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Review Article

Fasciolosis and tumour growth

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Summary

The data available about the interactions between fasciolosis and carcinogenesis are scanty and contradictory. According to some clinical observations, *Fasciola hepatica* could be a factor of potential neoplastic risk for humans and animals in natural conditions. Experimentally, however, two different phenomena are described: 1/ A stimulation of the tumour growth or hepatocyte's mitotic activity established in acute stage of fasciolosis and under the influence of *F. hepatica* metabolite products or implants. 2/ An inhibition of the tumour growth observed during the chronic stage of the helminthosis. Biologically active substances that act as inhibitors of cell proliferation and immunosuppressors have been isolated from *F. hepatica* tissues and *F. hepatica* infected host liver and particularly characterized. They have stronger growth-inhibiting effect compared with those isolated from healthy host liver. Immune, biochemical and gene mechanisms are probably involved in the interactions between fasciolosis and carcinogenesis. Future possibilities for preparing of recombinant or synthetic new growth inhibitors from helminth or infected host origin are suggested for application in tissue or organ transplantations and against autoimmune and tumour diseases.

Key words: *F. hepatica*; fasciolosis; carcinogenesis; tumour growth

Introduction

The oncogenic potential of *Fasciola hepatica* is not certain. According to Curley (2000), some of the other biliary parasites, such as *Fasciola hepatica* and *Schistosoma japonica*, do not appear to have the similar carcinogenic effects. But Tashev *et al.* (1961) and Genov *et al.* (1980) believe that the possibility of the development of primary liver cancer is provoked at the background of fasciolosis under specific conditions.

The interactions between fasciolosis and carcinogenesis are have not been investigated well and the received data are contradictory.

Clinical observations in humans and animals

In comparison with other helminthoses, in natural conditions fasciolosis is very rarely complicated with neoplastic degeneration in humans and animals. Chapman (1999) defines the chronic inflammation and the chronic alteration of the bile duct epithelium as a risk factor for the development of carcinogenesis of the bile ducts, but he considers that such a process is observed more rarely under *F. hepatica* infection, in comparison with *Clonorchis sinensis* and *Opisthorchis viverrini* infections. The risk of the development of malignant diseases at the background of helminthoses is lower in children than in adults.

The ectopic localization of *F. hepatica* is sometimes manifested with unspecific clinical symptoms and according to them may be wrongly attributed to a tumour or neoplasia. There are data about incorrect registration of the diagnosis of human fasciolosis in endemic or unendemic regions when the wrong primarily diagnosis has been tumour or neoplasia (Park *et al.*, 1984; Sapunar *et al.*, 1992). Nozais *et al.* (1998) report a case of fasciolosis with a double hepatic and gastric location initially diagnosed as primary stomach cancer with metastases in the liver. The peritoneal localization of the fasciolosis resembles peritoneal carcinomatosis (Rangheard *et al.*, 1999).

Gavinet *et al.* (1997) publish data about a case of tumour formed of hepatic distomatosis in a 49-year woman treated by steroids for connective tissue disease. She was admitted for a liver mass and epigastric pain. After the performance of right hepatectomy the presence of intraparenchymatous eggs was found and reflected the ectopic migration of a mature fluke into the hepatic parenchyma.

The inflammatory diseases of the liver represent an important subgroup of the focal liver alterations. They may re-

semble primary or metastatic liver neoplastic alterations and their clinical differentiation from the neoplastic tumours is very important, as the necessary approaches and treatment of the patient must be different (Gentile & Gentile, 1994). The correct diagnosis must be proved by specific parasitological, morphological and radiological investigations (Oto *et al.*, 1999).

But other authors suggest that *F. hepatica* belongs to the factors that represent neoplastic risk (Genov *et al.*, 1980; Gentile *et al.*, 1998). Sriurairatana *et al.* (1996) isolate human cholangiocarcinoma cell line (HuCCA-1) obtained from tumour of intrahepatic bile ducts with characteristics of adenocarcinoma, isolated from patient with fasciolosis. Maleewong *et al.* (1999) isolate specific antibodies against *Fasciola gigantica*, which have been used for the diagnosis of the parasitic disease in humans. Sera from patients with other parasitic infections, healthy volunteers and with cholangiocarcinoma are also analyzed. The data indicate possible correlation of antibodies to *F. gigantica* with cholangiocarcinoma.

