RAPID COMMUNICATION

New Drug Therapy of Amyloidoses: Resorption of AL-Type Deposits With 4'-Iodo-4'-Deoxydoxorubicin

By Luca Gianni, Vittorio Bellotti, Alessandro M. Gianni, and Giampaolo Merlini

Amyloidosis caused by monoclonal Ig light chains (AL) is characterized by the tissue deposition of paraproteins as insoluble fibrils that leads to organ dysfunction and death. After serendipitous observation of its efficacy, the new anthracycline 4'-ido-4'-deoxydoxorubicin (I-DOX) was evaluated in eight patients with biopsy-proven AL and symptomatic organ involvement who received 1 to 6 administrations of I-DOX at dosages of 15 to 100 mg/m². Five patients showed substantial clinical improvement concomitant with instrumental and physical evidence of response, and three patients presented objective evidence of amyloid resorption. The effects of I-DOX on amyloid deposits were not associated with cytotoxicity to the amyloidogenic clone. Five patients died of disease-related complications at 4 to 36 months; the remaining three are alive 29, 35, and 44 months after starting treatment. I-DOX caused short-lived granulocytopenia and minimal extra-hematologic side effects. The pharmacokinetics of I-DOX presented features exploitable for diagnosis in amyloidotic patients and documented the active metabolite in the cerebrospinal fluid. We conclude that I-DOX represents an important treatment option for subjects with AL amyloidosis and could be the prototype of a new class of drugs that interfere with and reverse the process of all types of amyloid deposition.

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were measured by high-performance liquid chromatography (HPLC) as previously described,\(^4\) with minor modifications.

**In vitro studies.** The possible effects of I-DOX on amyloid fibrils were investigated by studying urinary excretion of the amyloid precursor (light chain). Using the Western blot technique as previously reported,\(^9\) Amyloid fibrils used in the in vitro studies were extracted from an amyloidoma surgically removed from patient 1 before I-DOX therapy.

I-DOX binding to amyloid fibrils was evaluated by incubating 1 mg of fibril with 1 mL of \(10^{-7}\) mol/L I-DOX in 0.15 mol/L NaCl. After 1 hour the fibrils were washed three times with 1 mL saline and once with distilled water. The suspension of fibrils in water was allowed to dry on a glass microscope slide, and the characteristic anthracycline fluorescence was determined with a Leitz Diaplan microscope (Leitz, Wetzlar, Germany) equipped with a fluorescence stage, using an excitation filter at 485 nm and an emission filter at 610 nm. The amyloid nature of the fibrils was proved by staining a parallel slide with alkaline Congo red (BDH Chemicals, Poole, UK) followed by analysis with a polarized light microscope. Snap-frozen cryostat sections of brain affected by Alzheimer's disease were incubated with \(10^{-10}\) mol/L I-DOX for 20 minutes, washed, and examined by fluorescence microscopy.

**RESULTS**

I-DOX produced clinical benefits in five of the eight patients. All of them had a long history of symptomatic amyloidosis, with the exception of patient 4 (Table 1), who had a rapidly evolving disease with massive substitution of the liver and spleen, and of patient 7, who presented a rapidly progressing nephrotic syndrome. The therapeutic effect of I-DOX was immediate, substantial, and evident as instrumental and physical response in cases 1 and 8 (Table 1). In the other three responding patients (2, 3, and 5), the benefits appeared some weeks after treatment and persisted in subsequent months (median 6 months). The serendipitous observation of I-DOX effects in patient 1 prompted the present study.

