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Selection of DNA aptamers for digoxin and assaying of their binding profile and inhibitory effects

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Background and Aims: The aim of this study was designing DNA aptamers for digoxin, a widely-used drug, binding specific and selective with this cardiac glycoside.
Methods: Digoxin was coated onto the surface of streptavidin magnetic beads. DNA aptamers against digoxin were designed by Systematic Evolution of Ligands by Exponential enrichment method (SELEX) by 11 iterative rounds of incubation of digoxin-coated streptavidin magnetic beads with synthetic DNA library, DNA elution, electrophoresis and PCR amplification. The PCR product was cloned and sequenced. Binding affinity was determined using digoxin-BSA conjugate, coated onto ELISA plate. Inhibitory effect of anti-digoxin aptamer was conducted using isolated guinea-pig atrium.
Results: Three aptamers (D1, D2 and D3) were identified. Binding studies of fluorescein- labeled truncated (without primer binding region) D1 and D2 and full length D1 anti-digoxin aptamers were performed. Dissociation constants values were 8.2, 44.0 and 17.8 fmoles/µl, respectively. This is comparable to what other workers have obtained for interaction of monoclonal antibodies raised against digoxin. There was little difference in binding affinity between full length and truncated anti-digoxin D1 aptamer. D1 Anti-digoxin aptamer also inhibited the effects of digoxin on the isolated guinea-pig atrium. D1 Anti-digoxin aptamer distinguished between digoxin and ouabain in both tissue study and binding experiments.
Conclusions: Our finding indicated that D1 anti-digoxin aptamer can selectively bind digoxin. Further studies might show its suitability for use in digoxin assays and as a therapeutic agent in life-threatening digoxin toxicity.

Keywords: DNA aptamer; SELEX; Digoxin, Binding study