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Inhibiting effect of plasma from normal and tumour bearing mice on the mitotic rate of regenerating liver

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Summary. Plasma from normal mice and from mice bearing the ES2 transplatable malignant tumour was injected intraperitoneally at a dose of 0.01 ml/g body weight in partially hepatectomized mice. Control animals were injected with a solution of sodium citrate in saline. The recipients were killed at the first (14:00 hours/48 h). These times are the time of day and the number of h after partial hepatectomy and second (14:00 hours/72 h) peak times after partial hepatectomy. The number of colchicine metaphases per 1000 nuclei was determined for hepatocytes and litoral cells. A different effect was obtained with plasma from tumour-bearing compared with normal mice. Plasma from both sources when injected 26 h after partial hepatectomy (16:00 hours/26 h) inhibited the mitotic activity of hepatocytes at the next peak of regenerative activity (14:00 hours/48 h). The plasma from tumour-bearing mice also inhibited the peak on the following day (14:00 hours/72 h), whereas plasma from normal mice had no inhibitory effect and, indeed, a compensatory wave was observed at this time. Furthermore, plasma from tumour-bearing mice also showed an inhibitory effect at the first peak (14:00 hours/48 h) when injected at the time of partial hepatectomy (14:00 hours/00 h) or at 22 h before partial hepatectomy (16:00 hours/−22 h) whereas the injection of plasma from normal mice at these times had no inhibitory effect. In the litoral cells the injection of plasma from tumour-bearing mice made 22 h before hepatectomy (16:00 hours/−22 h) led to a stimulation of mitotic activity which was controlled at 14:00 hours/48 h. Injection made at the time of partial hepatectomy (14:00 hours/00 h) caused inhibition at 14:00 hours/48 h and injection at 26 h after partial hepatectomy (16:00 hours/26 h) had no effect. The three injections of normal plasma did not modify the mitotic activity of litoral cells at either 14:00 hours/48 h or at 14:00 hours/72 h.

Key words: Chalone – Circadian rhythms – Transplanted tumours – Plasma – Inhibiting effect – Regenerating liver

Introduction

We have recently reported an inhibitory effect of malignant tumour extracts on the mitotic rate of regenerating liver (Echave Llanos et al. 1985; Badrán et al. 1985b) and the detailed time pattern of this inhibitory effect for one of these tumours (Echave Llanos et al. 1986). The plasma from mice bearing these tumours also showed a clear inhibitory effect on the mitotic rate of regenerating liver (Badrán et al. 1985a).

The effect of plasma from normal or hepatectomized mice, injected or delivered by parabiosis or cross circulation, has been reviewed by Echave Llanos (1963) Croisille and Le Douarin (1965) and Bucher (1963) and more recently by Nadal (1979).

In the present paper we report differences between the time patterns of the inhibitory effect on the mitotic rate of regenerating liver, shown by plasma obtained from normal and tumour-bearing mice.

Materials and methods

Tumour. The tumour used for the present experiments is the ES2 transplantable malignant tumour described previously (Echave Llanos et al. 1986).

Plasma. The plasma was prepared by collecting blood from ten normal and ten tumour-bearing mice in 3% sodium citrate (final dilution 9:1) followed by centrifugation at 4,000 rpm at 0° C for 10 min. The plasma was immediately injected intraperitoneally into the recipients. All manipulations were performed under aseptic conditions and all the instruments were cooled before use.

Injections of plasma and saline. The plasma was administered in a dose of 0.01 ml plasma/g body weight. The controls received a solution of sodium citrate in saline only.

According to the results obtained previously with the tumour extract (Echave Llanos et al. 1986) either plasma or saline were given as a single intraperitoneal injection at three different times relative to partial hepatectomy, i.e. at 22 h *before* hepatectomy, *at* hepatectomy, or 26 h *after* hepatectomy. The animals were killed at the first (14:00 hours/48 h). These times are the time of day and the number of h after partial hepatectomy and second (14:00 hours/72 h) peak times of mitotic activity in the regenerating liver after partial hepatectomy.

Recipients. A total of 134 C3HS male mice, aged 90 days, were used as recipients. The selection, standardization and the procedure for partial hepatectomy of the animals, and the methods of determination of the mitotic activity were as described previously (Echave Llanos et al. 1986).

Results

The results are given in Fig. 1 and Table 1. Fig. 1 shows the mitotic rates in hepatocytes and litoral cells at the times of the first two peaks after partial hepatectomy and after the injection of normal and tumour-bearing mouse plasma at the three times described above.

