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Reconstitution of Recombinant Interleukin-2 (rIL-2): a Comparative Study of Various rIL-2 Muteins

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In a previous clinical study using a continuous infusion schedule of recombinant interleukin-2 (rIL-2) we noted a nearly complete loss of activity of EuroCetus rIL-2 when dissolved in 10 ml saline and infused at a very low rate through a plastic infusion device. In the present study, we demonstrated that the loss resulted from a concentration-dependent precipitation of rIL-2 in saline and adherence of the protein to the tubing material. These phenomena were not noted for four other rIL-2 muteins tested [Glaxo, Hoffmann-LaRoche, Amgen (2 muteins)]. EuroCetus rIL-2 was found to be completely soluble in water and 5% glucose.
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INTRODUCTION

THE BIOLOGICAL effects of recombinant interleukin-2 (rIL-2) depend on a variety of factors such as the dosage, schedule and route of administration [1]. Recently, it was demonstrated that the mode of administration of EuroCetus rIL-2 may dramatically influence its bioavailability [2–5]. Emphasis has been put on the addition of albumin to the solution to prevent adherence of rIL-2 to the tubing material [2, 4, 5]. We previously reported a nearly complete loss of bioactivity of EuroCetus rIL-2 when dissolved in a small volume of saline and infused at a low rate [3]. In this present study, we examined the cause and the extent of the loss of bioactivity of various rIL-2 muteins dissolved in 10 ml of saline and pumped slowly (0.5 ml/h) through a long infusion device and studied the effect of the addition of albumin. In addition, the solubility of EuroCetus rIL-2 in saline, glucose and water was tested.

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MATERIALS AND METHODS

Interleukin-2

The rIL-2 muteins provided as lyophilised powder were reconstituted in 1.2 ml sterile water (EuroCetus) or 0.9% saline (Hoffmann–LaRoche, Glaxo) according to the manufacturer's instructions. Both Amgen rIL-2 muteins were provided dissolved in 5% glucose. Before pumping, the muteins were diluted to the required rIL-2 concentration in 10 ml of the solvent to be tested.

Infusion device

The central venous access (Vascuport®) consisting of a titanium portal with a silicone membrane and a polyurethane

catheter of 20 cm was connected to the plastic syringe containing the rIL-2 solution by a 200–300 cm long polyethylene catheter. The syringe was fitted in a portable pump (Perfusor M®). The infusion rate was 0.5 ml/h. The internal volume of the entire infusion device was approximately 2.5 ml, and the duration of contact between the pumped fluid and the tubing material was 5 h.

Determination of IL-2

The bioactivity of IL-2 was determined by a standard bioassay [6] and the protein concentration was measured using the Pierce BCA Protein Assay Reagent®, which is a modification of the method originally described by Lowry [7, 8].

RESULTS

EuroCetus rIL-2 in saline

The results of the pumping experiments are listed in Table 1.

Running EuroCetus rIL-2 in saline through the infusion device for 24 h resulted in a nearly complete loss of bioactivity and protein content at wide concentration ranges. After the addition of 2% albumin there was still a 75% loss of bioactivity, indicating that adherence to the material of the infusion device may be only partially responsible for the loss. Thus we examined whether rIL-2 was retained in the residual volume of the syringe. At the highest concentrations readily resuspendable, aggregates were noted in the fluid remaining in the syringe. The rIL-2 concentration and bioactivity of this residual fluid were at least three times higher than that of the original solution, while at a concentration of 600 000 U/ml the bioactivity was only 25%. These data indicate that rIL-2 in saline may adhere to the plastic syringe at low concentrations, while at high concentrations EuroCetus rIL-2 precipitates.

The solubility of EuroCetus rIL-2 in saline was quantified at concentrations ranging from 600 000 to 1 800 000 U/ml. After 3–4 h a clearly visible and easily resuspendable precipitate was noted at each concentration. As indicated in Table 2, the protein concentration of the supernatant collected after centrifugation at 2000 rpm for 20 min was dramatically reduced when compared with the resuspended samples.

Table 2. The protein concentration (μg/ml) of EuroCetus rIL-2 dissolved in 1–1.5 ml saline in firmly resuspended samples and in supernatants of samples after centrifugation at 2000 rpm for 20 min*

Concentration (U/ml)	Calculated protein concentration (μg/ml)	Measured protein concentration Resuspended total	Supernatant
600 000	33.3	35	9
1 200 000	66.6	74	13
2 400 000	133.3	157	28

*The results are mean values of one test using two different EuroCetus rIL-2 batches.

EuroCetus rIL-2 in water and glucose (5%)

As indicated in Table 1, pumping of EuroCetus rIL-2 dissolved in water resulted in loss of bioactivity only at low rIL-2 concentrations. This loss could be prevented by the addition of 2% albumin.

Even at the highest concentration no precipitate was observed when EuroCetus rIL-2 was dissolved in water or glucose.

