Safety, Pharmacokinetics, and Tissue Distribution of Liposomal P-Ethoxy Antisense Oligonucleotides Targeted to Bcl-21

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ABSTRACT

Antisense oligonucleotides (oligos) have the ability to selectively block disease-causing genes, thereby inhibiting production of disease-associated proteins. However, their effectiveness has been limited by their low intracellular delivery. We had previously demonstrated that liposomes could increase the intracellular uptake of P-ethoxy oligos, hydrophobic analogs of phosphodiesters, and that liposomal Bcl-2 P-ethoxy antisense oligos (L-Bcl-2) could selectively inhibit Bcl-2 protein production, thereby inducing growth inhibition in Follicular Lymphoma cell lines. To understand the in vivo behavior of L-Bcl-2, we conducted a series of studies to evaluate the safety, pharmacokinetics, and tissue distribution of i.v. injections of L-Bcl-2 in normal rodents. Daily administration of 20 mg of L-Bcl-2/kg of body weight in 5 consecutive days had no adverse effects on renal or hepatic functions, nor on hematological parameters. Histopathology also did not reveal any significant changes in the morphology of the organs studied. In rats, the area under the curve of L-Bcl-2 reflects a two-compartment model of distribution with a biphasic plasma clearance. The \( T_{1/2a} \) and \( T_{1/2b} \) were approximately 8 min and 4.2 h, respectively, and the \( V_d \) was 79 ml, indicating a broad body distribution. The highest concentrations of L-Bcl-2 were found in spleen > liver > kidneys. These studies showed that in the schedules studied no significant toxicity associated with L-Bcl-2 was observed over 6 weeks, and that L-Bcl-2 could be widely distributed in the body.

Apoptosis, or programmed cell death, has been shown to be regulated by several signals, including the Bcl-2 gene. Bcl-2 interacts with other gene products, such as Bax, through the production of different dimers, which leads to the regulation of apoptosis. Bcl-2 homodimers block apoptosis whereas Bax homodimers promote apoptosis. Bax can bind to Bcl-2 protein and neutralize the ability of Bcl-2 to block cell death (Neilan et al., 1993; Reed, 1995). The ratio of Bcl-2 to Bax is thought to determine the susceptibility of a cell to death. Based on its function as a regulator of cell survival and its potential role in drug resistance, the Bcl-2 gene represents an ideal target for novel therapeutic strategies designed to improve the treatment of cancer (Miyashita et al., 1995).

Antisense oligonucleotides (oligos) can inhibit the expression of disease-associated proteins, such as Bcl-2 (Agrawal, 1996). When antisense oligos bind to the target mRNA, a DNA-RNA hybrid is formed, inhibiting the production of its corresponding protein. These antisense oligos can therefore facilitate the study of the role of individual proteins in cellular functions. However, their effectiveness as potential molecules has been limited by their low intracellular delivery (Cotter, 1997; Alama et al., 1997; Temsamani and Guinot, 1997).

We have previously shown that liposomal carriers can enhance the cellular uptake of oligos, including that of the nuclease resistant P-ethoxy oligos, a hydrophobic analog of phosphodiesters. P-ethoxy oligos were used because they could be incorporated into liposomes at \( \approx 95\% \) efficiency. Liposome-incorporated P-ethoxy antisense oligos can lead to selective inhibition of protein expression and cell growth in diverse cell lines (Tari et al., 1996; Tormo et al., 1998).

We showed that the Bcl-2/Bax protein ratio is important for the growth regulation of Follicular Lymphoma (FL) cell lines (Tormo et al., 1998). Bcl-2 down-regulation by liposomal Bcl-2 P-ethoxy antisense oligos (L-Bcl-2) leads to a decrease in the ratio of Bcl-2/Bax and an increase in apoptotic induction in transformed FL cells. Therefore, down-regulation of Bcl-2 protein provides a rationale for the molecular therapy of FL. As a prelude to clinical trials, it is important to un-

ABBREVIATIONS: oligos, oligonucleotides; FL, Follicular Lymphoma; L-Bcl-2, liposomal Bcl-2 P-ethoxy antisense oligos; BUN, blood urea nitrogen; SGOT, serum glutamic-oxaloacetic transaminase.
derstand the pharmacokinetics, tissue distribution, and safety of single and multiple i.v. administrations of L-Bcl-2 in rodents.