Plasma from normal mice produced no effect on the mitotic rate of hepatocytes when injected at the time of partial hepatectomy (14:00 hours/00 h) or 22 h before partial hepatectomy (16:00 hours/−22 h), but led to significant inhibition of the mitotic rate at the first peak (14:00 hours/48 h)

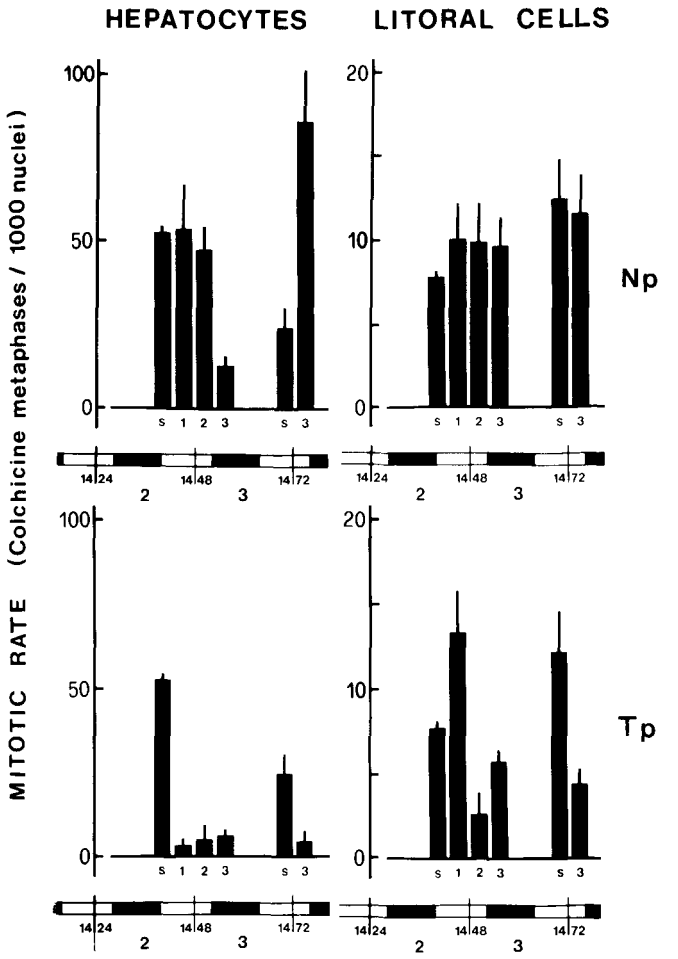

Fig. 1. Inhibitory effect of plasma from normal (NP) and tumour-bearing (TP) mice injected at different times on the mitotic rate (colchicine metaphases/1,000 nuclei) of hepatocytes and litoral cells of regenerating liver at the first (14:00 hours/48 h) and second (14:00 hours/72 h) peaks after partial hepatectomy, performed at 14:00 hours/00 h. s: saline. 1: injection made at 22 h *before* hepatectomy (16:00 hours/−22 h) 2: injection *at* hepatectomy (14:00 hours/00 h). 3: injection made at 26 h *after* hepatectomy (16:00 hours/26 h). In the lower part of the figure are indicated the illumination regimen (light/darkness 06:00 hours/18:00 hours), the time of day and time of regeneration and the days of regeneration. Black bars indicate the mean ±1 Standard error

when injected 26 h after partial hepatectomy (16:00 hours/26 h). At the time of the second peak (14:00 hours/72 h) animals injected with normal plasma at 16:00 hours/26 h showed much higher mitotic rates than control animals.

The injection of plasma from tumour-bearing mice led to significant inhibition of the mitotic rate of hepatocytes when given at any of the three times employed. When the injection was made 26 h after partial hepatectomy (16:00 hours/26 h) the inhibitory effect was still present at the second peak (14:00 hours/72 h).

