A Comparison of the Cardiopulmonary Effects of Continuous versus Bolus Infusion of Recombinant Interleukin-2 in Sheep

Frederick L. Clauser, Georgean G. DeBlois, Daniel E. Bechard, Randy E. Merchant, Angus J. Grant, Alpha A. Fowler, and R. Paul Fairman


ABSTRACT

The systemic administration of recombinant interleukin-2 (rIL-2) is used for the treatment of patients with far advanced cancer. However, treatment may be limited by a so-called "third space" syndrome. Whether these side effects are due to the total dose used or the method of administration is unclear. To define whether the continuous (Group 2) or bolus (Group 3) i.v. infusion of 9 × 10⁶ units/kg rIL-2 over 72 h is associated with similar toxicities, we established a chronic sheep model and monitored changes in systemic and pulmonary vascular pressures, cardiac function, and gas exchange. At 72 h lung lymph flow, lymph/plasma protein ratios, lung histology, and extravascular lung water were obtained. In both groups the infusion of rIL-2 resulted in an increase in high protein lung lymph flow, an increase in cardiac output, and a decrease in systemic vascular resistance. Large lymphoid cells were found by histology to be infiltrating the lung interstitium. In Group 2, in addition, there were mild pulmonary hypertension (pulmonary artery pressures increased from 14 ± 5 to 22 ± 6 mmHg (P < 0.05)), systemic hypotension [81 ± 7 compared to a baseline of 95 ± 6 mmHg (P < 0.01)], and worsening gas exchange. We conclude that a 72-h continuous or bolus infusion of equivalent doses of rIL-2 are associated with cardiopulmonary toxicity; however, pulmonary hypertension, systemic hypotension, and gas exchange are worse in animals receiving the continuous infusion.

INTRODUCTION

The systemic administration of rIL-2 alone, or in combination with autologous LAK cells, is a new experimental treatment for patients with far advanced cancer who have failed standard cancer therapy. Fifteen to 37.5% of these patients demonstrate objective primary and metastatic tumor regression (complete, partial, or minor) (1, 2). Unfortunately, systemic toxicity often develops with this therapy resulting in cessation of treatment. Toxicity includes an ill-defined "capillary leak" or "third space" syndrome manifested by dyspnea, interstitial pulmonary edema, systemic arterial hypotension, oliguria, and anasarca. Whether these side effects are dose related or dependent on the route and/or method of administration is unclear. rIL-2 has been administered by the i.v., s.c., i.p., and intrapleural routes. However, continuous or bolus i.v. infusions represent the most commonly used routes of administration (2, 3, 4–12).

To determine whether a difference in toxicity between bolus and continuous i.v. infusion existed, we established a chronic sheep model and monitored changes in systemic and pulmonary vascular pressures, cardiac function, gas exchange, and core

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1 The abbreviations used are: rIL-2, recombinant interleukin-2; LAK, lymphokine-activated killer; SBP, systemic blood pressure; RAP, right atrial pressure; PAP, pulmonary artery pressure; PWP, pulmonary wedge pressure; SVR, systemic vascular resistance; FiO₂, fraction of inspired oxygen; CO, cardiac output; QL, lymph flow; L/P, lymph/plasma; EVLW, extravascular lung water; DLW, dry lung weight; A-DO₂, alveolar-arterial oxygen difference.

SVR = SBP - RAP

CO

Thermodilution CO was measured in triplicate using an Edwards cardiac output computer (Edwards Co., Tustin, CA) and the mean value reported. Core temperature was obtained by this device. Arterial blood gas tension and pH were measured on an Instrumentation Laboratory 513 blood gas analyzer (Instrument Labs, Lexington, MA) calibrated daily.

To be included in the study, animals had to meet the following hemodynamic and gas exchange criteria: (a) SBP > 80 mm Hg; (b) PAP, < 20 mm Hg; and (c) PaO₂ > 60 torr on FiO₂, 0.21. Measurements were obtained at 0 (baseline), 24, 48, and 72 h. Infusion of the experi.
mental agents were begun immediately after obtaining base-line measurements.