Materials and Methods

Animals, Lipids, and P-Ethoxy Oligos. Lewis rats weighing approximately 400 g and ICR mice weighing 25 to 30 g were obtained from Harlan Spraque-Dawley (Indianapolis, IN). 1,2 Dioleoyl-sn-glycero-3-phosphocholine and P-ethoxy oligos were purchased from Avanti Polar Lipids (Alabaster, AL) and Oligos Etc. (Wllsonville, OR), respectively. An oligo specific for the translation initiation site of human Bel-2 mRNA: 5’CAGCGTGCGCCATCCCTCC3’ was used as the Bel-2 antisense oligo.

Labeling of Bel-2 Oligos with [32P]. Bel-2 oligos (480 µg) were labeled overnight at 37°C with 0.5 mCi of [γ-32P]ATP at the 5’ end by 500 U of T4 polyonucleotide kinase obtained from Boehringer Mannheim (Indianapolis, IN), in 350 µL of T4 kinase buffer similar to that described previously (Tari and Lopez-Berestein, 1997). A final 25% of dimethyl sulfoxide (v/v) was included in the labeling reaction to ensure that P-ethoxy oligos remained soluble during the labeling reactions. After the labeling reaction was carried out, oligos were twice filtered through a Microcon-3 filter (Amicon, Beverly, MA) to separate the labeled oligos from free [γ-32P]ATP. Typically, 99% of the radiolabel was associated with the oligos described (Tari and Lopez-Berestein, 1997).

Liposome Preparation. L-Bcl-2 were prepared as described (Beham et al., 1997; Tormo et al., 1998) by mixing 1,2 dioleoyl-sn-glycero-3-phosphocholine with Bel-2 P-ethoxy antisense oligos (20:1, mol/mol) in the presence of excess t-butanol. The mixture was vortexed, frozen in an acetone/dry ice bath, and then lyophilized. The lyophilized preparation was hydrated with normal 0.9% saline. We had previously determined that the incorporation efficiency of P-ethoxy oligos in liposomes was ≥5% (Tari and Lopez-Berestein, 1997).

Extraction of 32P-Labeled Bcl-2 Oligos from Whole Blood and Tissues. Whole blood (0.1 ml) or tissue (50–100 mg) samples were digested with 0.5 to 1.0 ml of hyamine hydroxide/ethanol (1:2, v/v) at 60°C overnight followed by decolorization with 0.5 ml of 30% (w/w) H2O2. Then, 15 ml of liquid scintillation cocktail was added to the samples. The amount of 32P associated with the samples was counted by liquid scintillation.

Pharmacokinetics. Male Lewis rats weighing approximately 400 g were anesthetized with sodium thiopental (35 mg/kg of body weight). The right femoral artery and left femoral vein were surgically exposed and cannulated. Rats were injected i.v. with L-Bcl-2 at a dose of 10 mg of oligos per kg of body weight. Arterial blood samples of approximately 0.3 ml each were withdrawn at 5, 10, 20, 30, 60, 90, 120, 180, and 240 min after injection. After each withdrawal, the catheter was flushed with sodium heparin 1:1000 (v/v). An aliquot of the injected dose was maintained as a control sample. Whole blood samples were extracted and assayed for 32P radioactivity by liquid scintillation. Pharmacokinetic parameters were determined by nonlinear regression analysis (Rstrip; Micro Math, Inc., Salt Lake City, UT). The data were best fit to a two-compartment model:

\[ C_T = A e^{-\alpha t} + B e^{-\beta t} \]

where \( C_I \) equals concentration at time \( T \), and \( A \) and \( B \) are the constants for distribution and elimination, respectively, and \( T \) is time. \( T_{1/2a} \) and \( T_{1/2b} \) were calculated from ln \( 2/\alpha \) and ln \( 2/\beta \), respectively. \( C_0 \) was calculated from the above equation at time zero; therefore, \( C_0 \) equals \( A + B \). \( V_a \) was calculated as the ratio of initial dose to \( C_0 \).

Safety Studies. Groups of 10 mice each were administered, via tail vein, a single dose or 5 daily doses of L-Bcl-2 (injection volume, 0.2 ml). Blood samples were obtained 1, 2, and 6 weeks postinjection and analyzed for hematological parameters and for hepatic and renal function. Hematological parameters include complete blood counts and platelets counts. Serum glutamic-oxaloacetic transaminase (SGOT) and alkaline phosphatase assays were performed to test for hepatic functions. Creatinine and blood urea nitrogen (BUN) assays were used to test for renal functions.