Table 1. Action of plasma from normal and tumour-bearing mice on the mitotic activity (Mitotic index: Colchicine metaphases/1,000 nuclei) in regenerating liver hepatocytes and sinusoid litoral cells populations controlled at the first 14:00 hours/48 h and second (14:00 hours/72 h) peak times. %I: percent of inhibition. %S: percent of stimulation. p: Statistical significance (Student's "t-test"). $\bar{x} \pm SE$ (n): Mean ± 1 standard error (n: sample size)

Time	Saline	Normal plasma				Saline	Tumour plasma			
TD/TR	$\bar{x} \pm SE$ (n)	$\bar{x} \pm SE$ (n)	%I	%S	p	$\bar{x} \pm SE$ (n)	$\bar{x} \pm SE$ (n)	%I	%S	p
Injection	Hepatocyte mitotic index 14:00/48 h									
16:00/−22	47.2± 9.4 (5)	53.1±13.2 (5)	–	–	–	66.3± 8.8 (6)	3.1±1.3 (6)	95	–	0.001
14:00/00	42.7±20.4 (6)	46.6±13.7 (6)	–	–	–	59.2±11.6 (4)	4.8±4.0 (5)	92	–	0.001
16:00/26	48.4± 7.2 (12)	12.4± 2.6 (12)	74	–	0.001	48.4± 7.2 (12)	5.8±1.8 (12)	88	–	0.001
Injection	Hepatocytes mitotic index 14:00/72									
16:00/26	23.9± 5.9 (6)	84.8±14.6 (6)	–	282	0.001	23.9± 5.9 (6)	4.6±2.4 (6)	81	–	0.01
Injection	Litoral cells mitotic index 14:00/48									
16:00/−22	6.5± 0.8 (5)	9.9± 2.0 (5)	–	–	–	4.3± 0.5 (6)	13.2±2.4 (6)	–	302	0.01
14:00/00	7.2± 1.3 (6)	9.6± 2.3 (6)	–	–	–	7.2± 0.7 (4)	2.5±1.3 (5)	66	–	0.05
16:00/26	10.8± 1.5 (12)	9.4± 1.7 (12)	–	–	–	7.6± 0.4 (12)	5.5±0.7 (12)	–	–	–
Injection	Litoral cells mitotic index 14:00/72									
16:00/26	12.1± 2.3 (6)	11.3± 2.2 (6)	–	–	–	12.1± 2.3 (6)	4.4±0.8 (6)	36	–	0.01

No change was observed in the mitotic rate of litoral cells at 14:00 hours/48 h or at 14:00 hours/72 h after injection of plasma from normal mice at any of the three times employed.

The injection of plasma from tumour-bearing mice made 22 h before partial hepatectomy (16:00 hours/−22 h) produced significant stimulation of mitotic activity in litoral cells at 14:00 hours/48 h whereas injection at the time of partial hepatectomy (14:00 hours/00 h) led to significant inhibition. No effect was observed on the mitotic rate of litoral cells at 14:00 hours/48 h when the injection was given 26 h after partial hepatectomy but there was significant inhibition at the second peak (14:00 hours/72 h).

Discussion

Our experiments have shown differences between plasma from tumour-bearing compared with normal mice in the pattern of their inhibitory effect on the mitotic rate of hepatocytes and litoral cells in regenerating liver.

Based on the different time patterns of reactions obtained, we believe that several explanations are possible. The lack of an apparent circadian time-dependance in the effect of the injections (Echave Llanos et al. 1986) does not exclude a possible circadian-time dependence in the effects either of several short-lived factors acting in given phase of the cell cycle, or of a single long-lived factor remaining inactive in the circulation until the appropriate sensitive phase of cell cycle arrives.

The effect of plasma from tumour-bearing mice on regenerating liver is similar to that of crude tumour extract (Echave Llanos et al. 1986), not only in the time patterns observed but also in the maximum lag of 60 h for these effects to occur. This is true for hepatocytes as well as for litoral cells, and we believe that this similarity supports the hypothesis that the responsible factors present in the plasma of tumour-bearing mice originate in the tumours.

The inhibitory effects of extracts from transplanted hepatomas have been described recently (Badrán et al. 1985b) and we have documented similar inhibitory properties in extracts of other transplanted hepatomas of different degrees of differentiation as well as some mammary carcinomas (unpublished results). Similar effects have been reported for human rhabdomyosarcoma (Fryling et al. 1985; Iwata et al. 1985) and in a human colonic carcinoma cell line (Levine et al. 1985). It would be interesting to determine whether plasma from animals bearing these other tumours shows an inhibitory effect on the mitotic rates of cell populations in regenerating liver. We have started this line of research and our preliminary results support this possibility (Badrán et al. 1985a). Experiments designed to analyze the cell population specificity of the inhibitory effect of tumour extracts and of plasma from tumour-bearing mice are now under way in our laboratory.

