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In vivo effect of chloroquine on platelet aggregation in anesthetized rats

Sir,

In vivo platelet aggregation was studied by a platelet count ratio (PCR) technique. Following the intravenous administration of collagen or ADP to rats the mean PCR was lower in controls than in two groups administered graded doses of chloroquine ($p<0.05$ and 0.01 respectively). Chloroquine inhibits platelet aggregation *in vivo* in rats.

Previous reports on the effect of chloroquine on platelet aggregation were based on *in vitro* and *ex vivo* studies where aggregation inducers and chloroquine were added to isolated platelets, or aggregation inducers added to platelets withdrawn from chloroquine-treated human volunteers.[1-3] Since not all the factors that affect aggregation *in vivo* may be available *in vitro* or *ex vivo*, the effect of chloroquine on platelet aggregation *in vivo* has been examined.

Rats were randomly assigned into a control or two test groups (n=6). The control group was administered 0.9% NaCl (1 mL/kg, ip). The first test group was given ADP at a dose of 8.6 mg/kg, ip[1] while the second test group was administered a higher dose of chloroquine (40 mg/kg, ip). After one hour, collagen (1 mg/kg, iv) was administered under urethane anesthesia (1.5 g/kg, ip) to all groups to induce platelet aggregation *in vivo*.

Blood (1 mL/rat) was taken by cardiac puncture for estimation of platelet aggregation.This was measured by a PCR technique[4] in which a lowering of the count ratio signifies an increase in platelet aggregation and vice versa. These experiments were repeated using another aggregation inducer, ADP (90 µg/kg, iv) and normal saline (1 mL/kg, iv). The doses of ADP and collagen were slightly higher than those reported for rabbits[5] since preliminary studies showed that lower doses were ineffective. Serum chloroquine concentration was estimated by the method of Prauty and Kuroda.[6]

Mean serum chloroquine concentrations one hour after administration were 5.06±1.29 mg/L and 10.98 ±3.75mg/L (mean±SD; $p<0.01$) in rats administered chloroquine at doses of 8.6 mg and 40 mg/kg respectively (n=5).

In the rats given i.v. collagen, the PCR were 0.283±0.165, 0.560± 0.175 and 0.694±0.193 in the control, first and second test groups respectively. The ratios for the two test groups were significantly higher ($p<0.05$ and 0.01) than that of the control group. Results after ADP were similar. Platelet count ratios following the infusion of normal saline were 0.818± 0.094; 0.830±0.073 and 0.876±0.070 for control, first and second test groups respectively. The ratios obtained with saline were not significantly different between the three groups (Figure 1).

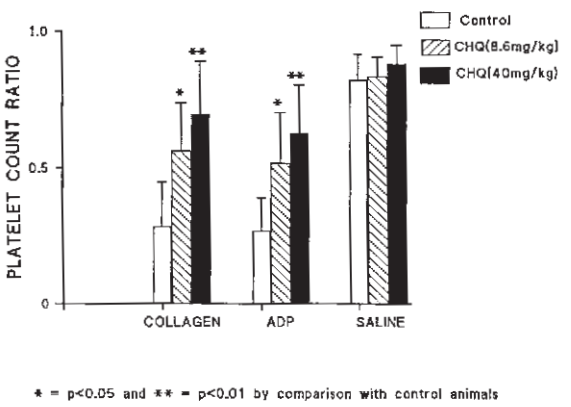

Figure 1. Platelet count ratio in rats administered collagen, adenosine diphosphate (ADP) or normal saline (iv) following ip administration of normal saline (1 mL/kg) to control rats (n=6) and chloroquine diphosphate to test rats at either 8.6 mg/kg (n=6) or 40 mg/kg (n=6). Values are represented as means±SD.

Based on *in vitro* and *ex vivo* studies some investigators have concluded that therapeutic concentrations of chloroquine have a negligible effect on platelet aggregation and are not a significant risk to patients with compromized hemostasis.[1] However, *in vitro* and *ex vivo* studies may not reflect *in vivo* events since some endogenous aggregation inducers and inhibitors from non platelet sources may be reduced or unavailable.

