Both AP-1 and NF-κB Seem to be Involved in Tumour Growth in an Experimental Rat Hepatoma

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Abstract. Daily treatment of rats bearing Yoshida AH-130 ascites hepatoma with the nuclear factor kappa-B (NF-κB) and activator protein-1 (AP-1) double inhibitors SP100030 and SP100207 at a dose of 5 mg/kg and 10 mg/kg of body weight, respectively, resulted in a clear inhibition of tumour growth. The decrease was not related to an altered cell cycle distribution of the tumour cell population suggesting a merely necrotic effect. The results presented confirm that both transcription factors are involved in the growth of the experimental tumour system used, suggesting that both signaling cascades play a very important role in the signaling of tumour cell proliferation. This could, in future, allow for the development of new therapeutic strategies for cancer patients.

In order to define successful approximations for the pharmacological treatment of different types of tumours, a good knowledge of the different intracellular signalling pathways linked to the proliferation process is essential. Previous studies have demonstrated an involvement of both nuclear factor kappa-B (NF-κB) and activator protein-1 (AP-1) transcription factors in tumour growth in both animals and humans (1-2). The Yoshida AH-130 rat ascites hepatoma is a suitable model system for studying the mechanisms involved in fast growing neoplasms. Indeed, this transplantable sarcoma grows exponentially from day 2 after transplantation and causes the death of the animals by day 12 (3-4).

Bearing this in mind, the main objective of the present work was to test if blockage of the two transcription factors, namely of NF-κB and AP-1, was able to interfere with the growth of the tumour in AH-130 hepatoma-bearing rats. The effects on the growth and cell cycle distribution in rat Yoshida AH-130 ascites hepatoma were examined using two different NF-κB and AP-1 double inhibitors, SP100030 and SP100207. These compounds have previously been used in pre-clinical studies involving arthritis (5), and host versus graft disease (6). In both experimental conditions, the double inhibitors have clearly proven to be anti-proliferators for T-cells (7).

Materials and Methods

Animals. Male Wistar rats (Interfauna, Barcelona, Spain), of 5 weeks of age, were used in the different experiments. The animals were maintained at 22±2˚C with a regular light-dark cycle (light on from 08:00 a.m. to 08:00 p.m.) and had free access to food and water. The food intake was measured daily. All animal manipulations were made in accordance with the European Community Guidelines for the Use of Laboratory Animals.

Tumour inoculation and treatment. Rats were divided into two groups, namely controls and tumour hosts. The latter received an intraperitoneal inoculum of 108 AH-130 Yoshida ascites hepatoma cells obtained from exponentially growing tumours (3). Both tumour and nontumour bearing animals were subdivided into four groups according to treatment. The treated groups were injected subcutaneously with either a daily dose of SP100030 (5 mg/kg body weight) or SP100207 (10 mg/kg) dissolved in 1% carboxymethyl cellulose (carrier). The non-treated groups received an equivalent injection of the carrier alone. On day 7 after tumour transplantation, the animals were weighed and anesthetized with an intraperitoneal (i.p.) injection of ketamine/xylazine mixture (3:1) (Imalgene® and Rompun® respectively). The tumour was harvested from the peritoneal cavity and its volume and cellularity evaluated.

Flow cytometry. Flow cytometric analysis was carried out using an Epics Elite flow cytometer (Coulter Electronics Corporation, Hialeah, FL, USA). Excitation took place using a standard 488 nm air-cooled argon-ion laser at 15 mW power. The instrument was set up with the standard configuration. Forward scatter (FSC), side scatter (SSC) and propidium iodide red fluorescence (675 nm...
by means of Student's Statistical analysis.

Chemical Co. (St. Louis, MO, USA). SP100030 and SP100207 obtained either from Roche S.A. (Barcelona, Spain) or from Sigma Biochemicals.

The cell cycle analysis was carried out using Multicycle Software (Phoenix Flow Systems, San Diego, CA, USA). The data of the cell distribution are expressed in % of the total number of cells in the cycle. Experimental groups: T1, tumour-bearing rats treated with SP100030; T2, tumour-bearing rats treated with SP100207.

Data are means±SEM, with the number of animals indicated in parentheses. Statistical significance of the results (Student’s t-test): treatment vs. none: *p≤0.05; **p≤0.01. Experimental groups: T1, tumour-bearing rats treated with SP100030; T2, tumour-bearing rats treated with SP100207.

Statistical analysis. Statistical analysis of the data was performed by means of Student’s t-test.

Results

The results presented in Table I clearly show that both inhibitors exerted a similar effect on tumour growth. Administration of SP100030 reduced tumour volume (by 11%), tumour cell density (by 16%) and total cell number (by 16%). Similarly, administration of SP100207 also reduced volume (by 20%), tumour cell density (by 12%) and total cell number (by 17%).

The results presented in Table II suggest that the inhibitors did not seem to affect the cell cycle distribution (G0/G1 and G2/M) nor produce aneuploid peaks (not detected).

Discussion

These results suggest that both these transcription factors are involved in the growth of the rat ascitic hepatoma AH-130.

Table I. Effects of in vivo SP100030 (5 mg/kg) and SP100207 (10 mg/kg) treatment on tumour growth in rats bearing Yoshida AH-130 ascites hepatoma.

<table>
<thead>
<tr>
<th>Volume (ml)</th>
<th>Cell density (10⁶/ml)</th>
<th>Total cells (x10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control tumour</td>
<td>75±1.7 (4)</td>
<td>91±2.2 (5)</td>
</tr>
<tr>
<td>T1</td>
<td>67±2.0 (4)*</td>
<td>76±4.0 (5)*</td>
</tr>
<tr>
<td>T2</td>
<td>60±2.6 (5)**</td>
<td>80±1.1 (5)**</td>
</tr>
</tbody>
</table>

Data are means±SEM, with the number of animals indicated in parentheses. Statistical significance of the results (Student’s t-test): treatment vs. none: *p≤0.05; **p≤0.01. Experimental groups: T1, tumour-bearing rats treated with SP100030; T2, tumour-bearing rats treated with SP100207.

The involvement of NF-κB in tumour growth has extensively been reported (8-10). In fact, previous studies from our laboratory using curcumin, a natural NF-κB inhibitor, clearly showed a reduction of tumour growth following administration of turmeric in the same experimental rat tumour model (11). Similarly, a large number of studies suggest an involvement of the AP-1 signalling cascade in tumour growth both in experimental and human tumours (12-15).

In elucidating the possible mechanism(s) involved in the antitumoral effects observed, the cell cycle distribution of the tumour cell population lead us to conclude, therefore, that the effects of the compounds were based on induction of cell necrosis.

In conclusion, the results presented here suggest that the family of double inhibitors used may have a potential therapeutic use in the treatment of both animal and human tumours but additional pre-clinical studies are required in other tumour types.

References


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