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Effects of liposomes with polyisoprenoids, potential drug carriers, on the cardiovascular and excretory system in rats

Olga Gawrys [a], Marta Polkowska [a], Malwina Roszkowska-Chojecka [a], Katarzyna Gawarecka [b], Tadeusz Chojnacki [b], Ewa Swiezewska [b], Marek Masnyk [c], Marek Chmielewski [c], Janina Rafałowska [d], Elżbieta Kompanowska-Jezierska [a,*]

[a] Department of Renal and Body Fluid Physiology, M. Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland
[b] Department of Lipid Biochemistry, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland
[c] Institute of Organic Chemistry, Polish Academy of Sciences, Warsaw, Poland
[d] Department of Experimental and Clinical Neuropathology, M. Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

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ABSTRACT

Background: The unpredictable side effects of a majority currently used drugs are the substantial issue, in which patients and physicians are forced to deal with. Augmenting the therapeutic efficacy of drugs may prove more fruitful than searching for the new ones. Since recent studies show that new cationic derivatives of polyisoprenoid alcohols (APrens) might exhibit augmenting properties, we intend to use them as a component of liposomal drug carriers. In this study we investigate if these compounds do not *per se* cause untoward effects on the living organism.
Methods: Male Sprague–Dawley rats received for four weeks daily injections (0.5 ml *sc*) of liposomes built of dioleoyl phosphatidylethanolamine (DOPE), liposomes built of DOPE and APren-7 (ratio 10:1) or water solvent. Weekly, rats were observed in metabolic cages (24 h); blood and urine were sampled for analysis; body weight (BW) and systolic blood pressure (SBP) were determined. After chronic experiment, kidneys and heart were harvested for histological and morphometric analysis.
Results: The 4-week BW increments were in the range of 97 ± 4 to 102 ± 4%, intergroup differences were not significant. Microalbuminuria was the lowest in the group receiving liposomes with APren-7 (0.22 ± 0.03 mg/day). Water and food intake, plasma and urine parameters were similar in all groups.
Conclusions: Newly designed liposomes containing APren-7 did not affect functions of the excretory and cardiovascular systems, and renal morphology; therefore we find them suitable as a component of liposomal drug carriers.

Introduction

A majority of currently used drugs display, in addition to the therapeutic action, definite side effects which are often severe and unpredictable; the difference between the therapeutic and harmful dose may be quite small. This may necessitate monitoring drug concentration in the patient's serum, which is cumbersome and increases the cost of treatment [9]. In light of the recent literature of the subject, a search for substances which augment the therapeutic efficacy of existing drugs may prove equally fruitful as the development of the new ones. Drug delivery systems (DDS) which include many different conjugates, such as polymeric micelles, liposomes, hybrid carriers and polyplexes (complexes of the polymer–DNA type) [1,4] may provide better penetration of drugs across biological membranes which results in faster access to the interior of cells and, in general, to areas distant from the lumen of blood vessels. Currently, liposomal formulations which facilitate the penetration through biological membranes are widely used as carriers of pharmacologically active substances [24]. Cationic lipids of various structures have been suggested to enhance the

Abbreviations: APren, cationic derivative of polyisoprenoid alcohol; BW, body weight; DDS, drug delivery systems; DOPE, dioleoyl phosphatidylethanolamine; DPPC, dipalmitoylphosphatidylcholine; Ht, haematocrit; LV/BW, left ventricular – to – body weight ratio; NO, nitric oxide; NO_x, excretion of nitric oxide metabolites; PE, phosphatidylethanolamine; P_{Na}, plasma sodium concentration; P_{osm}, plasma osmolality; SBP, systolic blood pressure; *sc*, subcutaneously; UAE, urinary albumin excretion; U_K, concentration of potassium in urine; U_{Na}, concentration of sodium in urine; U_{osm}, urine osmolality.

* Corresponding author.
E-mail address: ellakomp@gmail.com (E. Kompanowska-Jezierska).

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efficacy of liposomal drug and nucleic acids delivery [14,21]. Recent studies show that cationic derivative of polyprenol tentatively named APren, new semi-synthetic derivative of polyisoprenoid alcohol, might be successfully used as component of liposomal formulas used for lipofection [18].

Polyprenols are linear polymers built of 5 up to 150 isoprene units. Their postulated cellular functions comprise involvement in cell response to environmental stress [26]. It is known that polyprenols and their phosphorylated derivatives increase the permeability and fluidity of model membranes and intensify the fusion of model membranes [26]. A new type of semi-synthetic cationic polyisoprenoid derivatives has been obtained recently. Taking advantage of their lipofecting properties [18] new drug carriers based on liposomes containing these derivatives of polyisoprenoid alcohols were designed. From among various naturally occurring polyprenols, the one containing seven isoprene units was subjected to chemical modifications to obtain heptaprenyltrimethylammonium iodide (APren-7), to be used as a component of liposomal drug carrier. Our aim was to investigate if this compound does not *per se* cause untoward effects on the living organism.