To analyze the experimental conditions of the "in vivo" assay system, it is essential to have a thorough knowledge of the system when studying the effect of purified growth factors. This is particularly true with respect

to the times when the factors are injected and the controls are made, which must be related to the well-known circadian variations in growth variables (Echave Llanos 1967; Clausen and Elgjo 1984). These variables behave normally (Echave Llanos et al. 1971; Surur et al. 1985) only when partial hepatectomy is made at the optimal time in a circadian period (Souto and Echave Llanos 1985). The results obtained with normal plasma are a good example of this requirement. The apparent stimulation obtained at the second peak (14:00 hours/72 h) when the injection is made 26 h after partial hepatectomy, is, in fact, a compensatory wave following the previous inhibition observed at the first peak (14:00 hours/48 h). This result reproduces that of Weinbren (1959) and was recognised as a possible source of error as early as 1962 (Echave Llanos 1967).

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References

Bade EG, Sadnik IL, Pilgrim C, Maurer W (1966) Autoradiographic study of DNA synthesis in the regenerating liver of the mouse. Exp Cell Res 44:676–678

Badrán AF, Moreno FR, Echave Llanos JM (1985a) Effect of the plasma of tumour bearing mice on liver growth. Comunic Biológicas 4:1–6

Badrán AF, Moreno FR, Echave Llanos JM (1985b) Effect of tumour extracts on liver growth. Hepatocarcinomas SSlH and SSlK. Comunic Biológicas 4:143–149

Baserga R (ed) (1981) Tissue growth factors. Handbuch Exp Pharmacology, vol 57. Springer, Berlin Heidelberg New York

Bucher NLR (1963) Regeneration of mammalian liver. Int Rev Cytol 15:245–300

Clausen OPF, Elgjio K (1984) Epidermal growth inhibitors (chalones) show circadian stage dependent effects on coll cycle progression in mice. In: Haus E, Kabat HF (eds) Chronobiology 1982–1983. S Karger, Basel, pp 150–154

Croissille Y, Le Douarin NM (1965) Development and regeneration of the liver. In: de Haan R, Ursprung D (eds) Organogenesis. Holt Rinehart Winston, New York

Echave Llanos JM (1963) Importancia del ritmo circadiano en el estudio de los factores tisulares de crecimiento durante la regeneracion hepatica. Rev Soc Argent Biol 39:256–279

Echave Llanos JM (1967) Liver tissue growth factors and circadian rhythms in liver regeneration In: Their H, Rytömaa T (eds) Control of cellular growth in adult organism. Academic Press, London New York, pp 209–220

Echave Llanos JM, D'Aloissio MD, Souto M, Balduzzi R, Surur JM (1971) Circadian variations in DNA synthesis, mitotic activity and cell size of hepatocyte population in young immature male mouse growing liver. Virchows Arch [Cell Pathol] 8:309–317

Echave Llanos JM, Moreno FR, Badrán AF (1985) Effect of an indifferentiated fast growing tumour extract on hepatocytes of regenerating liver. Comunic Biológicas 3:401–408

Echave Llanos JM, Badrán AF, Moreno FR (1986) Inhibiting effect of a hepatoma extract on the mitotic rate of regenerating liver. Virchows Arch [Cell Pathol] 51:17–27

Fryling M, Iwata KK, Johnson PA, Knott WB, Todaro GJ (1985) Two distinct tumour cell growth inhibiting factors from a human rhabdomiosarcoma cell line. Cancer Res 45:2695–2699

Iwata KK, Fryling CM, Knott WB, Todaro GJ (1985) Isolation of tumour cell growth inhibiting factors from a human rhabdomiosarcoma cell line. Cancer Res 45:2689–2694

Levine AE, McRae LJ, Hamilton OA, Brattain DE, Yeoman LC, Brattain MG (1985) Identification of endogenous inhibitory growth factors from a human colon carcinoma cell line. Cancer Res 45:2248–2254

Nadal C (1979) Control of liver growth by growth inhibitors (Chalones) In Mechanism of toxic action on some target organs. Arch Toxicol [Suppl] 2:131–142
Souto M, Echave Llanos JM (1985) The circadian optimal time for hepatectomy in the study of liver regeneration. Chronobiology Int 2:169–175
Sporn JM, Roberts AB (1985) Autocrine growth factors and cancer. Nature 513:745–747
Surur JM, Moreno FR, Badrán AF, Echave Llanos JM (1985) Variations in DNS index and Mitotic index in hepatocytes and sinusoid litoral cells of adult intact male mouse liver along a circadian time span. Chronobiol Int 2:161–168
Weinberg RA (1985) The action of oncogenes in the cytoplasm and nucleus. Science 230:770–776
Weinbren K (1959) Regeneration of the liver. Gastroenterology 37:657–668

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