Lymph Collection. After 72 h of treatment, animals were reanesthetized and ventilated with an FIO2 and positive end expiratory pressure of 0.6 and 8-10 cm H2O, respectively. Through a right-sided thoracotomy, a silastic catheter was inserted into the main efferent duct of the caudal mediastinal lymph node (13), secured with suture, and externalized through the sixth or seventh intercostal space. The node was ligated caudal to the pulmonary ligament and any visible lymph vessels were ligated and resected. The node was then immersed in the same fixative. The tissue was infiltrated with glycol methacrylate and cut on a microtome. The 2-µm sections from each Group 2 and 3 animals were frozen, cut at 8-µm in a cryostat, and stained with α-naphthyl acetate. Electron microscopy was performed using a Phillips electron microscope.

EVLW/Blood free/DLW. The left lung was removed from the chest, weighed, and homogenized in a blender. Aliquots were centrifuged at 800 × g for 45 min and 150 µl of the supernatant analyzed for hemoglobin concentration. Hemoglobin concentration was also determined on simultaneously collected pulmonary artery blood. Aliquots of lung tissue and supernatant were dried to a stable weight in a microwave oven. The formula of Pearce et al. (15) was used to determine EVLW/DLW.

Recombinant Interleukin-2. rIL-2 packaged in premixed sterile vials was kindly supplied by the Cetus Corporation, Emeryville, CA. The rIL-2 was reconstituted using 0.9% NaCl. Lots of rIL-2 were tested by limulus amoeboocyte lysate assay to detect endotoxin and found to contain less than 0.45 EU/ml. The formula of Pearce et al. (15) was used to determine EVLW/DLW.

Results

Systemic Vascular Changes (Table 1). SVR fell and CO increased significantly without a significant change in SBP at 24, 48, and 72 h in Group 1 (excipient) and 3 (bolus rIL-2) animals. In Group 2 (continuous rIL-2), SVR fell to the same degree but CO increased to a lesser (but not statistically significant) degree compared to Groups 1 and 3. As a consequence, SBP fell significantly by 48 h in Group 2.

Pulmonary Vascular, Gas Exchange, and Core Temperature Changes (Table 1). RAP, PAP, PWP, PaO2, A-aO2 gradient, and core temperature did not change significantly at any time in Group 1 animals. There were no significant changes in RAP (data not shown) or PWP in Group 2. There was a significant increase in PAP at 72 h in Group 2 compared to baseline and Group 1 and 3 animals (P < 0.05). PaO2 fell and A-aO2 gradient widened significantly at 48 and 72 h in Group 2 and at 72 h in Group 3 animals. In addition, PaO2 and A-aO2 in Group 2 animals were significantly different from Group 3 animals at 48 and 72 h (P < 0.05). Core temperature was significantly elevated compared to base-line values in Groups 2 and 3 at 72 h (P < 0.05).

Lymph Flow, Lymph/Plasma Protein Ratios, and EVLW/DLW (Table 2). QL was 1.6 ± 0.4 ml/15 min and L/P protein ratio was 0.69 ± 0.13 in Group 1 animals. QL was significantly increased in Groups 2 and 3 (5.6 ± 3.2 and 6.7 ± 5.1 ml/15 min, respectively) compared to Group 1. QL was not significantly different between Groups 2 and 3. There was a trend for the L/P protein ratios in Groups 2 and 3 to be higher than Group 1 values but this never reached the level of statistical significance. EVLW/DLW ratios in Group 2 and 3 animals were significantly higher than in Group 1 (P < 0.01). EVLW/DLW ratios were similar in Groups 2 and 3.

