Rapamycin Even When Combined With Cyclosporine Attenuates Tumor Growth But Does Not Induce Regression of Established Walker Sarcomas

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ABSTRACT

Purpose. To investigate the effect of rapamycin (Rapa) on growth or regression of Walker tumor used alone or in combination with CsA and MMF.

Methods and results. Wistar rats received water (control) or Rapa or CsA 1 day before and daily after tumor inoculation. On day 10, tumor volume (TV) was smaller among Rapa (6.8 ± 2.7 cm³) versus control (14.9 ± 4.2 cm³, P < .001) or CsA (13.9 ± 3.0 cm³, P < .0001) treatment groups. Tumor growth was greatly inhibited (TI) by Rapa (−49.3%). Tumor weight (TW) was significantly (P < .001) lower in Rapa (3.7 ± 1.2 g) versus CsA (8.8 ± 2.1 g) or control (7.3 ± 2.0 g) animals. An additional set of rats received water or Rapa or CsA 1 day before tumor inoculation. On day 10, TV and TW were lower among Rapa (3.8 ± 1.5 cm³) and Rapa + CsA (3.1 ± 1.2 cm³) and Rapa + MMF (4.6 ± 2.7 cm³) groups compared with controls (10.9 ± 3.8 cm³, P < .0001). TI was −52.1% in Rapa, −68.5% in Rapa + CsA, and −63% in Rapa + MMF. A further set of rats received either water or Rapa on the day 4 after tumor inoculation. On day 10, tumor growth and TW among the Rapa and control groups were similar.

Conclusion. Rapamycin greatly inhibited tumor growth when used alone or with CsA or MMF, but did not produce an effect on a well-established Walker sarcoma.

Organ transplantation is a treatment modality for patients with end-stage organ diseases. Improved allograft survival has been followed by long-term complications that may limit patient survival. Among the most serious complications is a high risk of recurrent neoplasms and the development of de novo cancer. The risk of developing cancer is increased three to fourfold among transplant recipients compared with the general population.1 The incidence varies according to geographic region, the type of organ, and the time elapsed after transplantation. The calculated risk of developing cancer at 10 years posttransplantation is approximately 14%,2 increasing to 22% at 20 years.3 One approach to address this problem is to identify drugs with effective immunosuppressive but low proneoplastic, or even antineoplastic, properties. Rapamycin, a macrocyclic fermentation product that blocks cell cycle progression from phase G1 to S by inhibiting signal transduction pathways,4,5 has been screened against a panel of tumor cell lines by the National Cancer Institute (United States). It showed good activity against mammary, colon, brain, and ependymoblastoma tumors.6 These findings led us to investigate the effects of rapamycin (Rapa) on Walker carcinosaoma outgrowth and regression in a rat model to determine whether this effect was influenced by the addition of cyclosporine microemulsion (CsA) or by mycophenolate mofetil (MMF).

MATERIALS AND METHODS

Tumor nodules from tumor-bearing rats were excised, gently homogenized in a hand-operated tissue grinder, and suspended in sterile Lactated Anger solution with gentamycin. The suspension was adjusted to 1 × 10⁶ viable tumor cells/mL. A freshly prepared
cell suspension of 1.0 mL was implanted by subcutaneous injection into the right axilla of 4- to 8-week old male Wistar AF rats. The animals housed in cages were given food and mineral water ad libitum. Tumor measurements were performed daily; body weight was recorded on alternate days. On day 10 after tumor implantation animals were sacrificed by aorta puncture. The tumors were excised and weighed to calculate tumor weight (WT) and percent tumor inhibition (TI). CsA blood levels were measured using a monoclonal-specific fluorescence immunoassay (TDX, Abbott).

Evaluation
As subcutaneous tumors arose in the animals, caliper measurements were performed on their length and width (in millimeters) to calculate tumor volume (TV). On day 10 median tumor weight (WT) and percent tumor inhibition (TI) were calculated using the formula:

\[
TV = \frac{\text{length} \times \text{width}^2}{2}
\]

\[
TI = 100 - \frac{T}{C}\%
\]

\[
\frac{T}{C}\% = \frac{\text{MTW (treatment group)}}{\text{MTW (control group)}} \times 100
\]

where T≠C% is treatment/control.

Immunosuppressants
Immunosuppressants used were: CsA microemulsion oral solution, 10 mg/kg/d daily; MMF, 10 mg/kg daily; Rapa oral solution, 1.5 mg/kg daily.

**Experiment I**
Thirty male Wistar rats were allocated to three groups: control (Cont) mineral water \( (n = 10) \); Rapa \( (n = 10) \); CsA \( (n = 10) \). Drugs were started 1 day before tumor inoculation and administered PO over 10 days.

**Experiment II**
Thirty-six Wistar rats were allocated to four groups: control mineral water \( (n = 9) \); Rapa \( (n = 8) \); CsA + Rapa \( (n = 9) \); MMF + Rapa \( (n = 9) \). All drugs were introduced 1 day before tumor inoculation and administered PO over 10 days.

**Experiment III**
Seventeen Wistar rats were allocated to two groups: control mineral water \( (n = 7) \) versus Rapa \( (n = 10) \). Drug was introduced on day 4 after tumor inoculation and administered over 7 days.