Tissue Distribution. Liposomes containing 32P-labeled Bel-2 antisense were injected i.v. into 15 mice weighing an average of 30 g each. Mice were divided into three groups of five mice each, and sacrificed at 4, 24, and 48 h. A separate group of five mice was used as untreated control. Organs were dissected and 50- to 100-mg samples (lungs, heart, kidneys, liver, and spleen), 150 µL of blood, and 200 µL of bone marrow from each mouse were weighed and processed, and the radioactivity was counted in a scintillation counter. The data are expressed as mean ± S.D. micrograms of oligos per gram of tissue.

Histopathology. Six weeks after the final single- or multiple-dose injection, all the mice were sacrificed, and blood and tissue samples were collected. Heart, lungs, spleen, kidneys, and liver were fixed by immersion in neutral-buffered 10% Formalin solution. The tissues were embedded in paraffin blocks from which 2- to 4-μm sections were cut and stained with H&E.

Results

Single-DOse Toxicity Studies. Mice tolerated the highest concentration of L-Bcl-2 (40 mg/kg of body weight) that

### Table 1

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematological Parameters&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC (× 10&lt;sup&gt;3&lt;/sup&gt;/µl)</td>
<td>8.0 (1.9)</td>
<td>6.3 (1.9)</td>
<td>7.3 (2.8)</td>
<td>5.6 (1.9)</td>
<td>6.4 (2.2)</td>
</tr>
<tr>
<td>RBC (× 10&lt;sup&gt;6&lt;/sup&gt;/µl)</td>
<td>8.5 (0.5)</td>
<td>8.1 (0.5)</td>
<td>8.1 (0.4)</td>
<td>7.7 (0.5)</td>
<td>6.4 (2.2)</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>15.4 (0.8)</td>
<td>14.4 (0.9)</td>
<td>14.8 (0.6)</td>
<td>14.3 (1.1)</td>
<td>15.0 (0.8)</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>43.6 (1.6)</td>
<td>42.0 (2.2)</td>
<td>41.6 (1.4)</td>
<td>40.5 (2.0)</td>
<td>41.9 (1.8)</td>
</tr>
<tr>
<td>Lymphos (%)</td>
<td>84.7 (6.2)</td>
<td>82.1 (3.4)</td>
<td>79.8 (7.8)</td>
<td>82.9 (4.8)</td>
<td>80.4 (3.9)</td>
</tr>
<tr>
<td>Platelets (× 10&lt;sup&gt;3&lt;/sup&gt;/µl)</td>
<td>1025.0 (189)</td>
<td>1253.2 (243)</td>
<td>1382.7 (150)</td>
<td>970.0 (290)</td>
<td>1096.3 (207)</td>
</tr>
<tr>
<td>Renal and Hepatic Functions&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.7 (0.0)</td>
<td>0.6 (0.1)</td>
<td>0.5 (0.7)</td>
<td>0.6 (0.9)</td>
<td>0.5 (0.0)</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>30.0 (2.7)</td>
<td>29.9 (8.2)</td>
<td>27.0 (4.4)</td>
<td>26.0 (2.1)</td>
<td>28.1 (2.6)</td>
</tr>
<tr>
<td>SGOT (IU/L)</td>
<td>353.5 (347)</td>
<td>184.7 (105)</td>
<td>321.3 (251)</td>
<td>156.3 (53)</td>
<td>223.0 (192)</td>
</tr>
<tr>
<td>Alkaline Phosphatase (IU/L)</td>
<td>98.7 (40.7)</td>
<td>118.4 (39.9)</td>
<td>128.4 (34.6)</td>
<td>108.7 (30.7)</td>
<td>115.5 (50.9)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Five male and five female ICR mice were used.

<sup>b</sup> All parameters and functions were expressed as mean values (±S.D.).

<sup>c</sup> WBC, white blood cells; RBC, red blood cells.
could be administered in the maximal 0.2-ml volume, which represents approximately 10% of total intravascular volume in mice. No overt evidence of toxicity was noted in the hematological values nor in the biochemical results from tests of hepatic and renal function performed 1 (Table 1), 2 (Table 2), or 6 weeks (Table 3) postinjection.

**Multiple-Dose Toxicity Studies.** The five-dose administration of L-Bcl-2 at individual doses of 7.5 to 20 mg/kg of body weight did not result in significant abnormalities in hematological parameters, nor in renal and hepatic function tests performed 2 (Table 4) or 6 (Table 5) weeks postinjection.

