Radiol Oncol 1996; 30: 20-4.

Incidence, latency period, and survival of mice bearing 20-methyl cholantrene induced tumours do not change after Cyclosporin treatment

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Cyclosporin (CsA) is a very effective immunosuppressive drug. It is widely used in the treatment of allograft recipients as well as in other immune-dependent diseases. One of the important side effects of immunosuppression is unexpected and increased appearance of tumours. Development of solid tumours in CsA treated patients is rare and poorly explained. The authors developed an experimental system for studying the effects of high doses (250μg and 1000 μg, 25 doses each mouse in 90 days) of CsA on the development of experimental 20-methyl cholantrene induced tumours in mice. Latency period, time of survival from tumour appearance and overall survival time from the onset of the experiment are reported. Data were compared with those obtained in a control group of animals receiving saline instead of CsA. Based on the presented experiment it has been concluded that CsA treatment does not exert statistically significant effect on any of the measured parameters.

Key words: carcinoma - chemically induced; methyl cholantrene; cyclosporine; mice

Introduction

Cyclosporin (CsA) is an already well established immusuppressive agent.[1] It is a cyclic polypeptide consisting of 11 amino acids. CsA inhibits the development of cell-mediated reactions such as allograft immunity, delayed cutaneous hypersensitivity, experimental allergic encephalitis, graft-versus-host disease and T cell dependent antibody production.[2,3] It is an effective inhibitor of interleukin 2 (I1-2) production and release.[4] CsA blocs the resting lymphocytes in G_0 or G_1 phase of the cell cycle. Its activity is specific and reversible. It does not depress haemopoesis and has no effect on the function of phagocytic cells. Still the drug should be used with precaution, because it may affect normal immune response in patients and has some unpleasant side effects. The most frequent side effects are: hypertrichosis, tremor, impaired renal function, hypertension, hepatic dysfunction, fatigue, gastrointestinal disturbances, burning sensations of hands and feet.[5] Among the expected side effects is an increase in the occurrence of malignancies and lymphoproliferative disease development.[6,7] The reported number and distribution of these was similar as

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UDC: 616-006.6:615.277.4

in patients on conventional immunosuppressive therapy.[8–11] Little is known about the development of solid tumours after CsA treatment.[12, 13] The aim of our study was to test the effect of high-doses CsA treatment on latency period, incidence and survival of tumour bearing mice with 20-methyl cholantrene induced solid subcutaneous tumours.[14, 15]

Materials and methods

Animals

In all the experiments HanNmRi mice were used. Mice entered the experiment at the age of 2 months. They were kept in conventional animal facilities and fed with normal prefabricated food. They received food and water ad libitum. At the beginning of the experiment they were without evident signs of disease.

Tumour induction

Treatment with 20-methyl cholantrene was used for tumour induction. 20-methyl cholantrene was weighted out and dissolved in olive oil in concentration 10 mg/ml and given to the animals in 0.1 ml doses. The substance was injected subcutaneously into the right hind leg. The animals were examined for the tumour growth weekly.

Cyclosporin treatment

After the injection of 20-methyl cholantrene the animals received CsA two times weekly for 90 days (altogether 25 doses). CsA was given to both treated groups (each consisting of 20 animals) at two different dosage regimes. The mice of the first group received 250 μg/dose and the mice of the second group 1000 μg/dose of CsA (Sandimmin, Sandoz, Switzerland). The mice in the third group (20 animals) received saline instead of CsA.

Detection of the tumour growth

Once weekly the animals were examined for tumour growth by palpation. As soon as the tumour was detected the latency time was recorded. From then on the mice were monitored for the survival time. The data were analysed with the statistical computer program ANOVA (Quattro Pro for Windows 5.0, Borland International, Inc.)

Results

Latency periods of tumour detection are documented in Figure 1. The first tumours appeared on Day 56 and the last ones on Day 161. The average latency period for the control group was 85.94, for the group treated with 250 μg/dose of CsA, 91 days, and for 1000 μg/dose treated group 85.61 days. Tumours appeared at almost the same frequency in all groups tested. Statistics have (ANOVA One Way) proved that there is no difference in latency period between the groups tested ($p > 0.05$).

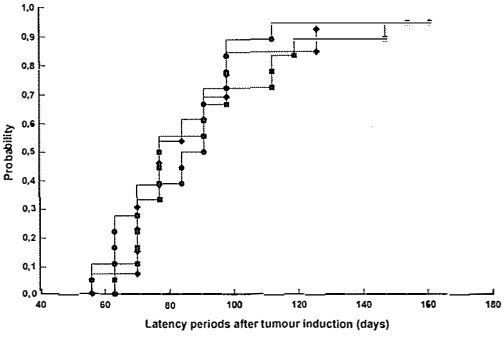

Figure 1. Latency periods of 20-methyl cholantrene induced tumours in the controls, 250 μg/dose and 1000 μg/dose CsA treated groups of mice. The first tumours appeared on Day 56 and the last ones on Day 161. The average latency period for the control group (-●-) was 85.94, for the group treated with 250 μg/dose of CsA (-■-) 91 days, and for the group treated with 1000 μg/dose (-◆-) 85.61 days. Tumours appeared at almost the same frequency in all groups tested. Statistics (ANOVA One Way) have proved that there is no difference in latency period between the groups tested ($p > 0.05$).

Mice died from 19 to 91 days after tumour detection (Figure 2). The average survival time after tumour occurrence for the control group was 37.5, for the group receiving 250 μg/dose of CsA 35.27 days and for 1000 μg/dose treated

group 38.84 days. There was no statistically significant difference between the groups tested ($p > 0.05$).