More of the available data about observations of malignant tumours in naturally infected animals (mainly cattle) are very old. Galvez and Maglajlic (1956) report data from different authors for cases of liver cancer in naturally *F. hepatica* infected cattle with fasciolosis but correlation between these diseases has not been proved for certain. Primary polymorphocellular anaplastic cholangiocarcinoma, within the little liver bile ducts, has been observed combined with biliar cirrhosis, fibrose cholangitis and pericholangitis at the background of chronic fasciolosis. The extrahepatal ducts have not been involved, distant metastases in other organs have not been detected.

Vitovec (1974) find out hepatocellular carcinoma in cattle under fasciolosis and its relationship to biliary cirrhosis of fasciolar origin.

Cornick (1988) reports a case of a 5-year-old intact male llama (*Llama glama*) with gastric squamous cell carcinoma and generalized metastasis at the background of natural *F. hepatica* infection. Weight loss, anorexia and cachexia have been the presenting clinical signs.

Experimental data

The experimental investigations of the oncogenic effect of fasciolosis on the host tissues are contradictory and not systematic. The only available data are those about two opposite phenomena described in experimentally *F. hepatica* infected hosts: growth stimulating and growth inhibiting effects.

Growth stimulating effect

An increase of the mitotic activity of hepatocytes is established after the treatment with *F. hepatica* extract or implant (Foster, 1981; Ginovker, 1979; Ginovker & Bychkov, 1991).

A stimulation of the experimental diethylnitrosamine induced tumour growth is proved histologically, autoradiographically and statistically at the background of acute stage of fasciolosis (Tsocheva, 1986; Kandekar, 1989; Tsocheva *et al.*, 1990a). Growth stimulating factor is isolated from the metabolite products of *F. hepatica* and investigated on cell cultures (Krustev *et al.*, 1987).

Proline is believed to represent a chemical effect of the liver fluke *F. hepatica*, and to provoke bile duct hyperplasia. However, when investigating the potential promoting effect of proline on experimental bile duct cancer development, a lack of promoting effect has been established (Thamavit *et al.*, 1994).

The CYP2A5 isozyme is known to be a participant in the metabolism of some carcinogens which are common polluters of the environment in the developing countries where the parasitic infections are prevalent. It is found an increased activity of this enzyme in the liver of *F. hepatica* infected mice (Montero *et al.*, 1999).

Some new molecular and genetic investigations may reveal other mechanisms of the interaction fasciolosis – carcinogenesis. Gentile *et al.* (1998) investigate the possibilities of *F. hepatica* to provoke mutagenic events in the host tissues. When using Big Blue ® transgenic mouse assay, the authors found out that lacI mutations were twofold increased in the cells from *F. hepatica* infected mice compared with the control animals. The data present that the biological infections may enhance the genes' alteration in the surrounding host tissues. The presence of an aggressive inducing inflammation liver fluke *F. hepatica* could induce mutagenic events in tissues of mice (Motorna *et al.*, 2001). The spectrum of the mutations in the liver of *F. hepatica* infected animals shows a significant increase in complex changes and multiple mutations (18.2 %) when compared to those from uninfected control animals (2.8 %).

In the publications, theories are discussed about the involvement of the immune system in cancer and the possible

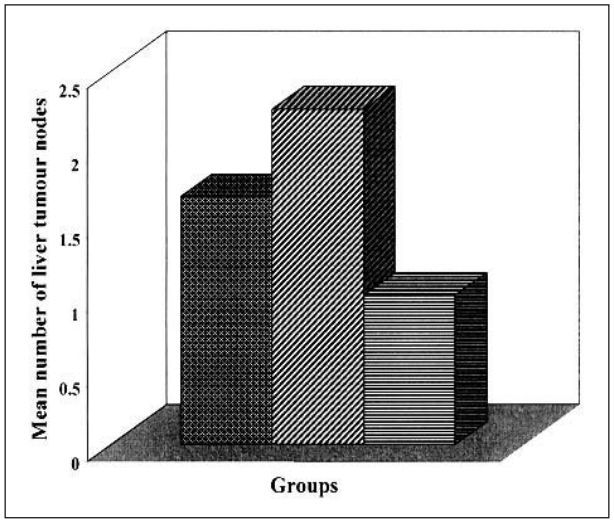

Fig.1. Mean number of liver tumour nodes after diethylnitrosamine treatment of rats during infection with *F. hepatica* (Tsocheva, 1986)

