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Uptake of amitriptyline and nortriptyline with liposomes, proteins, and serum: Implications for drug detoxification

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Abstract

Liposomes composed of DOPG and DMPC were studied for their ability to sequester amitriptyline and nortriptyline under physiological conditions. The liposomes reduced the free drug concentration in protein mixtures and in human serum, but the drug uptake efficiency of liposomes was reduced in the presence of plasma proteins, perhaps due to adsorption of proteins on the liposomes. The reduction was significantly more for the pure DOPG liposomes. The 50:50 DMPC:DOPG liposomes (0.72 mg lipid/mL) reduced the free amitriptyline concentration by 50–60% in the presence of 7% proteins (4% albumin (w/w), 2% fibrinogen (w/w), 1% globulins (w/w)). In human serum, the free drug reduction was 35–70% with the same 50:50 liposomes (0.72 mg lipid/mL). The liposomal systems were equally efficient at sequestering nortriptyline, which is a major metabolite of amitriptyline. The drug binding to liposomes in the presence of serum proteins is also quick and reversible and the likely mechanism of drug sequestration is adsorption of drug on the surface of liposomes. Accordingly, the drug uptake increases with increased charge and lipid loading. Even though the serum proteins reduced the effectiveness of the liposomes at sequestering the drug, the 50:50 DMPC:DOPG liposomes may be effective at treating amitriptyline overdose patients.

Keywords: Liposomes; Overdose; Amitriptyline; DMPC; DOPG; Protein; Albumin; Fibrinogen; Globulin; Isotherms

1. Introduction

Amitriptyline is a tricyclic antidepressant (TCA) commonly involved in overdose cases. As opposed to selective serotonin re-uptake inhibitors (SSRI), which are antidepressant medications developed in more recent years with milder side effects and higher toxic dosage levels, TCA's are often toxic at low dosages. This is especially true when taken by young children, where one or two pills can cause acute toxicity [1]. Furthermore, overdose cases involving TCA's can be fatal, and have been statistically shown to result in longer hospital stays than overdose cases with other antidepressant drugs [2]. Still, a large number of individuals continue to use TCA's to treat common depression disorders. Current methods of overdose treatment include gastric lavasion, dialysis, or activated charcoal. These methods have proven to be unsuccessful in many circumstances, due primarily to the large amount of time needed to render them effective and the tendency for amitriptyline to be highly bound to serum proteins. Accordingly, superior alternative methods of overdose treatment have been explored.

Previously, liposomes composed of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and 1,2-dioleoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (DOPG) were shown to sequester significant amounts of amitriptyline *in vitro* [3]. Effects of drug concentration, liposomal charge, and pH were explored, and it was shown that the charged liposomes can sequester almost 95% of amitriptyline at physiological concentrations. A majority of the experiments reported in [3] were conducted in PBS, which is not a good mimic for *in vivo* conditions. The goals of this study were to test the suitability of the charged liposomal systems at treating amitriptyline overdose under physiological conditions. The major difference between PBS and the blood is the presence of plasma proteins, which also bind a significant amount of drug. Thus, in this study we focused on the effect of plasma proteins on the sequestration, or temporary removal from solution by complexation, of amitriptyline by liposomes.

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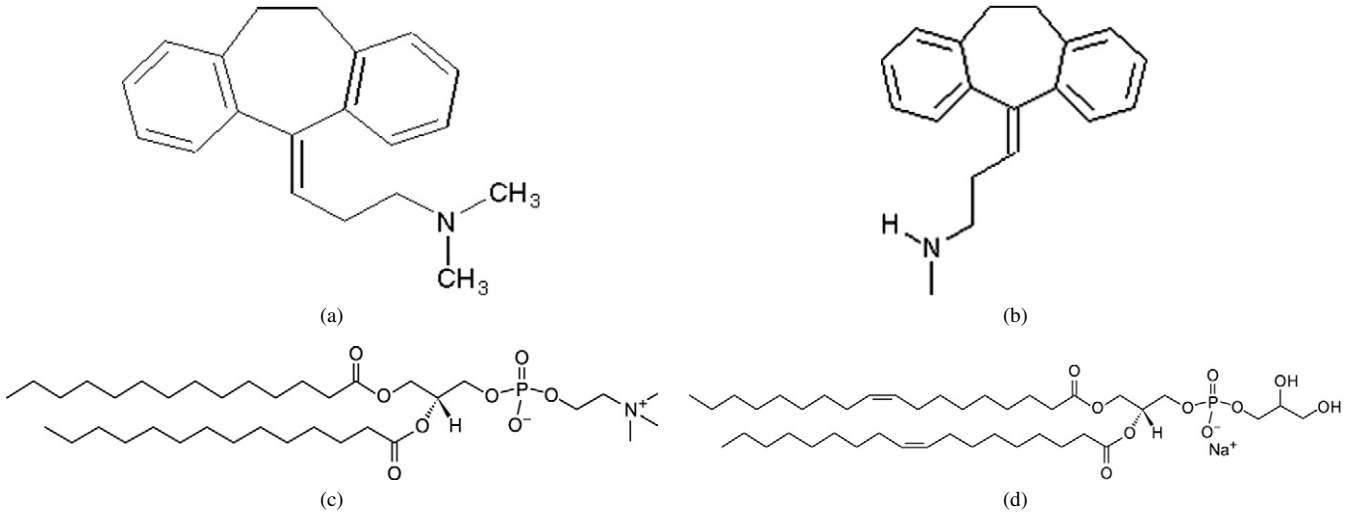