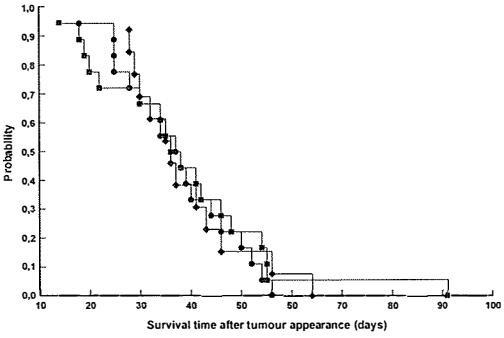

Figure 2. Survival time after tumour appearance. Mice died from 19 to 91 days after tumour detection. The average survival time after tumour occurrence for the control group (-●-) was 37.5 days, for the group recciving 250 μg/dose of CsA (-■-) 35.27 days, and for group treated with 1000 μg/dose (-◆-) 38.84 days. There was no statistically significant difference between the groups tested (ANOVA One Way) ($p > 0.05$).

In Figure 3 the time from the injection of carcinogen to animals' death is presented. Mice began to die on Day 77, and were all dead by Day 193, except for one individual in the control group, and one individual in the group treated with 1000 μg/dose of CsA. There were no statistically significant differences between groups ($p > 0.05$).

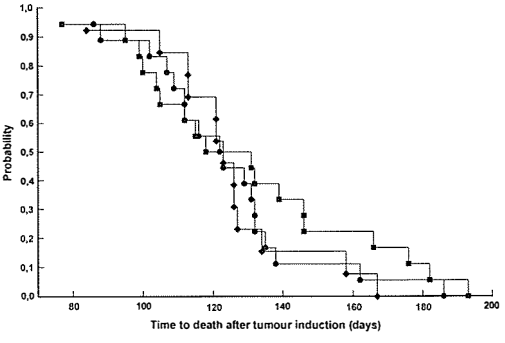

Figure 3. Time from the injection of 20-methyl cholantrene to animals' death. Mice began to die on Day 77 and were all dead by Day 193, except for one individual in the control group (-●-) and one individual in the group treated with 1000 μg/dose of CsA (-◆-). There were no statistically significant differences between groups tested (ANOVA One Way) ($p > 0.05$).

It is interesting to notice that in the control group one animal survived without tumour and one mouse died intercurently, while in the group treated with 250 μg/dose of CsA two mice died intercurrently; in the group treated with 1000 μg/dose of CsA one animal survived without tumour and six died intercurrently (data not presented).

Discussion

In the case of CsA, which has no direct genotoxic effect, tumour promotion is probably dose-dependent.[1,16] This observation led to the idea to test the possible role of CsA in a model of 20-methyl cholantrene induced solid subcutaneous tumours in mice.[14,17] It is allready known that CsA inhibits synthesis and excretion of Il-2, but how this function is performed remains unclear.[18] From the pharmacological studies it is known, that CsA binds to lymphocytes, leukocytes and to a variety of cell lines in a specific, saturable and reversible way.[19,20] In whole blood, CsA is mainly associated with erythrocytes which contain the Cyclosporin-binding protein ciclophilin. For cell membrane binding a membrane glycoprotein gp170 is important. It is physiologically expressed in kidney and liver cells and in multi-drug resistant tumour cells.[21] CsA in cells inhibits the gene transcription for Il-2 synthesis. The gene promoter is the most likely part of the gene affected. In contrast to generally accepted inhibitory effecs on Il-2 synthesis, CsA inhibits the transcription of a variety of other activation-induced cytokines and proto-oncogens. It is less known that CsA inhibits cell growth of several tumours. Concentrations in the range of 10 μM inhibit tumour cell proliferation.[22] These concentrations may either inhibit the protein syn-

thesis or are allready cytotoxic. Those tumours that grow only in the presence of growth factors are the most inhibited by CsA. In conclusion, CsA is not mitogenic and exerts no proliferative effect on any of the observed experimental system. In genotoxicity tests it was shown that it is devoid of any mutagenic properties.

Carcinogenicity of CsA was tested in a variety of animals. In mice (OF-1 strain) fed with 0, 1, 4 and 16 mg/kg of CsA for 78 weeks, an increased mortality of mice was established only for high-dose treated females.[23] Malignant lymphomas were found both in controls and treated animals at a high incidence, without statistically significant effect of the drug treatment.[1,3,10] An interesting finding was that CsA may alter the type, frequency and distribution of spontaneously occurring osteomas in OF-1 mice.[1] Mice treated with N-methyl-N-nitrosourea had a reduced latency period for the development of tumours, however the type and distribution of tumours were not influenced by CsA administration.[24,25] In rats, CsA allowed a faster development of gastrointestinal tumours induced by N-methyl-N-nitro-nitrosoguanidin treatment.[26] There is an interesting report on protective effect of CsA on 3-methylcholantrene-induced lymphomas by oral administration in the rat.[27] In summary, all the data cited until now have failed to demonstrate that CsA as a single agent posses a carcinogenic potential for induction of solid tumours. But on the contrary, CsA was shown to enhance the local growth as well as metastasis of immunogenic tumours[28] and lymphoproliferative malignances.[1,3,6,7,10]

Our data demonstrate a negligible influence of CsA treatment (even at high doses) on 20-methyl cholantrene induction of solid subcutaneous tumours in mice. We did have a higher incidence of intercurrent deaths of mice in the high-dose treated group, but the differences in latency period, the percentage of induced tumours and the survival time after tumour appearance between control mice and the CsA treated mice were statistically nonsignificant.

Acknowledgements

We gratefully acknowledge the help in preparation of the manuscrip to Mrs. Olga Shrestha and for designing of the graphs to Miss. Maja Čemažar, dipl. biol.. This work was supported by Ministry of Science and Technology Republic Slovenia Grant No. J3-5301-0381-94.

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