**Statistical Analysis**
All values were expressed as means ± SD. Statistical analysis was performed using SPSS Data Editor software (SPSS for Windows software). Significance was defined as \( P < .05 \). Groupwise comparisons were made by ANOVA univariate (Tukey) and multivariate assays.
RESULTS

Experiment I

On day 10, TV was smaller among Rapa (6.8 ± 2.7 cm³) versus control (14.9 ± 4.2 cm³, P < .001) or CsA animals (13.9 ± 3.0 cm³, P < .0001, Fig 1). Tumor growth was greatly inhibited (TI) by Rapa (−49.3%) but not by CsA. TW was also significantly lower among Rapa (3.7 ± 1.2 g) than CsA (8.8 ± 2.1 g, P < .001) or control hosts (7.3 ± 2.0 g, P < .001). Body weight was similar in the three groups both before (Cont, 141.3 ± 19.0 g; Rapa, 139.3 ± 16.3 g; CsA, 146.7 ± 21.8 g) and after treatment (Cont, 152.1 ± 22.8 g; Rapa, 146.4 ± 19.8 g; CsA, 159.4 ± 19.9 g; P = .085). Tumor metastasis was not observed in any group. Mean CsA-blood levels were 307.9 ± 142.3 ng/mL.

Experiment II

On day 10, TV was lower in Rapa (3.8 ± 1.5 cm³), Rapa + CsA (3.1 ± 1.2 cm³) or Rapa + MMF (4.6 ± 2.7 cm³) groups compared with control animals (10.9 ± 3.8 cm³; P < .0001; Fig 2). TW was also lower in these groups (Rapa, 3.5 ± 1.2 g; Rapa + CsA, 2.3 ± 0.4 g; and Rapa + MMF, 2.7 ± 1.1 g vs Cont, 7.3 ± 1.4 g; P < .0001). TI was −52.1% in Rapa, −68.5% in Rapa + CsA, and −63% in Rapa + MMF. Body weight was similar in the four groups before (Cont, 125.3 ± 12.1 g; Rapa, 128.0 ± 16.4 g; CsA + Rapa, 138.6 ± 11.5 g; MMF + Rapa, 138.7 ± 14.5 g) and after treatment (Cont, 135.1 ± 23.0 g; Rapa, 145.8 ± 22.3 g; CsA + Rapa, 130.1 ± 17.0 g; MMF + Rapa, 138.7 ± 14.5 g; P = .542). Tumor metastasis were not observed in any of the groups. Mean CsA-blood levels were 345.9 ± 194.9 ng/mL.

Experiment III

On day 10, TV in Rapa (13.2 ± 3.2 cm³) was similar to control hosts (14.9 ± 7.4 cm³; P = .831). Similarly TW, was not different (Rapa, 5.2 ± 1.6 g vs Cont, 6.3 ± 3.2 g; P = .229). Body weight was similar in the four groups before (Cont, 177.3 ± 28.0 g; Rapa, 162.6 ± 26.7 g) and after treatment (Cont, 188.1 ± 18.0 g; Rapa, 169.6 ± 24.8 g; P = .138). Tumor metastasis were not observed in either group.

DISCUSSION

The results of this study demonstrate that rapamycin displays antitumor activity against Walker sarcoma. The antitumor activity of rapamycin has been previously demonstrated using human rhabdomyosarcoma, neuroblastoma, and Epstein-Barr virus-transformed B lymphocyte cells in vitro7–9 and in B16 melanocarcinoma, colon 38 tumors, CD8F1 mammary tumors, and U251 glioblastoma brain tumors in vivo.6,10 Dilling et al7 were among the first to demonstrate the antiproliferative action of rapamycin in three of four lineages of pediatric rhabdomyosarcoma showing that this inhibition was dependent on the presence of the IGF-1R.

Shi et al11 proposed induction of apoptosis as the mechanism of the inhibitory action of rapamycin on IL2-depen-
dent murine T-cell lines, human promyelocytic leukemia HL-60, and human ovarian cancer SKOV3, findings that were corroborated by Seufferlein and Rozengurt in lung small cell carcinomas, where G1 blockade was due to p70/p85 kDa S6 kinases. Inhibition of the G1 phase by rapamycin, as shown by Metcalfe et al., led to increased apoptosis and/or inhibition of tumor cell growth. Another mechanism contributing to the antineoplastic action of rapamycin is blockade of tumor angiogenesis as proposed by Guba et al.

The inhibitory effect of rapamycin observed in this model is maintained even when CsA added into the treatment regimen. The persistence of rapamycin’s antitumor activity even in combination with CsA has been reported in other experimental settings, namely, B lymphoma and renal cancer cells. The sensitivity of tumor cell lines to rapamycin is variable. The present study shows that the drug does not produce regression of established tumors, which may justify the need to combine other antitumor agents with rapamycin.

In conclusion the present studies show that in rapamycin greatly inhibited tumor growth when used alone or in combination with cyclosporine microemulsion or MMF, but had no effect on a well-established Walker sarcoma.

REFERENCES