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**Table 1. Characteristics of the Patients Studied and Effects of I-DOX Treatment**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Clinical Manifestation</th>
<th>Previous Treatment</th>
<th>Treatment With I-DOX</th>
<th>Effects of I-DOX</th>
<th>Survival From Diagnosis (present status)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45</td>
<td>M</td>
<td>PS: 2 Severe skin involvement, impairment of gait, macroglossia impairing speech, IVS: 13 mm</td>
<td>No</td>
<td>100 mg/m²</td>
<td>PS: 1 Gait improved, speech improved, IVS: 11 mm, urinary excretion of light-chain fragments</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>49</td>
<td>M</td>
<td>Serum creatinine: 451 (\mu)mol/L, urinary protein 9.5 g/d</td>
<td>Alkylating agents</td>
<td>80 mg/m² &amp; 4 wk</td>
<td>No effect on renal function, stable clinical condition</td>
<td>36</td>
</tr>
<tr>
<td>3</td>
<td>57</td>
<td>M</td>
<td>Autoimmune involvement causing diarrhea (8-10/d) and weight loss (15 kg/6 mos), urinary protein 7 g/d</td>
<td>Alkylating agents</td>
<td>80 mg/m² &amp; 4 wk</td>
<td>PS: 1 Diarrhea reduced, weight gain (5 kg), urinary protein 6 g/d</td>
<td>44</td>
</tr>
<tr>
<td>4</td>
<td>42</td>
<td>F</td>
<td>Massive hepatomegaly, ascites and anasarca, terminal liver failure, serum AP: 1,225 U/L</td>
<td>No</td>
<td>30 mg/m²/wk &amp; 4 wk</td>
<td>Serum AP: 395 U/L, stabilization of clinical condition for 4 mos</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>49</td>
<td>F</td>
<td>PS: 2 Severe nephrotic syndrome, urinary protein 16 g/d</td>
<td>Alkylating agents</td>
<td>30 mg/m²/wk &amp; 2 wk</td>
<td>PS: 1 Urinary protein 5-6 g/d for 6 mos then 10-12 g/d</td>
<td>35</td>
</tr>
<tr>
<td>6</td>
<td>54</td>
<td>F</td>
<td>Congestive heart failure (NYHA class III), IVS: 11 mm</td>
<td>No</td>
<td>30 mg/m²/wk + 15 mg/m²/wk + 20 mg/m²/wk</td>
<td>Congestive heart failure IVS: 8 mm</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>48</td>
<td>M</td>
<td>Severe nephrotic syndrome, urinary protein 13 g/d</td>
<td>No</td>
<td>30 mg/m²/wk &amp; 4 wk</td>
<td>Urinary protein 7.5 g/d</td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td>70</td>
<td>M</td>
<td>Sacral amyloidoma (d 10 cm) infiltrating the skin (11 X 1 cm) causing pain, (^{131})I-SAP uptake by liver, spleen, amyloidoma</td>
<td>Alkylating agents and ionizing radiation</td>
<td>30 mg/m²/wk &amp; 2 wk + 20 mg/m²/wk &amp; 2 wk</td>
<td>Shrinking of amyloidoma to 8 cm and skin infiltrate to 5 X 0.6 cm, resolution of pain, (^{131})I-SAP uptake markedly reduced</td>
<td>29</td>
</tr>
</tbody>
</table>

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Abbreviations: PS, performance status (WHO); IVS, heart interventricular septum thickness; AP, alkaline phosphatase; \(^{131}\)I-SAP, labeled serum amyloid P component; q, every.

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and deserves detailed description. The patient presented with skin involvement that caused widespread thickening and papulae, macroglossia that severely impaired speech, and interventricular septum thickening (13 mm). Stiffness and pain from amyloid deposition made him unable to use his hands, and bulky amyloid deposits in his thighs seriously limited his gait. I-DOX was administered as part of a program of high-dose chemotherapy to be followed by autologous BMT with in vitro and in vivo purging with murine monoclonal anti-idiotypic antibodies. Within 48 hours of receiving I-DOX the patient experienced polyuria, dramatic amelioration of the severe skin involvement, and sudden improvement in general performance. He could again flex his fingers, walk long distances (2 km), and work full time. The rapid regression of macroglossia was concomitant with such an improvement in speech that the patient resumed teaching at the university. The thickness of the interventricular septum decreased from 13 mm to 11 mm in 2 weeks. Western blot analysis of the urine collected 48 hours after the infusion of I-DOX showed light-chain fragments of the same size as those present in amyloid fibrils extracted from a small amyloidoma excised from the left thigh (Fig 1). The extracted fibrils avidly bound to I-DOX after 1-hour incubation in a $10^{-7}$ mol/L solution (Fig 2). Concentrations of the serum and urine monoclonal component only transiently decreased after I-DOX. The clinical improvements persisted for 6 months, then the patient’s condition slowly deteriorated. He died suddenly of cardiac arrest 31 months after diagnosis and 10 months after treatment with I-DOX.

