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Beneficial cardio-renovascular effects of a low-molecular-weight heparin-derivative on adriamycin-induced glycosaminoglycanuria and tissue lipid abnormalities

P.R. Deepa, P. Varalakshmi *

Department of Medical Biochemistry, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani, Chennai 600 113, India

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Abstract

The present work includes a study on the glycosaminoglycanuric condition induced by adriamycin (ADR, a chemotherapeutic agent) and the accompanying secondary hyperlipidemia, wherein the treatment with a low-molecular-weight heparin-derivative (LMWH), certoparin, is evaluated for its protective role (if any) on these parameters. Two groups of male albino rats of the Wistar strain (140 ± 10 g) received a single intravenous injection of adriamycin (7.5 mg/kg), and one of these groups was treated with a low-molecular-weight heparin-derivative (Certoparin Sodium, Troparin®; 300 μg/day/rat s.c.), commencing on day 8, for a week. Urinary total glycosaminoglycans excretion of the untreated ADR-induced group was found to increase on the 8th and the 15th days of observation, when compared with the controls. The LMWH treatment commencing on day 8 resulted in minimising the glycosaminoglycans (GAGs) excretion by day 15 ($p < 0.001$). Plasma, cardiac, hepatic and renal lipids (cholesterol, triglycerides and phospholipids) showed a sharp increase in the pathologic group, along with a rise in plasma LDL and VLDL cholesterol and drop in HDL cholesterol levels, paralleled by abnormal activities of the enzymes involved in lipid metabolism. LMWH treated group showed a normalised lipid profile and the activities of the lipid-metabolising enzymes was close to that of controls. It is concluded herein that adriamycin administration resulted in severe nephropathy manifested by increased glycosaminoglycanuria and abnormal lipid metabolism, and that LMWH treatment afforded substantial protection by restoring glomerular structure and function, and normalised the plasma and tissue lipid levels, lipoprotein profile and the activities of lipid-metabolising enzymes.

Keywords: Low-molecular-weight heparin; Adriamycin; Cytotoxicity; Nephrotic syndrome; Glycosaminoglycan; Hyperlipidemia

1. Introduction

A single high-dose injection of adriamycin (ADR) induces in rats a nephrotic syndrome characterised

* Corresponding author. Tel.: +91 44 24925548; fax: +91 44 24926709.
E-mail addresses: dipa_pr@mailcity.com (P.R. Deepa), drvlakshmi@yahoo.com (P. Varalakshmi).

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by heavy proteinuria, hypoalbuminemia, hypercoagulability, and hyperlipemia with hypercholesterolemia (Bertani et al., 1982; Bizzi et al., 1983). The hyperlipidemia associated with the nephrotic syndrome has long been recognized. There have been reports of patients with urinary protein loss showing accelerated atherosclerosis, which may be directly related to the hyperlipidemia (Berlyne and Mallick, 1969; Curry and Roberts, 1977). In a recent work (Malarkodi et al., 2003), adriamycin nephropathy was reported to be associated with hyperlipidemia. Hyperlipidemia associated with nephrotic syndrome is a complex disorder involving abnormalities of both synthesis and catabolism of lipoproteins most likely induced by the glomerular barrier defect (resulting in proteinuria) and the secondary reduction in serum oncotic pressure that occurs as hypoalbuminemia ensues. Therefore, therapy that reduces proteinuria and increases serum albumin concentration can ameliorate hyperlipidemia (Hutchison, 1993). In our earlier work, we reported that the low-molecular-weight heparin-derivative, certoparin ameliorated both the proteinuric as well as the hypoalbuminemic consequence of ADR-induced nephrotic syndrome (Deepa and Varalakshmi, 2003a). The present work explores the possible effects of the exogenous administration of heparin-derivative, certoparin on the ADR-induced glycosaminoglycanuria and secondary hyperlipidemia.

Adriamycin cytotoxicity in the present work serves as a single platform to study secondary hyperlipidemia associated with nephrotic syndrome as well as the lipid status in the other target organs of cytotoxicity, namely, heart and liver. Here, the effect of LMWH on the altered lipid metabolism has been evaluated. Low-molecular-weight heparins (LMWHs) are fragments of commercial grade heparin, produced by either chemical or enzymatic depolymerization, and are potentially more advantageous than heparin due to their reduced hemorrhagic to antithrombotic ratio, reduced risk of bleeding, greater bioavailability at low doses, longer half life and more predictable anticoagulant response at fixed doses (Green et al., 1994). Some salient anti-lipemic properties of heparin (Engelberg, 1980) prompted the present investigation on the low-molecular-weight heparin-derivative, certoparin.

2. Materials and methods

2.1. Drugs and chemicals

Adriamycin (Doxorubicin Hydrochloride, Adrim; Dabur Pharmaceuticals, New Delhi, India) and low-molecular-weight heparin (Certoparin Sodium, Troparin®; Mfd by Biochemie GmBH, Austria and marketed by Novartis India Ltd., Mumbai, India) have been used in the present study. All other chemicals and solvents used in the present experimentation were of analytical grade.

2.2. Experimental animals and treatment

Male albino rats of Wistar strain (140 ± 10 g) were housed under standard conditions of temperature ($23 \pm 1\,^{\circ}$C), relative humidity ($55 \pm 10\%$), and 12 h light/12 h dark cycle and were given food and water ad libitum. Experimental animals were handled according to the University and institutional legislation, regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India (IAEC No. 02/OGP-2/011/03).

Group I served as the control. Groups II and IV comprised of rats that were administered adriamycin (7.5 mg/kg) as a single injection intravenously through the tail vein. While group II was left untreated, group IV comprised of rats given the single adriamycin injection followed by low-molecular-weight heparin treatment commencing 1 week later. Low-molecular-weight heparin (Certoparin Sodium, Troparin®; average molecular weight of 4200–6200) was administered subcutaneously at a dosage of 300 μg/day/rat for 7 days. Group III rats received only LMWH treatment for 7 days, serving as the LMWH drug control. At the end of the 2-week experimental period, the body weight of the rats was recorded. The animals were then sacrificed and the heart, liver and kidney tissues were excised.

2.3. Estimation of total glycosaminoglycans (GAGs)

Total GAGs was determined by the method of Whiteman (1973) with slight modifications (Hwang et al., 1988). This method is based on the interaction of cationic dye alcian blue 8GX with acidic glycosamino-