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Acknowledgements—This work is supported by a grant of the Fédération Nationale des Centres de Lutte Contre le Cancer and Les Comités Départementaux (Région Midi-Pyrénées) de la Ligue Nationale Contre le Cancer.

Pergamon

European Journal of Cancer Vol. 30A, No. 4, pp. 525–527, 1994

0959–8049/94 $7.00 + 0.00

0959-8049(93)E0096-9

H7, an Inhibitor of Protein Kinase C, Inhibits Tumour Cell Division in Mice Bearing Ascitic Ehrlich's Carcinoma

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We have previously shown that H7, an inhibitor of protein kinase C (PKC), inhibits proliferation of several cell lines as well as of primary cultured cells from human tumours. The aim of this work was to assess whether H7 is able to prevent the division of tumour cells in mice bearing Ehrlich's ascitic carcinoma. The LD_{50} of H7 injected intravenously was 61 mg/kg and 94 mg/kg for starved and fed mice, respectively. Acute intraperitoneal injection of 100 mg/kg of H7 decreased the number of mitoses in tumoral cells from ascitic fluid of mice bearing the carcinoma. The reduction was maximal (approximately 50%) after 90 min and then the number of mitosis rose due to a decrease in H7. Continuous delivery of H7 from miniosmotic pumps implanted on the backs of the mice reduced the number of mitoses by approximately 65%, and the effect was maintained for approximately 24 h. The effect cannot be maintained for longer because H7 is unstable at body temperature. These results indicate that inhibition of PKC can block division of tumour cells in carcinoma-bearing animals, and support the idea that inhibitors of PKC could be useful for the clinical control of proliferation of certain tumours.

Key words: protein kinase C, H7, Ehrlich's carcinoma, cell division, tumour proliferation, ascites
Eur J Cancer, Vol. 30A, No. 4, pp. 525–527, 1994

INTRODUCTION

PROTEIN KINASE C (PKC) plays an important role in transmembrane signal transduction and in the control of many cellular responses, including cell proliferation and differentiation, gene expression and tumour promotion [1]. The involvement of PKC in the control of cell growth was initially suggested by the finding that PKC constitutes the receptor for the mitogenic phorbol esters, which are tumour promoters [2]. The role of PKC in the regulation of cellular growth is also supported by the fact that certain growth factors mediate their mitogenic effects in part through a cascade of phosphatidyl inositol hydrolysis and activation of PKC [3, 4]. It has also been shown that PKC activity is overexpressed in aflatoxin-transformed cells [5]. Moreover, PKC activity is increased in human tumour carcinoma and adenoma [6], and the expression of different isoforms of PKC is altered in human astrocytomas/brain tumours, melanomas and

other tumours, and it has been suggested that this alteration plays a role in the malignant progression [7–10]. These findings suggest that inhibitors of PKC could be useful in the clinical treatment of tumour proliferation. We have previously shown that 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H7), an inhibitor of PKC, induces differentiation and inhibits proliferation of neuroblastoma cells in culture [11–13], and also arrests proliferation of other cell types, primary cultures of different tissues and human brain tumours in culture [14, 15]. The aim of this work was to assess whether this inhibitor is able to prevent tumour proliferation in animals. We tested the effect on the proliferation of Ehrlich's ascitic carcinoma in mice. This is a well-known tumour model which allows easy sampling and analysis of the effects of H7. The results showed that acute or continuous administration of H7 reduces the number of mitosis of tumour cells in ascitic fluid of mice bearing the carcinoma.

MATERIALS AND METHODS

Toxicity of H7

Male Swiss mice weighing 18–26 g were used. H7 was dissolved in dimethylsulphoxide to give a concentration of 350 mg/ml. The solutions to be injected were prepared by diluting this solution with water to give the concentration required. All mice were injected intravenously in the tail vein with 100 μl of the corresponding solution. All control mice injected with the solvent survived without any ill effects. A group of mice was starved for 20 h before injection and another group was not. Ten to thirty mice were injected for each dose of H7 assayed.

Treatment of mice bearing the Ehrlich's ascitic carcinoma with H7

Acute administration of H7. Two groups of 10 mice were inoculated intraperitoneally (i.p.) with 0.5 ml of Ehrlich's carcinoma ascitic fluid. After 5 days, a sample of the ascitic fluid was taken as a control and then 10 mice were injected i.p. with 100 mg/kg of H7, and additional samples were taken at 30, 60, 90, 120 and 180 min. Samples were stained with Giemsa and May-Grünwalds stains, and the number of mitoses, metaphases, prophases, telophases and anaphases were counted under the microscope. At least 1000 cells were counted for each preparation.