In addition to patient 1, I-DOX produced measurable reduction of amyloid deposits in two patients. In patient 6, with overt clinical amyloid heart involvement, echocardiography showed a reduction of the thickness of the interventricular septum. Patient 8 presented an amyloidoma (diameter 10 cm) that destroyed part of the left sacrum and ilium and infiltrated the gluteus, where a palpable mass 11 x 1 cm caused piercing pain that required chronic analgesic therapy. The amyloidoma had grown in the prior 20 months despite radiotherapy with 50 Gy and chemotherapy with cyclophosphamide. I-DOX (two weekly doses of 30 mg/m^2, followed by two weekly doses of 20 mg/m^2) caused a major reduction of the palpable mass to 5 x 0.6 cm, and of the bone amyloidoma to 8 cm in diameter as assessed by nuclear magnetic resonance. The pain completely disappeared and the patient was able to go off all analgesic drugs. Before therapy, imaging with $^{131}$I-SAP showed uptake by the amyloidoma, liver, and spleen. The $^{131}$I-SAP study was repeated 1 month and 14 months after treatment with I-DOX. As illustrated in Fig 3, there was a marked regression of the amyloid deposits 14 months after the last I-DOX dose even though the serum amyloidogenic precursor (IgG κ) persisted unmodified. The comparison of imaging with $^{131}$I-SAP was performed in three more patients (5, 6, and 7). There was no significant change at 3 to 4 weeks after treatment with I-DOX, and a definite, although relatively modest, increase of amyloid deposits after 18 months in patient 5. In patient 2, who suffered from severe renal failure with nephrotic syndrome and initial heart involvement (interventricular septum thickness 14 mm, normal ≤10 mm), I-DOX did not affect renal function. However, after treatment with I-DOX his general condition remained satisfactory for 11 months, without progression of heart involvement, notwithstanding that his serum monoclonal component remained in the range of 9 to 12 g/L. In patient 3 I-DOX produced a significant reversal of clinical symptoms, including reduction of the diarrhea caused by autonomic nerve involvement and progressive weight gain. In patient 5, who had a severe nephrotic syndrome caused by renal amyloidosis, a single course of I-DOX was associated with a decrease in proteinuria from 16 to 6 g/d that persisted for 6 months. In none of the patients whose urinary protein excretion decreased (3, 5, and 7), was there a concomitant increase of serum creatinine.

In patients 4, 6, and 7 the expected natural history of the disease was not affected despite objective amyloid resorption in patient 6 and temporary reduction of urinary protein excretion in patient 7. Already irreversible organ damage caused by amyloid infiltration could explain the clinical inefficacy.
in patients 4 and 6, whereas a single I-DOX course might have been insufficient to overcome the possibly strong amyloidogenic potential of patient 7's light chain. Overall, three patients are alive at 29, 35, and 44 months after starting treatment with I-DOX, whereas five have died of disease complications.

Toxicity. The main toxicity was reversible, clinically uneventful granulocytopenia (Table 2). The onset of neutropenia was rapid (median time from treatment to nadir 12 days, range 8 to 16) and short-lived (median time from treatment to recovery 14 days, range 9 to 22), and was not clearly dose-related as it is in cancer patients. Neutropenia was never associated with infection or febrile episodes. As already reported, anemia and thrombocytopenia were of minor importance. Transient nausea was reported by three patients. Because anthracyclines may have cardiotoxic effects, all patients were thoroughly investigated to rule out this possibility. A comparative analysis of the left ventricular ejection fraction before and after therapy did not show any significant, consistent, or substantial modification in any of the patients. Patient 4 died in shock with acute total heart failure as the terminal event. The left ventricular ejection fraction, measured 1 day before the last dose of I-DOX, was normal (69%) and equal to that measured before the start of anthracycline therapy (66%). Postmortem examination...
showed multiple amyloid deposits that massively involved the liver, spleen, lungs, kidneys, and the adrenocortical glands, and may explain her death as the result of disease progression.