▩ – Rats treated with diethylnitrosamine: ▨ – Rats injected with diethylnitrosamine during the acute stage of fascioliasis; ▤ – Rats treated with diethylnitrosamine during the chronic stage of fascioliasis

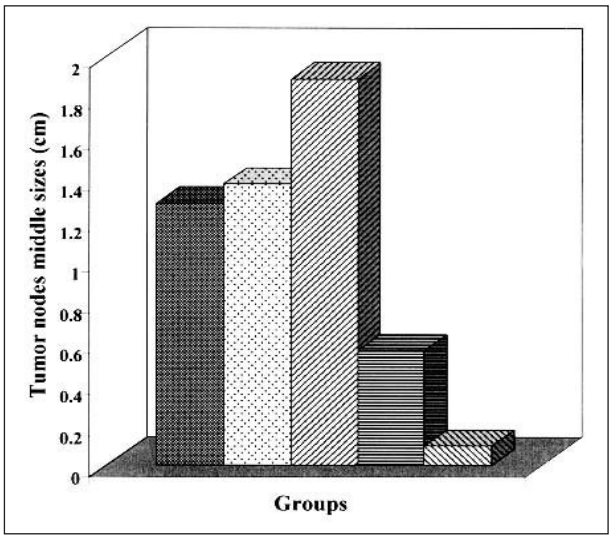

Fig. 2. Middle sizes of solid hepatoma-22 tumour nodes in *Fasciola hepatica* infected and tumour bearing C3H mice (cm) (Tsocheva-Gaytandzhieva *et al.*, 2002)

▩ – Solid hepatoma-22 bearing C3H mice; ▩ – Simultaneously solid hepatoma-22 transplanted and *Fasciola hepatica* infected C3H mice; ▨ – *F. hepatica* infected mice transplanted with solid hepatoma-22 during the acute stage of the disease; ▤ – *F. hepatica* infected mice transplanted with solid hepatoma-22 during the chronic stage of the disease; ▧ – *F. hepatica* infected and reinfected mice transplanted with solid hepatoma-22 during the chronic stage of the reinfection

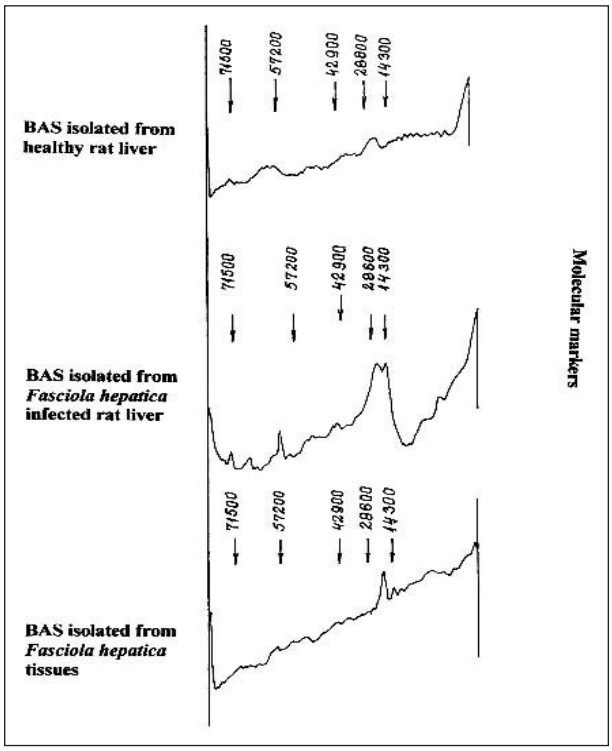

Fig. 3. SDS-PAGE electrophoregrame of biologically active substances isolated from rat liver and *Fasciola hepatica* tissues at the background of commercial molecular markers (Tsocheva *et al.*, 1995a; Tsocheva *et al.*, 1995b)

relationship between the mammalian inflammatory response and parasite-associated cancers (Gentile & Gentile, 1994).