Fig. 1. Structures of drugs and lipids used for drug uptake studies with liposomes. (a) Amitriptyline; (b) nortriptyline; (c) 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) lipid; (d) 1,2-dioleoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (DOPG) lipid.

Additionally, we sought to understand the role of liposome–protein interactions in drug overdose treatment, and to apply that knowledge to further optimize our liposomal systems. Uptake of nortriptyline, the major metabolite of amitriptyline, was also investigated with similar liposomal suspensions. A primary characteristic of each species considered in this study was net charge. At the physiological pH of 7.4, both amitriptyline and nortriptyline exist predominantly in their positively charged forms. DMPC has no net charge, whereas DOPG bears a negative charge. Previous studies showed that liposomes composed of more negatively charged lipids sequestered more amitriptyline than those with neutral lipids [3]. Accordingly, we have studied liposomes composed of 50:50 molar ratio of DMPC and DOPG, as well as pure DOPG lipids. The structures of both drugs and both lipids are shown in Fig. 1. In addition to investigating the suitability of liposomes for overdose treatment, we have investigated binding isotherms for amitriptyline with albumin, fibrinogen, and globulins. These studies have revealed some very interesting behavior which is particularly evident at low drug concentrations, a regime which has not been explored in detail by previous investigators.

Several studies have been done in recent years involving drug detoxification with nanoparticles. These include the work of Varshney et al. [4,5], Underhill et al. [6], Petrikovics et al. [7], Deo et al. [8], and Fisar [9]. Perhaps the most relevant work to our research, published a short time ago, was done by Dhanikula et al. They have reported results from experiments using nanoparticles for the sequestration of amitriptyline and other drugs [10,11]. While their studies are similar to ours, there are also several important differences. In one study, they used oil-filled lipid nanocapsules for drug sequestration, rather than small uni-lamellar liposomes [10]. They hypothesized that the oil-filled nanocapsules bind to drug or other molecules based on oil–drug affinities, as opposed to the charge–charge interactions suspected to be important in our case. They also studied the uptake of haloperidol, docetaxel, and paclitaxel, rather than amitriptyline. Their nanocapsules were made from lipids other than DMPC or DOPG, which are the lipids used in our case. In a second study, they used spherulites and nanocapsules to do *in vitro* experiments involving amitriptyline, as well as *in vivo* experiments using amitriptyline-intoxicated rat hearts [11]. Spherulites are similar to nanocapsules and liposomes, with one key difference. They have numerous concentric bilayers surrounding their core, rather than a single layer. They reported a maximum amitriptyline uptake of $97.3 \pm 4\%$ in their *in vitro* experiments in the presence of 3% albumin. However, they used an initial amitriptyline concentration of roughly 0.20 μM for their *in vitro* experiments, which is below the reported therapeutic dose range for amitriptyline, and well below the concentration range of 1–3 μM reported to be relevant for overdose treatment [12]. Likewise, only 3% albumin was tested in their study, which is significantly smaller than the 7% protein present in human blood. Also, they did not investigate the effects of fibrinogen and globulins, which are also present in significant amounts in human blood. Perhaps most importantly, their lipid loading was 2.5 mg lipid/mL, whereas our maximum loading was only 0.72 mg lipid/mL. The primary mechanism for uptake in our study was hypothesized to be charge–charge interactions, whereas their uptake was driven by concentration gradients and/or pH gradients. Lastly, we also studied the uptake of nortriptyline, the most notable metabolite of amitriptyline.

2. Materials and methods

2.1. Materials

Methanol, chloroform, Dulbecco's phosphate buffered saline (PBS) without calcium chloride and magnesium chloride, bovine serum albumin (BSA), fibrinogen from bovine plasma, γ-globulins from human blood, human serum from male plasma, nortriptyline hydrochloride, and amitriptyline hydrochloride were purchased from Sigma Aldrich. 0.45 μm nylon syringe filters, YM30 centrifugation filters (30,000 molecular weight cut-off), and YM10 centrifugation filters (10,000 molecular weight cut-off) were purchased from Fisher Scien-