Continuous administration of H7. For continuous delivery of H7, miniature implantable pumps Alzet 2002 from Alza (Palo Alto, California, U.S.A.) were used. Eight mice were inoculated i.p. with 0.5 ml of Ehrlich's carcinoma ascitic fluid. After 5 days, a sample of the ascitic fluid was taken as a control. The pump was filled with a solution of H7 (125 mg/ml), prepared as above, and implanted in the back of the mice. The mini-osmotic pump released 0.5 μl/h. Samples of the ascitic fluid were taken after 1, 3, 5, 9, 13, 17, 21, 25, 33, 41, 49 and 60 h, and stained as above. The number of mitoses, metaphases, prophases, telophases and anaphases were counted as above. One thousand cells were counted for each preparation.

RESULTS

The toxicity of H7 was tested in fed or starved mice by injection of 100 μl of different solutions in the tail vein. The results obtained are shown in Figure 1. The LD_{50} calculated from these data was 61 mg/kg for starved mice and 94 mg/kg for fed mice.

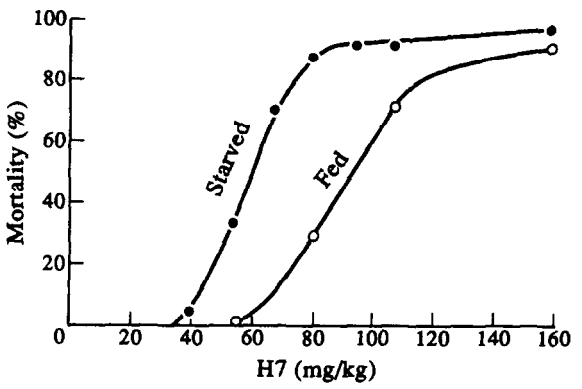

Figure 1. Toxicity of H7. Groups of 15–30 mice were injected in the tail vein with 100 μl of solutions of H7 to give the indicated dose of H7/kg body weight. The experiments were carried out in fed mice and in mice starved for 20 h before injection of H7. A total of 212 mice were used.

On the bases of these results, we decided to test the effect of a single i.p. injection of 100 mg/ml of H7 on the proliferation of Ehrlich's ascitic carcinoma. Two groups of 10 mice were inoculated with the carcinoma and after 5 days a sample of the ascitic fluid was taken to count the number of mitoses. Then 10 mice were injected with H7 and samples were taken at different times (Figure 2). Survival of the treated animals was similar and slightly longer than for controls. As shown in Figure 2, injection of H7 markedly decreased the number of mitoses; the effect was maximal (approximately 50%) at 90 min, and was maintained at 2 h, but the number of mitoses increased at 3 h.

We have recently shown that continuous administration of H7 by delivery from mini-osmotic pumps implanted in the back of

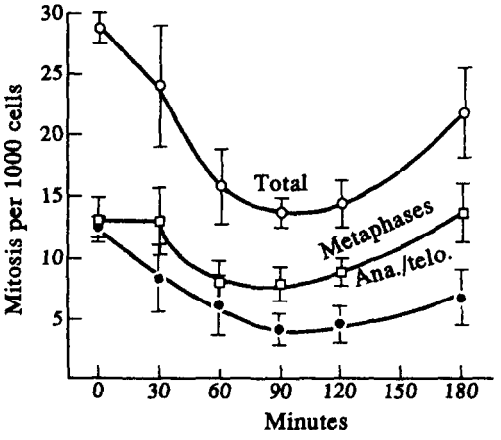

Figure 2. Effect of acute administration of H7 on tumour cell division. Ten mice bearing ascitic Ehrlich's carcinoma (see Materials and Methods) were injected intraperitoneally with 100 mg/kg of H7. Samples of the ascitic fluid were taken before and at different times after injection of H7. The number of mitoses, prophases, metaphases, anaphases and telophases (Ana./telo.) were counted in at least 1000 cells from each animal per point. Standard deviations are given for each point. Values for total mitoses are statistically different from controls ($P \leq 0.001$) at all times except 30 min. For metaphases, values are different from controls ($P \leq 0.01$) at 60, 90 and 120 min. For anaphases and telophases, all values are different from controls ($P \leq 0.001$).