Growth inhibiting effect

A growth inhibition of diethylnitrosamine induced experimental liver carcinogenesis is established morphologically and statistically during the chronic stage of fasciolosis in rats (Fig. 1). The growth inhibiting effect of the mature *F. hepatica* is also confirmed on hepatoma – 22 bearing mice (Fig. 2). Immunological and biochemical pathogenic mechanisms have been supposed. Biologically active substances (BAS) (thermostabile and thermolabile), inhibitors of cell proliferation, of parasite or host origin, are isolated from *F. hepatica* tissues and the infected host liver and spleen, characterized and investigated *in vivo* on tumour bearing mice (Tab. 1) and *in vitro* on normal and tumour cell cultures (hepatocyte, lymphocyte, hepatoma MC29 and myeloma cells) (Tabs. 2, 3, 4).

The effects of BAS isolated from *F. hepatica* tissues and

Table 1. Changes in some indexes of the cell proliferation *in vivo* in ascite leiomiosarcoma bearing BALB/c mice after the application of the thermostabile BAS isolated from *Fasciola hepatica* infected rat liver (Tsocheva & Topashka-Ancheva, 1992)

Groups	Thymidine index (%)	Mitotic index (%)	Tumour volume (ml)
1. Controls – mice injected with salt solution	36.36 ± 1.14	45.12 ± 2.32	0.64 ± 0.11
2. Tumour bearing mice injected once with BAS isolated from normal rat liver	34.75 ± 1.57 P > 0.5	44.03 ± 3.26 P > 0.5	2.12 ± 0.42 P < 0.01
3. Tumour bearing mice injected once with BAS isolated from *F. hepatica* infected rat liver	32.08 ± 0.89 P < 0.02	46.51 ± 2.28 P > 0.5	2.53 ± 0.54 P < 0.01
4. Tumour bearing mice injected three-fold with BAS isolated from normal rat liver	38.86 ± 2.43 P > 0.5	30.56 ± 3.53 P < 0.01	0.32 ± 0.11 P < 0.05
5. Tumour bearing mice injected three-fold with BAS isolated from *F. hepatica* infected rat liver	21.21 ± 1.01 P < 0.001	22.60 ± 3.17 P < 0.001	0.28 ± 0.05 P < 0.01
Groups 4 / 5	P < 0.01	P < 0.05	P < 0.05

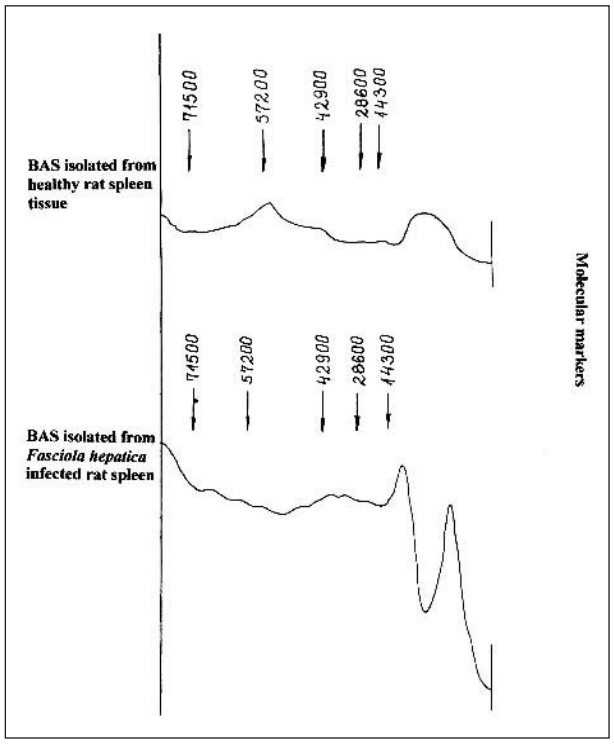

Fig. 4. SDS-PAGE electrophoregrame of biologically active substances isolated from *F. hepatica* infected rat spleen at the background of commercial molecular markers (Tsocheva-Gaytandzhieva *et al.*, 1999)

F. hepatica infected host livers are stronger than the effects of the substances isolated from the healthy rat liver. The strongest growth inhibiting effect is manifested by BAS isolated from *F. hepatica* tissues. The thermolabile BAS have the properties of immunosuppressors and specific inhibitors of cell proliferation with tissue specific and species non-specific activities.