**Pharmacokinetics.** The clearance of L-Bcl-2 from plasma was found to closely fit a two-compartment mathematical model (correlation $r^2 > 0.98$). The initial distribution phase occurred over the first 10 min after injection ($T_{1/2a} = 8.1 \pm 2.4$ min). The terminal phase half-life ($T_{1/2b}$) was 235.56 ± 128.2 min (Table 6). The immediate apparent $V_d$ was higher (79.74 ± 18.93 ml) than the total blood volume for rats of this size.

**Tissue Distribution.** L-Bcl-2 were found in various organs, such as heart, kidney, liver, lung, and spleen (Table 7). However, at 4 and 24 h, the highest tissue concentrations of L-Bcl-2 were detected in liver and spleen, but at 48 h, lung showed the highest concentration. Four hours after injection, L-Bcl-2 concentrations in liver and spleen ranged between 30 and 40 $\mu$g/g of tissue, whereas L-Bcl-2 concentrations in heart, lung, and kidneys ranged between 18 and 22 $\mu$g/g of tissue. In brain, the range was always less than 4.2 $\mu$g/g of tissue. By 48 h, L-Bcl-2 concentrations in liver and spleen had decreased to 8 to 10 $\mu$g/g of tissue, as compared with 5 to 16 $\mu$g/g of tissue in heart, lungs, and kidneys. A much lower concentration of L-Bcl-2 (0.04–0.15 $\mu$g/femur) could be detected in the bone marrow but it remained constant throughout the period range (48 h). In blood, the level was less than 7.2 $\mu$g/ml at all time points measured.

**Histopathology.** No microscopic lesion that could be attributed to the experimental L-Bcl-2 treatments was found in any animal. Incidental lesions observed in occasional mice from all groups included lymphoid hyperplasia in the spleen, hyperplasia of various lymph nodes, myeloid hyperplasia in the red pulp of the spleen, bronchoalveolar adenoma in the lung, and focal nephrosis. It has been reported that these incidental lesions are normal for this strain of mice (Altman, 1985).

**Discussion**

Our results indicate that neither significant adverse effects nor organ toxicity were associated with i.v. injections of L-Bcl-2. L-Bcl-2 could be safely administered up to 20 mg of oligos per kg of body weight daily for 5 consecutive days. This is a higher concentration than that used by Tari et al. (1998), who under the same conditions, tested a concentration of 75 mg of liposomal P-ethoxy oligos per kg of body weight. However, in the Tari et al. studies, even though they used liposomal P-ethoxy antisense oligos, their oligos were targeting to the BCR-ABL gene, which is absent in normal mice. On the contrary, in this study, we used L-Bcl-2, which targets to the Bcl-2 gene. This gene is expressed in normal mouse tissues. Therefore, the Tari et al. studies only evaluated the in vivo effects of liposomal P-ethoxy oligos whereas our studies were designed to evaluate the in vivo effects of Bcl-2 protein inhibition. We feel that the toxicity of an oligo cannot be generalized and that it is necessary to assess the potential safety profile of the inhibition of each individual gene. The potential use of L-Bcl-2 as a therapeutic agent is also greatly affected by its safety profile. These studies are of particular value because the Bcl-2 gene is well conserved among mammalian species (Tsujimoto et al., 1985). We therefore decided to study the safety profile of L-Bcl-2 in normal mice.

The effectiveness of antisense oligos as therapeutic agents is also dependent on their pharmacokinetics, tissue disposition, stability, and elimination characteristics (Agrawal et al., 1995, 1997). We found that the pharmacokinetics and tissue distribution of L-Bcl-2 are very similar to those of other liposomal compounds (Zou et al., 1993; Lopez-Berestein et al., 1994; Leonetti et al., 1996). The plasma clearance rate of L-Bcl-2 is biphasic; the $T_{1/2a}$ is within 8 min whereas the $T_{1/2b}$ is within 4.2 h. These pharmacokinetic results differ from those reported by Raynaud et al. (1997) when they i.v. administered G3139, a phosphorothioate Bcl-2 antisense oligo, into mice. G3139 was observed to display triexponential kinetics, with $\alpha$, $\beta$, and $\gamma$ half-lives of 5 min, 37 min, and 11 h, respectively. High concentrations of L-Bcl-2 were found in the organs studied. Furthermore, L-Bcl-2 could remain in spleen, liver, and lungs for at least 48 h. This L-Bcl-2 accumulation pattern differs from those reported for free Bcl-2 antisense oligos in which the organs that had the high-