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Revised and accepted 29 Oct. 1993.

the mice can maintain the activity of the Na^+/K^+-ATPase in the sciatic nerve of diabetic mice for approximately 24 h [16]. We, therefore, decided to assess whether continuous administration of H7 to tumour-bearing mice can maintain a decrease in the number of mitoses during this period of time. Eight tumour-bearing mice were implanted with mini-osmotic pumps filled with a solution of H7. As shown in Figure 3, the number of mitoses was markedly reduced by administration of H7. The decrease was maximal (approximately 65%) at 3–5 h and was still high (approximately 60%) at 17 h. After 20 h, the number of mitoses increased again. The number of animals surviving at different times is also given in Figure 3.

DISCUSSION

The results shown in Figure 2 clearly indicate that a single administration of H7 inhibits division of tumoral cells in mice bearing the Ehrlich's carcinoma. The effect was rapid but transient. In cultured cells, the effect of a single administration of H7 was maintained for 48 h [13]; in contrast, the effect in animals was maximal at 90 min but decreased after 2 h. This is due to the rapid metabolisation or elimination of H7 in the mice. The kinetics shown in Figure 2 are in good agreement with a previous report showing that the effect of H7 on the activity of Na^+/K^+-ATPase in the sciatic nerve of diabetic mice is rapid but transient with a half-life of approximately 1 h [17].

When H7 was administered by continuous delivery from mini-osmotic pumps implanted in the backs of the animals, the decrease in the number of mitoses was maintained for longer periods of time (17 h) and increased again after 20 h (Figure 3). The loss of the effect of H7 at this time was expected on the basis of the instability of H7 in solution at this temperature [16]. Although after 17 h the number of mice remaining was too low for statistical purposes, the values obtained up to this time were significant and clearly showed that continuous administration of H7 can maintain the suppression of tumour cell division in mice bearing Ehrlich's ascitic carcinoma.

These results support the idea that PKC inhibitors can be useful for the clinical treatment of tumour proliferation, and are in agreement with two recent reports showing that a staurosporine derivative and UCN-01, inhibitors of PKC, have antitumour activity [18, 19]. Although H7 is not a suitable inhibitor due to its instability, new inhibitors with suitable pharmacokinetic properties could be beneficial in arresting tumour growth.

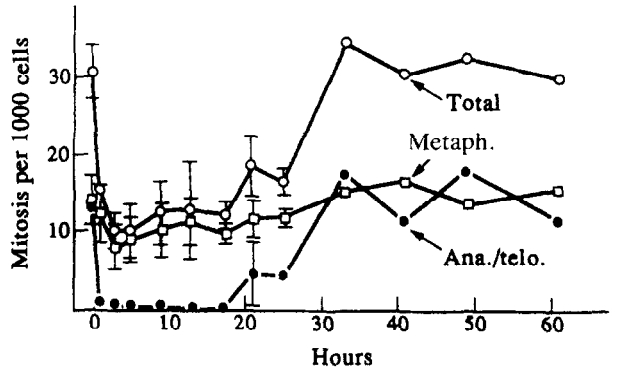

Figure 3. Effect of continuous administration of H7 on tumour cell division. Eight mice bearing ascitic Ehrlich carcinoma were implanted subcutaneously in the back with mini-osmotic pumps filled with H7 (125 mg/ml). The pumps were incubated for 4 h at 37°C in saline before implantation so that release of H7 began immediately after implantation. The pumps released 0.5 μl/h. Samples of the ascitic fluid were taken before and at different times after implantation of the pumps. The number of mitoses, prophases, metaphases (Metaph.), anaphases and telophases (Ana./Telo.) were counted in at least 1000 cells from each animal per point. The number of animals remaining alive were 8, 6, 3, 2 and 1 after 12, 16, 24, 32 and 60 h, respectively. For the control group, all animals remained alive at this time. Standard deviations are given. For points before 32 h, S.D. were less then the width of the point if they are not shown. For total mitoses and for anaphases and telophases the values were different from controls ($P \leq 0.001$) at all times up to 25 h. For metaphases only the values at 3 and 5 h were different from controls.

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Acknowledgement—This work was suppored by the Fondo de Investigaciones Sanitarias of Spain (93/0142).