fig.1	Fig. 2	Fig. 3	Fig. 4	Fig. 5	Fig. 6
-	-	-	4.25	-	4.25
-	-	4.55	-	4.55	4.55
4.60	4.60	-	-	-	-
-	-	-	4.65	4.75	-
-	-	4.95	-	-	-
5.00	5.00	-	-	-	5.00
-	-	-	5.05	-	-
-	-	5.20	-	5.20	-
-	5.40	-	-	-	-
-	-	-	-	-	5.55
-	-	-	5.70	-	-
-	-	-	-	5.75	5.75
-	-	-	6.00	-	-
6.05	-	6.05	-	-	-
-	6.25	-	-	-	-
-	-	6.35	-	-	-
-	-	-	6.45	6.45	6.45
-	6.90	-	-	6.90	6.90
6.95	-	-	-	-	-
-	-	-	7.05	-	-
-	-	7.10	-	-	-
7.30	-	7.35	-	-	-
-	-	-	-	-	7.40
-	-	-	7.45	-	-
-	7.50	-	-	-	-
7.70	-	-	-	-	-
-	-	8.00	-	-	8.00
-	8.30	-	-	8.30	-
-	-	-	-	-	8.35
-	-	8.65	8.65	-	-
-	-	-	-	8.90	8.90
-	-	-	9.05	-	-
9.30	-	9.20	9.30	-	-

Fig. 1. Relative radioactivity of [^{3}H]-fucose labelled glycoprotein fractions isolated from sera of healthy rats

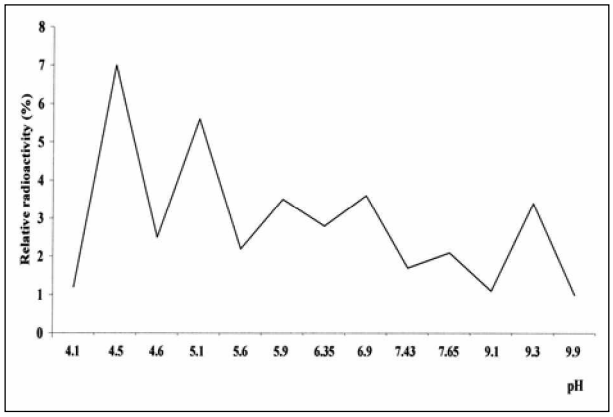

Fig. 2. Relative radioactivity of [^{3}H]-fucose labelled glycoprotein fractions isolated from sera of healthy rats

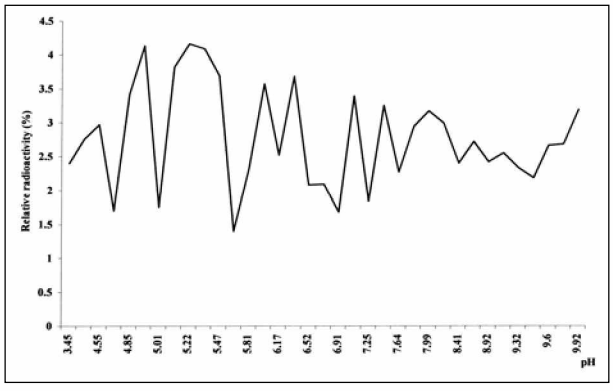

Fig. 3. Relative radioactivity of [^{3}H]-fucose labeled glycoprotein fractions isolated from sera of rats with acute fascioliasis

Fig.4. Relative radioactivity of [^{3}H]-fucose labeled glycoprotein fractions isolated from sera of rats with chronic fasciolosis

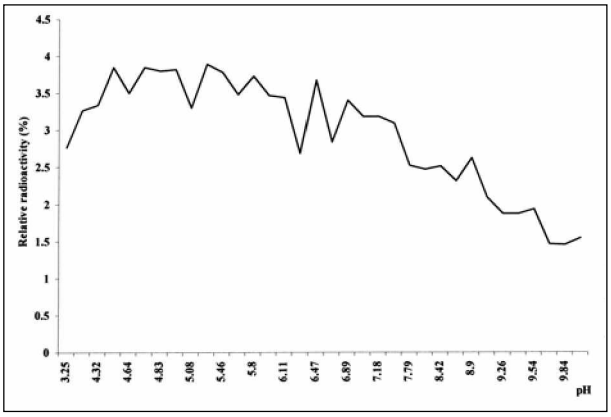

Fig. 5. Relative radioactivity of [^{3}H]-fucose labeled glycoprotein fractions isolated from sera of ascite Zajdela hepatoma bearing rats