### TABLE 2

Two weeks postinjection effects of a single dose of L-Bcl-2 in ICR mice

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Hematological Parameters&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Renal and Hepatic Functions&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WBC ($\times 10^3/\mu l$)</td>
<td>RBC ($\times 10^3/\mu l$)</td>
</tr>
<tr>
<td>0</td>
<td>9.5 (3.2)</td>
<td>8.0 (0.2)</td>
</tr>
<tr>
<td>10</td>
<td>6.4 (4.1)</td>
<td>7.9 (0.3)</td>
</tr>
<tr>
<td>20</td>
<td>7.9 (1.5)</td>
<td>8.0 (0.2)</td>
</tr>
<tr>
<td>30</td>
<td>5.9 (1.4)</td>
<td>8.0 (0.4)</td>
</tr>
<tr>
<td>40</td>
<td>7.0 (2.8)</td>
<td>8.2 (0.4)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Five male and five female ICR mice were used.

<sup>b</sup> All parameters and functions were expressed as mean values (± S.D.).
est Bcl-2 antisense accumulation were kidneys and liver (DeLong et al., 1997). This difference in L-Bcl-2 biodistribution could be attributed to the use of lipid-incorporated rather than free oligos. For example, when lipid carriers (Litzinger et al., 1996) or lipoproteins (de Smidt et al., 1991) were used as delivery vehicles for antisense oligos, oligos were mainly found to accumulate in liver and spleen, rather than in kidney, which is the main organ where free oligos accumulate. Backbone modification can also contribute to a change in the tissue distribution of oligos. Leonetti et al. (1996) found the greatest concentration of liposomes in liver rather than spleen when they used lipidosome-incorporated

TABLE 3

Six weeks postinjection effects of a single dose of L-Bcl-2 in ICR mice

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (×10⁹/µl)</td>
<td>5.5 (1.6)</td>
<td>5.3 (1.7)</td>
<td>6.2 (2.6)</td>
<td>5.3 (2.7)</td>
<td>5.5 (1.1)</td>
</tr>
<tr>
<td>RBC (×10⁹/µl)</td>
<td>8.6 (0.5)</td>
<td>8.4 (0.1)</td>
<td>7.8 (1.8)</td>
<td>7.3 (2.7)</td>
<td>8.8 (0.4)</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>14.7 (0.8)</td>
<td>14.5 (0.4)</td>
<td>13.7 (3.3)</td>
<td>12.7 (4.6)</td>
<td>15.3 (0.9)</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>42.2 (1.9)</td>
<td>41.9 (0.9)</td>
<td>38.7 (8.9)</td>
<td>36.5 (13.5)</td>
<td>43.5 (2.5)</td>
</tr>
<tr>
<td>Lymphos (%)</td>
<td>82.8 (7.1)</td>
<td>82.2 (7.3)</td>
<td>87.4 (6.9)</td>
<td>83.3 (29.5)</td>
<td>79.7 (7.4)</td>
</tr>
<tr>
<td>Platelets (×10⁹/µl)</td>
<td>1124.5 (277)</td>
<td>1238.1 (209)</td>
<td>1061.0 (319)</td>
<td>1133.2 (410)</td>
<td>1126.7 (287)</td>
</tr>
</tbody>
</table>

Renal and Hepatic Functions

| | Creatinine (mg/dL) | 0.7 (0.0) | 0.6 (0.0) | 0.6 (0.0) | 0.6 (0.0) |
| | BUN (mg/dL) | 25.3 (5.5) | 30.5 (3.0) | 32.1 (4.2) | 28.0 (3.9) |
| | SGOT (IU/L) | 335.5 (190) | 145.8 (28) | 407.0 (268) | 208.0 (85) |
| | Alkaline Phosphatase (IU/L) | 63.3 (21.7) | 84.6 (10.6) | 62.3 (26.3) | 60.0 (16.9) |

a Five male and five female ICR mice were used.

b All parameters and functions were expressed as mean values (± S.D.).