Pharmacokinetic studies. The plasma pharmacokinetics of I-DOX was studied in three patients and was significantly different from that described in subjects with solid tumors. In patient 4, who was treated with four consecutive doses of 30 mg/m², plasma retention of I-DOX increased after each of the first three doses, as shown by a shift in total body clearance, which became progressively slower (335, 214, and 153 L/h/m² after the first, second, and third administrations, respectively). The pharmacokinetic profiles of I-DOX and I-DOXOL after administration of 80 mg/m² in patients 2 and 3 were also at odds with the plasma anthracycline disposition measured in patients with solid tumors. In these two patients I-DOX (Fig 4) and I-DOXOL (data not shown) plasma concentrations during infusion were significantly lower than and outside the corresponding I-DOXOL plasma concentrations.

Table 2. Hematologic Toxicity of Treatment With I-DOX

<table>
<thead>
<tr>
<th>Dose (mg/m²)</th>
<th>Cycles (n)</th>
<th>Anemia (Hb g/dL) Median Range</th>
<th>Neutropenia (ANC × 10⁹/L) Median Range</th>
<th>Thrombocytopenia (platelets × 10⁹/L) Median Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>5</td>
<td>11.3 7.4-12.8</td>
<td>900 140-1,660</td>
<td>203 83-437</td>
</tr>
<tr>
<td>80</td>
<td>4</td>
<td>7.75 7.1-8.3</td>
<td>375 60-750</td>
<td>118.5 99-182</td>
</tr>
<tr>
<td>100</td>
<td>3</td>
<td>9.2 8.8-9.6</td>
<td>1,358 850-3,840</td>
<td>113 112-207</td>
</tr>
</tbody>
</table>

Abbreviations: Hb, hemoglobin; ANC, absolute neutrophil count.

DISCUSSION

AL-type systemic amyloidosis is a progressive disease with poor prognosis. Treatment options are mainly based on the use of anticancer agents to eradicate cells that produce the amyloid precursors or, subordinately, on the administration of colchicine or dimethyl sulfoxide (DMSO) to interfere with amyloid deposition or promote its resorption, respectively. However, alkylating agents improve clinical condition and survival in only a small fraction of patients. The use of more aggressive high-dose chemotherapy programs is substantially limited by poor tolerability and severe toxicity because of amyloid organ damage, although this approach could possibly prove feasible and effective in those few AL patients without severe organ damage. Colchicine was reported to improve life expectancy in a survival analysis with historical controls, but no clear-cut regression of organ involvement has been described.

Finally, DMSO is substantially ineffective in AL amyloidosis and its use caused serious problems of tolerability and compliance. In all these treatment modalities resorption of amyloid deposits is a rare event. Within this framework of long-standing clinical experience, the effects of I-DOX in patients with AL amyloidosis appear particularly relevant. We obtained evidence that I-DOX induced amyloid resorption in three patients, as indicated by measurable reduction of the thickness of the interventricular septum (patients 1, and 6), by massive urinary excretion of amyloid fragments (patient 1), and by the substantial reduction of a large amyloidoma and spleen and liver amyloid deposits (patient 8). The pattern of resorption was not consistent in all cases. The apparent urinary excretion of amyloid fragments was documented only in patient 1 (see Fig 1), despite the fact that we examined all subsequent patients with the same technique. Of note, he was also the only patient with severe skin involvement and the only one in whom the onset of the clinical improvement occurred very rapidly. A brief course of I-DOX inhibited progression of amyloidosis and improved the clinical condition of five patients (1, 2, 3, 5, and 8) for several months.

The potential impact of I-DOX treatment on patient survival cannot be assessed on the basis of the present pilot study. Ethical considerations prompted a selection of patients with progressive disease refractory to previous treatment (patients 2, 3, 5, and 8), with end-stage amyloid organ involvement (4 and 6) and/or rapidly progressing disease (4 and 7).
The median time from diagnosis of AL to treatment with I-DOX was 16.5 months (range 3 to 40 months). It is known that longer intervals from diagnosis to study entry are associated with better survival, whereas patients seen within 1 month of diagnosis have a dramatically poorer prognosis compared with all patients seen. Based on the above considerations, the patients selected for the present study could be expected to have an inherently longer and better survival independently of I-DOX treatment. A prospective controlled study of I-DOX versus chemotherapy in which patients are stratified by major prognostic factors, including time from initial diagnosis to initiation of therapy, is being designed to assess the full therapeutic potential of this new anthracycline and its impact on survival.