We have shown that a therapeutic dose of chloroquine inhibits platelet aggregation *in vivo* in rats and so, its use in patients with compromized hemostasis could be risky if the results are confirmed in humans. Conversely, chloroquine administration could be beneficial in the reduction of hyperaggregability of platelets in malaria[7,8] and in the prevention of thrombosis.

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Key words

Chloroquine, platelets, in vivo aggregation.

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Successful treatment of AA amyloidosis secondary to Hodgkin's disease with 4'-iodo-4'-deoxydoxorubicin

Sir,

A case of AA amyloidosis secondary to Hodgkin's disease is reported. After complete remission of the lymphoma, treatment with the drug 4'-iodo-4'-deoxydoxorubicin resulted in an improvement of the nephrotic syndrome and removal of amyloid from liver tissue. The drug could be a therapeutic option for secondary amyloidosis.

Secondary (AA) amyloidosis is known to be associated with a variety of diseases in which inflammation is a common feature.[1] Apart from control of underlying disease, currently there are no treatments able to remove amyloid from involved tissues. Preliminary reports on the use of the drug 4-iodo-4'-deoxydoxorubicin in primary (AL) amyloidosis seem encouraging.[2] We report here a case of AA amyloidosis secondary to Hodgkin's disease in which treatment with 4'-iodo-4-deoxydoxorubicin resulted in substantial improvement of clinical status and removal of fibrils as assessed by liver biopsy.

The patient was a 37-year-old male whose complaints were fatigue and significant maleolar edema. An abdominal ultrasound showed enlarged retroperitoneal lymph nodes and after biopsy, a diagnosis of Hodgkin's disease was made. From the blood analysis severe hypoproteinemia (4.2 g/dL), hypoalbuminemia (1.1 g/dL) and increased alkaline phosphatase (1163 U/L) were found as well as proteinuria (12 g/L). During pathologic staging, amyloid deposition was found in hepatic sinusoids (Figure 1). Immunohistochemical staining confirmed amyloid AA deposition. After complete staging the definitive diagnosis was mixed cellularity Hodgkin's disease stage II A with secondary amyloidosis. A renal biopsy was not performed due to an increased risk of bleeding; the nephrotic syndrome was attributed to amyloidosis.

After six cycles of COPP/ABV chemotherapy, a complete remission was achieved, assessed by computerized tomography. Nevertheless, proteinuria, hypoalbuminemia and edema persisted, probably due to renal deposition of amyloid. A repeat liver biopsy showed similar findings to those at diagnosis, with the same amount of amyloid deposition. Four months after complete remission, biochemical parameters and edema remained at similar levels.

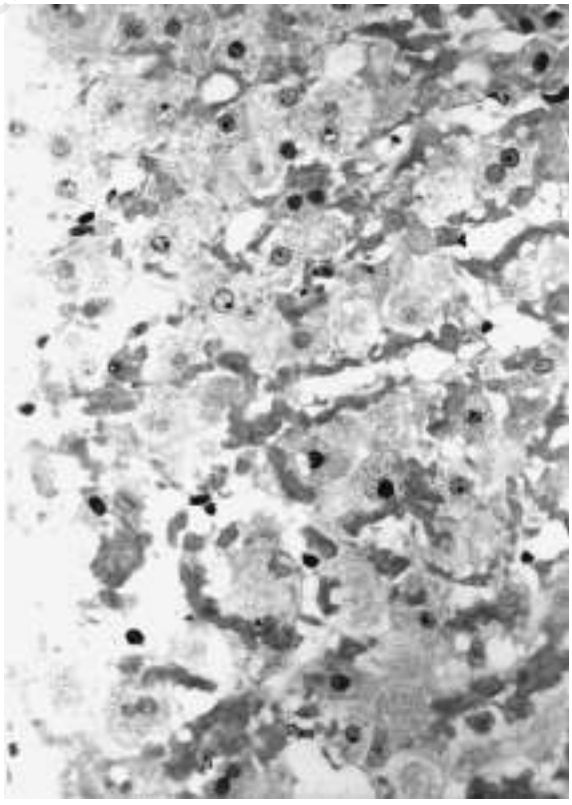

Figure 1. Liver biopsy showing extracellular amyloid deposition (Congo red, ×600).