TABLE 4

Two weeks postinjection effects of multiple doses of L-Bcl-2 ICR mice

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>0</th>
<th>7.5</th>
<th>15</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (×10⁹/µl)</td>
<td>7.5 (2.0)</td>
<td>6.4 (1.9)</td>
<td>6.1 (2.0)</td>
<td>7.2 (2.0)</td>
</tr>
<tr>
<td>RBC (×10⁹/µl)</td>
<td>8.4 (0.4)</td>
<td>8.2 (0.4)</td>
<td>8.9 (0.8)</td>
<td>5.7 (0.5)</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>14.6 (0.6)</td>
<td>14.1 (0.4)</td>
<td>14.7 (0.6)</td>
<td>14.4 (0.5)</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>41.7 (1.6)</td>
<td>41.6 (3.2)</td>
<td>43.8 (3.1)</td>
<td>42.7 (2.4)</td>
</tr>
<tr>
<td>Lymphos (%)</td>
<td>81.3 (3.7)</td>
<td>74.9 (8.5)</td>
<td>71.3 (7.3)</td>
<td>78.8 (8.1)</td>
</tr>
<tr>
<td>Platelets (×10⁹/µl)</td>
<td>1030.8 (286)</td>
<td>1044.7 (212)</td>
<td>1061.2 (296)</td>
<td>1020.3 (183)</td>
</tr>
</tbody>
</table>

Renal and Hepatic Functions

| | Creatinine (mg/dL) | 0.7 (0.0) | 0.7 (0.1) | 0.6 (0.0) | 0.6 (0.0) |
| | BUN (mg/dL) | 27.0 (4.4) | 28.4 (6.0) | 28.4 (5.6) | 25.2 (3.7) |
| | SGOT (IU/L) | 134.3 (64) | 136.8 (68) | 193.1 (96) | 131.8 (49) |
| | Alkaline Phosphatase (IU/L) | 69.9 (33.7) | 67.1 (21.9) | 68.4 (32.0) | 48.4 (41.3) |

a Five male and five female ICR mice were used.

b All parameters and functions were expressed as mean values (± S.D.).

TABLE 5

Six weeks postinjection effects of multiple doses of L-Bcl-2 in ICR mice

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>0</th>
<th>7.5</th>
<th>15</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (×10⁹/µl)</td>
<td>6.5 (2.5)</td>
<td>6.3 (2.5)</td>
<td>7.3 (2.9)</td>
<td>6.8 (2.5)</td>
</tr>
<tr>
<td>RBC (×10⁹/µl)</td>
<td>8.5 (0.3)</td>
<td>8.5 (0.5)</td>
<td>8.6 (0.5)</td>
<td>8.4 (0.7)</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>15.1 (0.6)</td>
<td>15.1 (0.3)</td>
<td>15.5 (0.8)</td>
<td>14.7 (1.2)</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>43.7 (2.2)</td>
<td>42.4 (1.2)</td>
<td>43.3 (2.0)</td>
<td>42.0 (2.8)</td>
</tr>
<tr>
<td>Lymphos (%)</td>
<td>81.2 (4.3)</td>
<td>73.2 (6.0)</td>
<td>80.6 (4.2)</td>
<td>82.2 (7.6)</td>
</tr>
<tr>
<td>Platelets (×10⁹/µl)</td>
<td>1154.5 (231)</td>
<td>1153.3 (302)</td>
<td>1165.3 (240)</td>
<td>1201.5 (239)</td>
</tr>
</tbody>
</table>

Renal and Hepatic Functions

| | Creatinine (mg/dL) | 0.7 (0.0) | 0.6 (0.1) | 0.6 (0.1) | 0.5 (0.0) |
| | BUN (mg/dL) | 28.2 (2.8) | 25.6 (5.5) | 28.2 (4.2) | 28.3 (5.6) |
| | SGOT (IU/L) | 252.9 (180) | 188.3 (89) | 256.4 (161) | 266.4 (176) |
| | Alkaline Phosphatase (IU/L) | 37.2 (22.1) | 20.8 (11.8) | 37.5 (11.5) | 47.1 (16.5) |

a Five male and five female ICR mice were used.

b All parameters and functions were expressed as mean values (± S.D.).
phosphorothioate oligos containing a modified internucleo-
side phosphate backbone.

We had reported previously that L-Bcl-2 leads to Bcl-2 pro-
tein inhibition and induces growth suppression in FL cell lines that
have the t(14;18) translocation. High concentrations of
L-Bcl-2 were found in spleen, liver, and, to a lower extent, bone
marrow, which are the organs where the tumor mass of FL is
mainly found. Because L-Bcl-2 does not induce any adverse
effects or organ toxicities in vivo in over 6 weeks on the sched-
ules studied, L-Bcl-2 may be safely used to inhibit the growth of
FL. These pharmacokinetic and tissue distribution studies have
allowed us to estimate the frequency and dose administration of
L-Bcl-2 in vivo. We are currently investigating the efficacy of
L-Bcl-2 against experimental FL models.

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