Lung Histopathological Changes. Sheep receiving the continuous and bolus infusions of rIL-2 had comparable pulmonary pathology. Both groups demonstrated a significant mononuclear cell interstitial infiltrate (Fig. 1, B and C). The majority of the interstitial cells failed to demonstrate any esterase activity when stained with α-naphthyl acetate and C). The majority of the interstitial cells failed to demonstrate any esterase activity when stained with α-naphthyl acetate.

Table 1 Systemic vascular, pulmonary vascular, gas exchange, and core temperature changes in Groups 1–3

<table>
<thead>
<tr>
<th>Group</th>
<th>H</th>
<th>SBP (mmHg)</th>
<th>CO (liters/min)</th>
<th>SVR (µm)</th>
<th>PAP (mmHg)</th>
<th>PWP (mmHg)</th>
<th>PaO2 (torr)</th>
<th>A-aO2 (torr)</th>
<th>Temperature (°C)</th>
</tr>
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<tr>
<td>1</td>
<td>0</td>
<td>90 ± 5</td>
<td>3.5 ± 0.6</td>
<td>25.7 ± 2</td>
<td>13 ± 5</td>
<td>5 ± 2</td>
<td>100 ± 7</td>
<td>17 ± 5</td>
<td>40.2 ± 0.3</td>
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<tr>
<td>2</td>
<td>24</td>
<td>88 ± 7</td>
<td>5.1 ± 1 4</td>
<td>17.3 ± 1.8</td>
<td>11 ± 3</td>
<td>5 ± 3</td>
<td>97 ± 6</td>
<td>20 ± 9</td>
<td>40.5 ± 0.3</td>
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<td>48</td>
<td>91 ± 8</td>
<td>5.3 ± 1.6</td>
<td>17.3 ± 2.2</td>
<td>15 ± 7</td>
<td>6 ± 4</td>
<td>98 ± 9</td>
<td>19 ± 7</td>
<td>40.2 ± 0.4</td>
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<tr>
<td>72</td>
<td>87 ± 10</td>
<td>5.7 ± 0.8</td>
<td>15.3 ± 2.2</td>
<td>12 ± 6</td>
<td>4 ± 2</td>
<td>101 ± 7</td>
<td>16 ± 5</td>
<td>40.3 ± 0.3</td>
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</tr>
<tr>
<td>2</td>
<td>0</td>
<td>95 ± 9</td>
<td>3.3 ± 0.5</td>
<td>28.5 ± 3</td>
<td>14 ± 5</td>
<td>6 ± 2</td>
<td>100 ± 5</td>
<td>15 ± 6</td>
<td>39.8 ± 0.5</td>
</tr>
<tr>
<td>24</td>
<td>90 ± 10</td>
<td>4.5 ± 0.6</td>
<td>20 ± 1.1</td>
<td>12 ± 6</td>
<td>6 ± 3</td>
<td>96 ± 7</td>
<td>19 ± 3</td>
<td>39.6 ± 0.4</td>
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<td>84 ± 6</td>
<td>4.9 ± 0.8</td>
<td>17.1 ± 1.5</td>
<td>16 ± 3</td>
<td>5 ± 2</td>
<td>84 ± 5</td>
<td>31 ± 4</td>
<td>40.2 ± 0.6</td>
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<td>72</td>
<td>81 ± 7</td>
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<td>16.9 ± 1.6</td>
<td>22 ± 6</td>
<td>5 ± 1</td>
<td>75 ± 6</td>
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<td>3</td>
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<td>3.2 ± 0.6</td>
<td>30 ± 3</td>
<td>12 ± 4</td>
<td>5 ± 2</td>
<td>105 ± 6</td>
<td>10 ± 4</td>
<td>39.6 ± 0.5</td>
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<tr>
<td>24</td>
<td>92 ± 9</td>
<td>4.8 ± 1.6</td>
<td>19.2 ± 1.4</td>
<td>14 ± 6</td>
<td>5 ± 2</td>
<td>102 ± 10</td>
<td>13 ± 6</td>
<td>39.8 ± 0.6</td>
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<tr>
<td>48</td>
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<td>18.5 ± 1.5</td>
<td>14 ± 8</td>
<td>4 ± 1</td>
<td>99 ± 8</td>
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<td>16.3 ± 2.2</td>
<td>15 ± 6</td>
<td>5 ± 2</td>
<td>93 ± 7</td>
<td>22 ± 6</td>
<td>40.8 ± 0.4</td>
<td></td>
</tr>
</tbody>
</table>