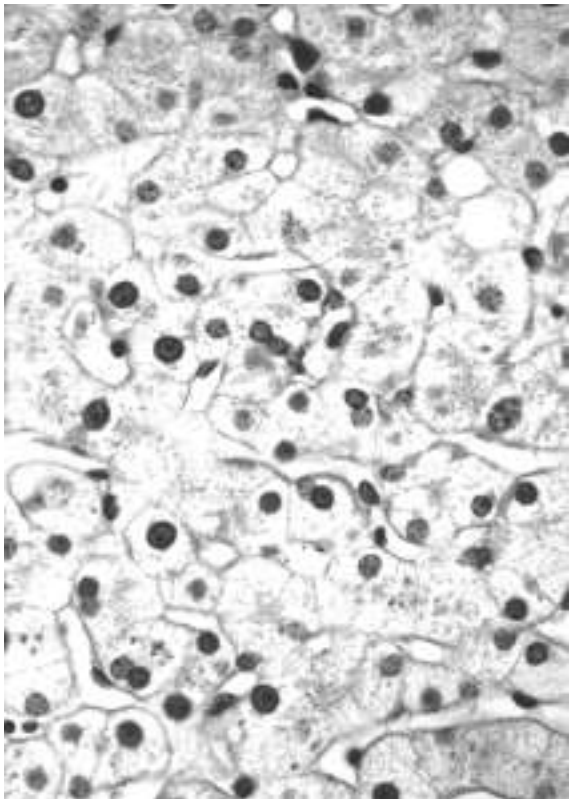

Figure 2. Liver biopsy; substantial removal of amyloid after treatment (PAS, ×600).

At that point, we started treatment with 4-iodo-4'-deoxydoxorubicin in an attempt to improve the patient's situation. Two weeks later, after four cycles of weekly administration at a dose of 30 mg/m^2, a new evaluation was performed. Increased albuminemia (2.5 g/dL) and proteinemia (4.8 g/dL), decreased alkaline phosphatase (711 U/L) and decreased proteinuria (5 g/L) were found. Fatigue and edema disappeared and a new liver biopsy showed substantial decrease in amyloid deposits (Figure 2). After one year of follow-up, the patient's status is similar, with hypoalbuminemia and proteinuria at levels comparable to those achieved at the end of therapy and no drug-related toxicity.

Initial reports of *in vitro* binding to amyloid fibrils[3] led to clinical studies[2] that suggest that 4-iodo-4'-deoxydoxorubicin might achieve not only blockage of amyloid deposition but also removal of fibrils from the extracellular matrix. The drug has been successfully used for the treatment of AL amyloidosis but to date, there are no reports of its use in AA amyloidosis.

The possibility of improvement after resolution of underlying Hodgkin's disease cannot be completely ruled out,[4] but the evolution of biological parameters was not uniform. No improvement was achieved four months after complete remission of the lymphoma, but proteinuria and edema dramatically changed after four cycles of therapy with 4-iodo-4'-deoxydoxorubicin. Thus, it is reasonable to think that the drug is responsible for partial resolution of the disease. In our opinion, use of this drug for the treatment of AA amyloidoses, as well as AL amyloidosis, should also be investigated.

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Key words

Amyloidosis AA, 4'-iodo-4'-deoxydoxorrubicin, Hodgkin's disease.

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Hepatitis C virus infection and mixed cryoglobulinemia in patients with lymphoproliferative diseases

Sir,

In the last few years hepatitis-C virus (HCV) has been implicated in the pathogenesis of diverse processes originating from B-clonal lymphoid proliferation, such as mixed cryoglobulinemia (MC) and B-cell non-Hodgkin's lymphomas (NHL).[1,2] However, other studies carried out in other geographic areas have not confimed these observations.[3] We, therefore, analyzed 95 patients affected by B-cell lymphoproliferative diseases (B-LPD), seen from October 1991 to December 1995 at the Hematology Department of the University Hospital of Zaragoza, Spain.

B-LPD was diagnosed on the basis of morphologic and immunologic evaluation of lymph nodes, bone marrow or peripheral blood specimens. All the processes were classified according the REAL classification.[4] Detection and characterization of cryo-