It is important to note that the drug was well tolerated by all patients, treatment was feasible, and the period of neutropenia was short enough not to entail a risk of infection.

Besides these clinical effects, our findings also offer insights into the possible mechanism of action of I-DOX.

Pharmacokinetic analysis of plasma concentrations of I-DOX and I-DOXOL showed very peculiar features in patients with amyloidosis. As already described in patients with solid tumors, I-DOXOL was the main anthracycline circulating in the plasma immediately after I-DOX administration. However, in the three patients with amyloidosis the pharmacokinetics were characterized by significantly lower I-DOX and I-DOXOL concentrations than those measured in patients without amyloidosis, and by a progressive increase of plasma retention of the anthracycline with each subsequent dose. This unusual drug disposition is reminiscent of the plasma kinetics of Congo red in amyloidotic patients. The dye was used in the past as a diagnostic tool because of its high affinity for amyloid. With several notable exceptions, a low serum retention (ie, fast clearance) of the dye after intravenous administration was observed in subjects with massive amyloid deposits. Similarly, the faster clearance of I-DOX in amyloidotic patients is consistent with the concept that the drug avidly binds to a target easily accessible from the blood or interstitial fluid, and that this target becomes progressively less available with subsequent treatments because of saturation or removal. In vitro studies documenting the marked affinity of I-DOX for AL amyloid fibrils (Fig 2) and for several other types of amyloid deposits, including Alzheimer's (Fig 5), support the hypothesis that such a target was represented by the in vivo amyloid deposits. Substitution of the C' 4-hydroxyl group with an iodine atom is the only structural difference between doxorubicin and its derivative I-DOX. Because doxorubicin only loosely binds to amyloid fibrils, the presence of iodine in the anthracycline structure may well be responsible for the homing of I-DOX and I-DOXOL to the amyloid.

The binding to amyloid deposits most likely plays a key role in the mechanism of action of I-DOX. We did not observe any significant and/or persistent modification of the serum or urinary concentrations of the monoclonal protein in any of the patients described in this report. Thus, the clinical effects could not be attributed to anthracycline cytotoxicity toward the clones producing the amyloidogenic precursor. I-DOX treatment induced mobilization and resorption of amyloid deposits; however, we were unable to induce in vitro dissolution of amyloid fibrils by the addition of I-DOX or I-DOXOL. This apparent discrepancy may be reconciled by assuming that the tight binding of iodinated anthracyclines in vivo makes amyloid deposits less accessible to amyloid precursors, slowing down or even preventing their deposition and facilitating existing proteolytic mechanisms for amyloid degradation and resorption. This hypothesis would explain the relatively long duration (median 6 months) of the clinical benefits derived from a few I-DOX administrations (patients 2, 3, and 5), and the slow resorption of amyloid deposits in patient 8. The acute clinical and laboratory effects induced by a single high dose of I-DOX in patient 1 indicate that in certain patients, for reasons that still need to be investigated, the mobilization and resorption of amyloid deposits could be very rapid.

In conclusion, I-DOX represents a new, feasible, and well-tolerated treatment option for AL amyloidosis. Its effects should be investigated further, preferably in patients at an earlier stage of the disease than those described in this report. The observation that the drug appears to have high tropism for amyloid deposits on which it exerts its action over time suggests that I-DOX should be used for brief courses of low weekly doses (30 mg/m² for 4 consecutive weeks). In addition, its mechanism of action is independent of its cytotoxicity, produces amyloid resorption, and is most likely caused by binding to amyloid fibrils. Thus, the affinity of I-DOX for all clinically relevant types of amyloid tested (AA, AL, ATTR, Aβ, Aβ2M) qualifies this new anthracycline as a prototype drug that is potentially useful for all amyloidotic diseases. With this in mind, the demonstration that I-DOXOL can be found in the CSF after systemic administration of I-DOX is surely a very attractive feature in the perspective of targeting amyloid deposits within the central nervous system, as in the case of Alzheimer's disease. Nontoxic analogs of I-DOX are presently being investigated to make this novel therapeutic approach available for all forms of amyloidosis.

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New drug therapy of amyloidoses: resorption of AL-type deposits with 4'-iodo-4'-deoxydoxorubicin

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