* Group 1, excipient; 2, rIL-2 at 9 × 105 units/kg bolus infusion; 3, rIL-2 at 9 × 105 units/kg bolus infusion.

‡ P < 0.01 compared to Group 1.

### Table 2 QL, L/P Protein, and EVLW/DLW of Groups 1–3

<table>
<thead>
<tr>
<th>Group</th>
<th>QL (ml/15 min)</th>
<th>L/P protein</th>
<th>EVLW/DLW</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.6 ± 0.4</td>
<td>0.69 ± 0.13</td>
<td>3.7 ± 0.1</td>
</tr>
<tr>
<td>2</td>
<td>5.6 ± 3.2</td>
<td>0.74 ± 0.16</td>
<td>4.2 ± 0.3</td>
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<tr>
<td>3</td>
<td>6.7 ± 5.1</td>
<td>0.75 ± 0.09</td>
<td>4.15 ± 0.3</td>
</tr>
</tbody>
</table>

* Group 1, excipient; 2, rIL-2 at 9 × 105 units/kg continuous infusion; 3, rIL-2 at 9 × 105 units/kg bolus infusion.

‡ P < 0.05 compared to Group 1.

§ P < 0.01 compared to Group 1.
INTERLEUKIN-2 IN SHEEP

Fig. 1. Medium-power view contrasting the pulmonary histology of sheep receiving excipient (A) to the sheep infused with the continuous (B) and bolus (C) doses of rIL-2. Note the lymphangitic distribution of the infiltrate in B and C. ILS, interlobular septum. Hematoxylin and eosin, x 200.

Fig. 2. The interstitial cells from rIL-2-treated sheep demonstrate a high nuclear:cytoplasmic ratio and prominent nucleoli. These cells are morphologically typical of activated lymphoid cells. Hematoxylin and eosin, x 850.

examination (Fig. 3) revealed large lymphoid cells within the alveolar capillaries and clustered within the alveolar interstitium. In contrast to the sheep treated with pharmacological doses of rIL-2, the lungs from the animals which received excipient revealed normal histology (Fig. 1A). In these animals the pulmonary interstitium was devoid of any cellular infiltrate and the alveolar walls appeared thin and delicate.

DISCUSSION

Continuous or bolus infusion of equivalent doses of rIL-2 over 72 h in sheep leads to increases in high protein lung lymph flow consistent with an increase in pulmonary microvascular permeability, pulmonary edema as reflected by an elevated EVLW/DLW ratio, increased cardiac output, and decreased systemic vascular resistance. Substantial alterations in lung histology occurred during both the continuous and bolus infusion of rIL-2. Histopathologically, neither qualitative nor quantitative differences could be discerned between the two groups.

In vivo, pharmacological doses of rIL-2 provoke a marked mononuclear interstitial infiltrate which has both the morphological and enzymatic features typical of large lymphoid cells (Figs. 1–3). These cells could represent LAK cells although we have no functional proof of this.

There is very little difference in the cardiopulmonary toxicity of continuous versus bolus infusion of equivalent doses of rIL-2 other than the worsening oxygenation, systemic hypotension, and mild pulmonary hypertension with the former method of administration. These differences may be mediated by the release of vaso- and bronchoactive substances such as prostaglandins, thromboxanes, lipooxygenases, platelet activating factor, serotonin, and histamine in response to rIL-2 (16). The gas exchange abnormality is consistent with ventilation-perfusion mismatching and is not due to the amount of pulmonary edema present since lung water was similar in both groups. Others have shown, in different models (17, 18), that there is a poor correlation between the degree of hypoxemia and the amount of pulmonary edema present.