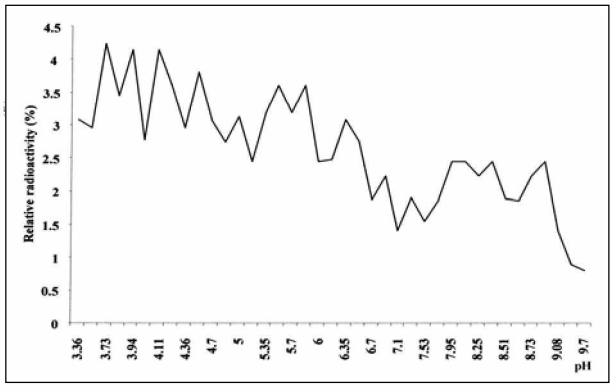

Fig. 6. Relative radioactivity of [^{3}H]-fucose labeled glycoprotein fractions isolated from sera of ascite Zajdela hepatoma bearing rats with chronic fascioliasis

Discussion

The results show that more peaks of radioactivity are obtained in the sera of *F. hepatica* infected rats compared to the controls. Peaks with isoelectric points 4.60, 5.00 and 6.05 are common both for the healthy and ill from fasciolois animals. Specific for acute fasciolosis are both the presence of the peaks at pI 5.20, 7.35, 8.00, 8.65 in the infected host sera, and the lack of peaks at pI 7.70, 9.30 in comparison with the sera of healthy animals. Specific for

the chronic stage of fasciolosis are both the presence of host sera peaks at pI 4.25, 5.70, 9.05 and the lack of peaks at pI 7.70, 9.30 in comparison with the sera of healthy animals.

The specific peaks of radioactivity of the serum glycolproteins established in the acute stage of fasciolosis may be a result mainly of the circulation of helminths secretory-excretory products in host serum, and those, detected in the chronic stage of fasciolosis maybe are mainly from host origin and probably are appeared as a result of the changed host metabolism or immune response under fasciolosis. It is proved by immunological tests that antigenic nature of some host polipeptides or glycoproteins is changed during *F. hepatica* development (Bennet & Threadgold, 1975; Hanna, 1980; Hanna & Trudgett, 1983; Dalton *et al.,* 1985). Literature data about the application of isoelectrofocusing in parasitology exist mainly concerning its usage for investigations of some enzymes from helminth or infected host tissue origins (Rhoads, 1988; Thalhofer & Hofer, 1989; Kristensen & Fried, 1991; Villalta *et al.*, 1992, 1993).

Ivanov *et al.* (1998) published data about characteristics of serum [^{3}H]-fucose labelled glycoproteins in ascite Zajdela hepatoma bearing rats. According to the authors, received results may be useful for explanation of typical changes in the processes of fucosylation of the glycoproteins in sera of Zajdela hepatoma bearing animals. The appearance of specific peaks with pI 5.40, 7.50, 8.30 and a lack of peaks at pI 7.70, 9.30 in the sera of ascite Zajdela hepatoma bearing animals compared to the controls is confirmed in the present study.

Specific for the materials from the combined groups are both the appearance of peaks at pI 4.25, 5.20, 5.55, 5.75, 8.0, 8.90 and the lack of peaks at pI 7.70, 9.30, in comparison with the sera of healthy or single treated animals. Interesting observation is the presence of similar peaks in the sera from animals of the combined group with acute fasciolosis and sera from tumour-bearing animals at pI 4.55, 5.00, 6.45, 6.90 and 8.30. Specific for the animals from the combined group with chronic fasciolosis is the presence of the peak at pI 5.55. This maybe one of the keys for the revealing of some mechanisms of the complex interactions between experimental fasciolosis and carcinogenesis (Tsocheva, 1986).

We suggest either that host liver cells metabolism is altered as a result of the parasites migration through the liver tissue, or that the penetration of biologically active substances is increased through the hepatocyte membrane during the parasitic disorder. May be some of these substances has properties of immunomodulators or inhibitors of the cell proliferation (Tsocheva & Topashka-Ancheva, 1992, Tsocheva *et al.*, 1992a, Tsocheva *et al.*, 1992b). Probably these substances are secreted in the host serum from heaptocytes of the infected host, act on the host immune system and the tumour cells, change them biochemically and cause the appearance of specific glycoproteins obtained in the present study.

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