The newly isolated thermolabile substances (fraction with a final concentration of ethanol between 70 % and 87 % (v/v) by Verly *et al.*, 1971) are characterized biochemically. Gel-chromatography on Sephadex G-75, SDS-PAGE electrophoreses, the establishing of the molecular weights and amino acid composition of the BAS are carried out (Tsocheva *et al.*, 1995a; Tsocheva *et al.*, 1995b; Tsocheva-Gaytandzhieva *et al.*, 1999).

All isolated thermolabile BAS are formed of two similar fractions (MW 14300 D and 57200 D, respectively), but their amino acid content differ from one another (Tsocheva *et al.*, 1995a; 1995b; Tsocheva-Gaytandzhieva *et al.*, 1999) (Figs. 3, 4; Tab. 5). The results for their molecular weights are close to the literature data about the BAS isolated from normal liver mammalian tissue by the similar method (Verly *et al.*, 1971). The BAS consist of 19 amino acids in different proportions and as they contain glucosamine and galactosamine, we suggest that they are glycoproteins (Tsocheva *et al.*, 1995a,b; Tsocheva-Gaytandzhieva *et al.*, 1999). This coincides with the literature data about the structure of some BASes (chalones) isolated from different mammalian tissues (Balazh, 1987; Balazh & Blazhek, 1982). As the newly isolated BAS are glycoproteins, [6-

Table 2. Incorporation of [^{3}H]-thymidine in hepatoma MC29 cell culture after treatment with the newly isolated BASes (Tsocheva *et al.*, 1992a)

Groups	Incorporation of [^{3}H]-thymidine (CPM*)	Inhibition of the cell proliferation (%)
Controls (cells untreated with the BASes)	56225 ± 6723	–
Cells treated with the BAS isolated from the healthy rat liver	20064 ± 3085	64.3
	P < 0.01	
Cells treated with the BAS isolated From the *F. hepatica* infected rat liver	11898 ± 5317	78.8
	P < 0.001	
Cells treated with the BAS isolated from *F. hepatica* tissues	4282 ± 590	92.4
	P < 0.001	

*CPM – counts per minute

Table 3. Incorporation of [^{3}H]-thymidine in myeloma cell culture after treatment with the newly isolated BASes (Tsocheva *et al.*, 1992a)

Groups	Incorporation of [^{3}H]-thymidine (CPM*)	Inhibition of the cell proliferation (%)
Controls (untreated with the BASes cells)	20846 ± 1045	–
Cells treated with the BAS isolated from the healthy rat liver	18728 ± 7146	10.2
	P > 0.1	
Cells treated with the BAS isolated from the *F. hepatica* infected rat liver	19376 ± 2090	7.1
	P > 0.1	
Cells treated with BAS isolated from *F. hepatica* tissues	15585 ± 7476	25.2
	P > 0.1	

*CPM – counts per minute

Table 4. Incorporation of [^{3}H]-thymidine in lymphocyte cell culture from healthy rat spleen after treatment with the newly isolated BASes (Tsocheva *et al.*, 1992b)

Groups	CPM* after using of different mitogens			
	Non-stimulated	PHA stimulated	PWM stimulated	LPS stimulated
Controls (untreated with the BASes cells)	4482 ± 417	13044 ± 1039	3699 ± 370	1604 ± 165
Cells treated with BAS isolated from the healthy rat liver	1749 ± 107 P < 0.02	2663 ± 569 P < 0.1	2594 ± 1280 P > 0.5	2077 ± 137 P < 0.1
Cells treated with BAS isolated from the *F. hepatica* infected rat liver	2363 ± 399 P < 0.05	1686 ± 464 P < 0.1	1824 ± 101 P < 0.05	1029 ± 137 P < 0.1
Cells treated with BAS isolated from *F. hepatica* tissues	2102 ± 339 P < 0.05	1612 ± 309 P < 0.1	2022 ± 923 P > 0.5	861 ± 230 P < 0.1