Our findings are compatible with those of Rosenstein et al. (19), Townley et al. (20), and Matory et al. (21). The latter investigators found, in mice, that the bolus injection of $10^6$ units/kg rIL-2 3 times a wk for 2 wk was not associated with hepatic or renal abnormalities. In contrast, a continuous i.v. infusion of equivalent doses of rIL-2 (approximately 18,000 units/kg/h) for 1 wk resulted in significant hepatopathology. In addition, histological analysis revealed lymphocytic infiltration of the liver, spleen, and lungs. They concluded that bolus rIL-2 infusion was the preferred method of administration to minimize side effects. Similarly, Lotze et al. (22) reported in human cancer patients that the total limiting dose (due to toxicity) was approximately $5 \times 10^6$ units/kg rIL-2 when administered by continuous infusion compared to $70 \times 10^6$ units/kg when given by bolus infusion.

West et al. (2) found that the continuous infusion of rIL-2 in patients with advanced cancer was associated with less toxicity (and equivalent therapeutic efficacy) than had been reported for patients receiving bolus infusions (1, 22). They concluded that continuous in comparison to bolus rIL-2 infusion was associ-
Fig. 3. Low-power electron micrograph showing a lymphoid cell within an alveolar capillary (right) and clustered within the alveolar interstitium (left). AS, alveolar space; AC, alveolar capillary. Uranyl acetate and lead citrate, × 7000.

ated with fewer side effects. In contrast to the present study and that of Matory et al. (21), West et al. (2) used much lower doses of rIL-2. The highest dose of rIL-2 administered to their patients was equivalent to 10⁵ units/kg/day which is approximately 3-fold lower than the doses used in the present study. These differences in experimental protocols may explain the discrepancies between the various studies.

That there are differences in toxicity between continuous and bolus infusion of rIL-2 is not surprising since there are pharmacological, distributional, and biological differences between these administrative modes. The serum half-life of bolus rIL-2 is very short, 6.9 min in humans and 1.5 min in mice (22, 23). With bolus infusion, rIL-2 blood levels are very high (3000 units/ml). Blood rIL-2 levels fall rapidly (within 30 min) to undetectable levels. In contrast, continuous infusion is associated with constant low rIL-2 blood levels. The clearance of bolus infusion of rIL-2 is consistent with a two-compartment model comprised of the plasma and extravascular space. Although both modes of infusion cause a marked fall in peripheral lymphocytes, the continuous infusion of rIL-2 eventually leads to a 2- to 16-fold increase of these cells in the peripheral blood. In addition, only the continuous infusion of rIL-2 leads to human IL-2 receptor-positive mononuclear cells appearing in the peripheral blood (22).

In contrast to findings in the isolated perfused rat lung (24), recent studies have shown that rIL-2 itself exhibits no direct toxicity against cultured vascular endothelium (25). The vascular leak syndrome appears to be celluloarly mediated, either by LAK cells or their release of esterases and performins, or through activation of host effector cells by rIL-2.

Finally, we chose to study rIL-2 infusion in sheep for the following reasons. (a) This is an accepted and sensitive model for differentiating various forms of increased permeability pulmonary edema from cardiogenic pulmonary edema (17, 26–28). (b) Others have reported results with short-term rIL-2 infusion in this animal model (29). (c) The cardiopulmonary responses in sheep are similar to those in humans after various insults (17, 26–28); and (d) we have extensive experience with this model.

From our study we conclude that continuous and bolus infusions of equivalent doses of rIL-2 are associated with cardiopulmonary toxicity. However, continuous infusion results in greater amounts of dysfunction as evidenced by the presence of mild pulmonary hypertension, moderate systemic hypotension, and worse gas exchange.

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REFERENCES


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