Among mammalian organs and tissues the kidneys are known to be particularly sensitive to drug-dependent toxicity which may damage the renal glomeruli and various structures and tissues located in the renal medulla, such as local tubule fragments, interstitium and vasa recta. The vulnerability of the medulla obviously depends on the usual process of concentration of numerous substances, including various drugs, which occurs in this region. In this study we focused on potential changes in kidney structure and function but also examined some indices of general health and behaviour (*e.g.* weight gain, activity) as well as selected status indices of the cardiovascular system.

The impact of APren-7 on living organisms was studied in male Sprague–Dawley rats; animals aged 5–6 weeks were used to determine, in addition, the possible influence of APren-7 on the development and maturation. Effects of subcutaneous injections of liposomes containing APren-7 were compared with those of liposomes built exclusively of commonly used dioleoyl phosphatidylethanolamine (DOPE), and those seen in rats receiving water solvent for liposome solutions.

Materials and methods

Preparation of heptaprenyltrimethylammonium iodide (APren-7)

This compound was obtained by modification of polyisoprenoid alcohols derived from birch wood following the procedure described earlier [18].

Preparation of liposomal suspensions for injection

Liposomal suspensions were prepared using lipid film hydration protocol. Briefly, to obtain *classical liposomes* (*L*), 55 μl of a solution of 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE, Sigma) in chloroform (90 mg/ml) was dried by stream of nitrogen gas to form a thin film on the bottom of the glass tube. The film was further dried by exsiccation under reduced pressure, overnight. Then water was added to hydrate the film and multilamellar liposomes were formed by vortexing each tube for at least 8 min. The obtained suspension was extruded few times through a 100 nm membrane to obtain unilamellar liposomes (LiposoFast extruder, Avestin Europe GmbH, Mannheim, Germany).

To obtain *liposomes with APren-7* (L + P), 50 μl of a solution of DOPE in chloroform (90 mg/ml) and 25 μl of a solution of APren-7 in chloroform (18 mg/ml) were mixed together (DOPE:APren-7 molar ratio 10:1) and then the procedure of further preparation was as described above.

Experimental animals

The experimental procedures were approved by the IV Ethical Committee, Warsaw. Male Sprague–Dawley rats, weighing 154 ± 3 g at the start of experiments, were fed *ad libitum* a standard diet (STD, 0.25% Na w/w, SSNIFF GmbH, Soest, Germany) and had free access to drinking water during the whole experiment. The animals were accustomed to the housing and measurement procedures during the week preceding the experiments. This was done to eliminate the stress associated with immobilization needed to measure systolic blood pressure (SBP); such stress is known to increase SBP *per se*.

Experimental protocols and measurements

During four weeks three groups of rats received daily injections (0.5 ml, *sc*) of freshly prepared solutions of classical liposomes (L, $n = 13$), liposomes with APren-7 (L + P, $n = 13$, 12 mg/kg), or water solvent (W, $n = 12$). At one-week intervals rats were placed for 24 h in metabolic cages (Tecniplast S.p.A. Buguggiate, Italy) to measure food and water intake, the weight of faeces, and urine volume and osmolality (U_{osm}), as well as the concentration of sodium (U_{Na}), potassium (U_K), albumin and nitric oxide metabolites. Also determined were body weight (BW) and SBP (tail-cuff method, Coda System, Kent Scientific Corporation, Connecticut, USA); blood was sampled for plasma osmolality (P_{osm}), plasma sodium concentration (P_{Na}) and haematocrit (Ht). In the end of the experiment all animals were anesthetized with intraperitoneal sodium thiopental (Sandoz GmbH, Kundl, Austria), 100 mg/kg, and the samples of the kidneys (for morphometric and histologic studies) and of the heart (for morphometric examination only) were harvested.

Analytical procedures

Urine volume was determined by gravimetric method, plasma and urine osmolality by freezing point depression (Osmomat® 030 M, Gonotec, Berlin, Germany), sodium and potassium concentration by flame photometry (PFP7/C, Jenway Ltd, Stone, UK), nitrites/nitrates using nitric oxide (total) detection kit (Enzo Life Sciences Inc., New York, USA), and albumin content using UAE immunoperoxidase assay for determination of albumin in rat samples (Immunology Consultants Laboratory, Inc., Portland, USA).

Statistics

Data are presented as means ± SEM. The significance of changes was evaluated by multivariate analysis of variance (ANOVA) with repeated measurements, followed by Newman–Keuls *post hoc* test (STATISTICA, version 10.0, StatSoft Inc.). The 4-week increments in the parameters measured were compared between groups by one-way ANOVA. The level of statistical significance was set at of $p < 0.05$.

Results

Body weight profiles over 4 weeks are shown in Fig. 1. The increase of the body mass was almost parallel for all three groups of animals analyzed. When expressed as per cent of the starting weight value (unlike in Fig. 1. where absolute values are given), the curves for L, L + P and W groups were superimposable. The 4-week BW increments were 109 ± 4, 97 ± 4 and 102 ± 4% in the respective groups (intergroup differences not significant).

Water and food intake, faeces weight, and urine volume, total solute, sodium and potassium excretion in four consecutive weeks are presented in Table 1. For most of the parameters measured the starting values (week 0) tended to be slightly higher in animals