Other rIL-2 muteins

After reconstitution each rIL-2 mutein was dissolved in 10 ml saline at a concentration of 33.3 μg/ml and pumped through the

Table 1. The effect of pumping EuroCetus rIL-2 through the infusion device

Solvent concentration U/ml	μg/ml	Running for 24 h Effluent from tubing device	Residuum in syringe	Passing quickly Effluent from tubing device
NaCl				
180 000	10	1%	—	100%
600 000	33.3	< 1%	< 25%	—
1 080 000	60	< 1%	—	—
1 800 000	100	< 1% (0 μg)	300% (350 μg)	—
3 000 000	166	< 10%	—	100%
4 320 000	240	< 1% (0 μg)	300% (700 μg)	—
Water				
1 200 000	66.6	25–50%	—	—
3 000 000	166	100%	—	—
4 320 000	240	100% (225 μg)	100% (270 μg)	—
NaCl + 2% albumin				
4 320 000		25%	—	—
Water + 2% albumin				
30 000		100%	—	—
108 000		100%	—	—
300 000		100%	—	—
Glucose				
4 320 000		100%	100%	—

EuroCetus rIL-2 was either pumped for 24 h or passed quickly (< 1 min) through the infusion device. The results of the bioactivity are expressed as percentage of the bioactivity of the samples taken before pumping. —, not determined.

infusion device in 24 h. In contrast to the observed loss of bioactivity of EuroCetus rIL-2, no loss of bioactivity was noted for Hoffmann–LaRoche, Glaxo and two Amgen rIL-2 muteins.

DISCUSSION

We found that the dramatic loss of bioavailability of EuroCetus rIL-2, when dissolved in 10 ml saline and infused slowly through a plastic infusion device, resulted from a concentration-dependent precipitation of rIL-2 in saline and adherence of the protein to the tubing material. Accordingly, in the package insert (June 1989) EuroCetus states that the product has to be dissolved in 5% dextrose with or without 2% albumin.

Other rIL-2 muteins tested [Hoffmann-LaRoche, Glaxo, Amgen (two different muteins)] could easily be dissolved in normal saline at a concentration of 33.3 μg/ml, and had no evident tendency to adhere to the tubing material. The various rIL-2 muteins differ only a little in amino acid sequence. In EuroCetus rIL-2 and in one of the Amgen rIL-2 muteins, cys^{125} has been replaced by serine and alanine, respectively. EuroCetus rIL-2 has the alanine at position one deleted, while Hoffmann–LaRoche, Glaxo and both Amgen rIL-2 muteins have an additional methionine at the N-terminal end. These changes may dramatically influence the biophysical characteristics. Phase separation studies demonstrated that EuroCetus rIL-2 is strongly hydrophobic and that the ala^{125} Amgen mutein is mildly hydrophobic, while muteins with an additional methionine but without replacement at cys^{125} are as hydrophilic as the non-glycosylated natural IL-2 [9].

In our experiments no difference in behaviour was observed between Amgen (ala^{125}) and the rIL-2 muteins without cys^{125} replacement. The aberrant behaviour of EuroCetus rIL-2 may, therefore, not only be determined by the cys^{125}→ser^{125} mutation but also by the alteration at the N-terminal end of the molecule.

The rIL-2 muteins with replaced cys^{125} have a strong tendency to form aggregates likely to adhere to solid material [9–11]. Our data indicate that the addition of albumin prevents adherence to the tubing material of EuroCetus rIL-2 dissolved in water. Other groups have also demonstrated the importance of adding albumin to EuroCetus rIL-2 dissolved in 5% glucose [2, 4, 5].

Based on this study, we strongly advocate testing the influence of the mode of administration on the bioavailability of biological agents before giving them to patients.

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Expression of the *mdr*1 Gene in Bone and Soft Tissue Sarcomas of Adult Patients

Ulrike Stein, Volker Wunderlich, Wolfgang Haensch and Peter Schmidt-Peter

The expression of the *mdr*1 gene was evaluated at the RNA level by northern and slot blot analysis, and at the protein level by immunohistochemistry, in a total of 29 bone and 32 soft tissue sarcomas. All patients, mainly adults, had not received previous chemotherapy. Of the tumours investigated, 69% were *mdr*1-positive. An intermediate *mdr*1 expression was observed most frequently, with the exception of osteosarcomas (high) and malignant fibrous histiocytomas (low). Detection of P-glycoprotein in selected tumours revealed consistent results. However, no conclusion can be drawn as yet regarding correlation of *mdr*1 expression and drug resistance in patients.

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INTRODUCTION

BONE AND soft tissue tumours constitute a major histogenetic class of neoplasms with rather high malignant potential. By using multi-agent, now well established chemotherapy, the past decade has seen major changes in treatment strategies for most of these tumours [1, 2].

Multidrug resistance (MDR), either intrinsic or acquired, often limits successful chemotherapy. It is mediated in humans by the *mdr*1 gene product P-glycoprotein [3]. Increased expression of the *mdr*1 gene is frequently observed in a variety of human tumours [4]. A few reports [5–7] cover *mdr*1 expression in bone and soft tissue sarcomas, mostly childhood cancers.