*CPM – counts per minute; PHA – phytohemagglutinin; PWM – pokeweed mitogen; LPS – lypopolysaccharide

Table 5. Amino acid content of thermolabile biologically active substances (BAS) isolated from *F. hepatica* tissues, livers and spleens of healthy and *F. hepatica* infected rats (Mol %) (Tsocheva *et al.*, 1995a; Tsocheva *et al.*, 1995b; Tsocheva-Gaytandzhieva *et al.*, 1999)

Materials			BAS isolated from *F. hepatica* tissues	BAS isolated from the healthy rat livers	BAS isolated from *F. hepatica* infected rat liver	BAS isolated from the healthy rat spleens	BAS isolated from *F. hepatica* infected rat spleens
Basic amino acids		Lys	5.17	10.25	18.55	9.12	5.80
		His	1.55	1.54	1.13	1.64	0.42
		Arg	0.75	2.29	0.17	0.95	1.63
		Σ	7.47	14.08	19.85	11.71	7.85
Acid amino acids		Met sulf	3.85	1.84	2.16	6.16	10.76
		Asp	12.00	5.13	6.58	13.02	14.66
		Glu	13.96	24.43	22.18	20.35	13.40
		Σ	29.81	31.40	30.92	39.53	38.82
Neutral amino acids	Polar	Thr	5.97	1.15	1.13	4.47	5.59
		Ser	4.31	2.17	3.18	6.65	7.12
		Gly	19.59	38.30	36.76	10.06	13.40
		Tyr	+	0.12	+	0.10	0.05
		Σ	29.87	41.74	41.07	21.28	26.16
	Nonpolar	Pro	7.35	1.15	1.30	3.98	4.75
		Ala	7.81	7.69	4.20	7.29	8.18
		Val	4.42	1.48	0.74	4.82	2.58
		Met	+	+	+	+	+
		Ile	3.68	0.51	0.28	2.89	2.48
		Leu	6.38	1.26	0.91	5.99	5.75
		Phe	+	0.15	+	2.51	3.43
		Σ	29.64	12.24	7.43	27.48	27.17
Galactosamine			3.21	0.36	0.40	+	+
Glucosamine			+	0.18	0.34	+	+

^{3}H]-fucose labelled sera glycoproteins from *F. hepatica* infected and Zajdela hepatoma bearing rats are investigated by isoelectrofocussing and specific radioactive peaks are detected (Tsocheva-Gaytandzhieva *et al.*, 2000).

Discussion

We suggest that the mature helminth *F. hepatica* consists of/or contains endogenous inhibitors of cell proliferation, similar to mammalians' with which it acts upon the host and uses them as a mean of autodefence during its parasitic way of life. The liver cells metabolism in *F. hepatica*-infected host is also changed as a result of the parasite migration through the liver tissue and secretion. Thus, either an activation of the endogenous production of inhibitors of cell proliferation is induced as a result of genetic mutations, or the permeability of some substances through the liver cell membranes is increased.

In the field of parasitology mainly non-specific inhibitors of cell proliferation (having many different cells for targets – e.g. cytokines) are investigated (Sher & Coffman, 1992; Brown *et al.*, 1994; Cervi *et al.*, 2001); it is known that

they take part in the host immune mechanisms. There are a few data for the specific growth inhibitors from parasite and host origin: except our data such BAS are isolated from *Taenia crassiceps* and *Ascaris suum* and tested on larvae cultures from these parasites (Kudrna & Prokopič, 1985).

In medicine some cytokines are applied in the treatment of autoimmune diseases like multiple sclerosis, rheumatoid arthritis, etc. (Park *et al.*, 2001). There already exist data about the treatment of different carcinomas with non-specific inhibitors of the cell proliferation in humans (Grimm, 2000). In addition, growth inhibiting, N-substituted oligopeptides from different mammalian tissues, which are specific growth inhibitors, are used for experimental tumour therapy (Elgjo *et al.*, 1998).

It is possible that the development of the recombinant technologies or the synthesis of some BAS – growth inhibitors – from helminth or infected host origin, will allow their application in the treatment of autoimmune diseases, malignant tumours or in the transplantations of tissues and organs.

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