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**Desenvolvimento de nanodispersões lipídicas contendo insulina para
tratamento tópico da doença do olho seco**

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RESUMO

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A doença do olho seco (DOS) é uma desordem multifatorial relacionada a um processo inflamatório das estruturas oculares. Ela afeta a produção do filme lacrimal e resulta em feridas na córnea que são de difícil cicatrização. Estudos recentes demonstraram que a insulina (INS) facilita o processo de cicatrização de feridas e restaura a produção de filme lacrimal em ratos diabéticos. No entanto, a INS é uma proteína que degrada com facilidade em meio biológico, carecendo de estratégias que melhorem sua biodisponibilidade para sua administração ocular. Neste contexto, lipossomas podem ser um veículo promissor para liberação tópica ocular da INS devido a sua biocompatibilidade, baixa toxicidade e alto tempo de residência na superfície ocular. Sendo assim, o objetivo do presente trabalho foi desenvolver lipossomas contendo INS para uso tópico no tratamento da DOS. Pelo método de hidratação do filme lipídico e homogeneização em homogeneizador de alta pressão, foram preparados lipossomas compostos por fosfatidilcolina de soja (PC), colato de sódio (NaC) e poloxamer 188 em tampão fosfato salino (PBS) 10 mM pH 7,4. A INS foi adicionada ao tampão PBS, usado para hidratação do filme lipídico, na concentração de 0,4 mg/mL. Os lipossomas foram esterilizados por filtração em membrana 0,22 μ m e caracterizados quanto ao tamanho de partícula e índice de polidispersão (Pdl) por espalhamento dinâmico de luz, potencial zeta, pH, eficiência de encapsulação (EE,%) e recuperação do fármaco (RF,%). Lipossomas de 125 ± 3 nm, Pdl de $0,27 \pm 0,01$, potencial zeta de $-11 \pm 1,5$ mV, pH 7,1, EE $32 \pm 2\%$ e RF $82 \pm 12\%$ foram obtidos. Imagens de microscopia eletrônica de transmissão e de força atômica permitiram a observação de vesículas esféricas e assimétricas na presença de INS, com diâmetro médio variando de 120 a 150 nm. Estudos realizados por espalhamento de raios-X a baixo ângulo (SAXS) mostraram que a bicamada lipídica dos lipossomas contendo INS apresentaram simetria, com espessura de 42 Å, e INS dispersa em populações compostas por 6 a 15 monômeros com tamanho hidrodinâmico variável. Armazenados a 4°C, os lipossomas mantiveram suas características físicas estáveis por até 60 dias; e à temperatura ambiente, não houve degradação da INS por pelo menos 72 h. O ensaio *in vitro* de irritação ocular HET-CAM demonstrou baixo potencial irritante para as formulações sem e com INS. Estudos em cultura de células de epitélio de córnea humana (HCE) imortalizada demonstraram que o tratamento com as formulações contendo 1 UI de INS não foram citotóxicos e não promoveram aumento de MMP-9 em relação aos controles. Observou-se, no entanto, aumento na produção de IL-6, o qual pode contribuir para a cicatrização de feridas. Desta forma, os lipossomas demonstraram ser um sistema de liberação promissor para a administração tópica ocular da INS para DOS e cicatrização de feridas. Palavras-chave: Doença do olho seco (DOS). Insulina (INS). Lipossomas.

1 INTRODUÇÃO

A doença do olho seco (DOS), também denominada síndrome do olho seco, ou ainda *Keratoconjuntivitis sicca*, é uma doença multifatorial que afeta a integridade do filme lacrimal, estando associada a um processo inflamatório crônico na superfície ocular (COURSEY et al., 2015). Sintomas como desconforto e irritação, visão distorcida ou embaçada, sensibilidade à luz, dor e fadiga ocular são comuns em pacientes portadores da doença, a qual chega a afetar de 5 a 30% da população, tendo maior prevalência em pessoas acima de 65 anos (DRY EYE WORKSHOP, 2007; DING; SULLIVAN, 2012; COURSEY et al., 2015; AZIZI et al., 2016; CWIKLIK, 2016; LANZA et al., 2016).

De papel fundamental na homeostase da superfície ocular, o filme lacrimal fornece umidade, nutrientes e oxigênio para estruturas oculares como a córnea. De maneira geral, qualquer alteração no volume, composição, distribuição ou *clearance* lacrimal, leva à instabilidade na lágrima e à instalação da DOS, que induz a perda da integridade ocular (FUJI et al., 2013; CWIKLIK, 2016). Sendo considerada multifatorial, a DOS pode estar relacionada a doenças autoimunes, como Síndrome de *Sjögren* e *diabetes mellitus*, disfunções hormonais e até mesmo ao envelhecimento (DING; SULLIVAN, 2012; COLLIGRIS et al., 2014; CWIKLIK, 2016; SHEN; MA, 2016). Embora seja bastante prevalente na população, o tratamento para a doença ainda é realizado de forma paliativa. As chamadas “lágrimas artificiais”, colírios compostos por soluções iso ou hipotônicas são ainda o tratamento convencional. Embora aliviem os sintomas mais incômodos, colírios lubrificantes envolvem alta frequência de instilação e nenhuma eficácia clínica no controle da doença.

Fármacos anti-inflamatórios, como corticoesteróides, são amplamente utilizados via tópica para tratar as condições inflamatórias envolvidas na DOS severa. O uso desses fármacos pode inibir os vários mediadores envolvidos na fisiopatologia da doença e reduzir alguns sintomas presentes na doença. Porém, esse tipo de terapia traz inúmeros efeitos adversos, como por exemplo, o aumento da pressão intraocular e formação de catarata no segmento posterior, restringindo seu uso para casos mais severos e por pouco tempo (ERDINEST et al., 2014; NAKAMURA et al., 2015). Nos EUA, o uso de ciclosporina incorporada em uma nanoemulsão (Restasis®), foi recentemente aprovado pelo FDA para tratamento tópico da DOS. Porém, por possuir efeito imunossupressor, a sua absorção sistêmica traz riscos à

saúde, além de aumentar a susceptibilidade a infecções oculares (LAIBOVITZ et al., 1993). Sendo assim, uma nova terapia que pudesse combinar efeitos secretagogos a efeitos protetores na GL e superfície ocular seria uma alternativa promissora para os pacientes portadores de DOS.

Neste contexto, estudos recentes sugerem que a insulina (INS), o hormônio anabólico envolvido no metabolismo da glicose, tem efeitos secretagogos em glândula lacrimal (GL) de animais experimentais, protetor contra danos oxidativos em estruturas oculares (MODULO et al., 2009), além de auxiliar no processo de cicatrização de feridas de córnea, tanto em animais (BARTLETT et al., 1994; ROCHA et al., 2002; CUNHA et al., 2005; ZAGON et al., 2007; ALVES et al., 2008; YU et al., 2010), quanto em humanos (BASTION; LING, 2013). Apesar de não ter seu mecanismo elucidado nessas estruturas oculares, a INS possui receptores descritos em glândulas lacrimais, tecidos neurais e vasculares da retina, incluindo epitélio de córnea e conjuntiva (IM et al., 1986; ROCHA et al., 2002; MODULO et al., 2009; YU et al., 2010).

Sendo assim, considerando a relevância e a incidência cada vez mais crescente da DOS, e ainda a falta de estudos concretos a respeito de novas terapias para o tratamento desta desordem, estudos com formulações inovadoras contendo INS tornam-se promissores. A administração por via tópica dessas formulações é naturalmente preferida, mas apresenta desvantagens, como o rápido *clearance* lacrimal e o baixo tempo de residência na superfície ocular que resultam em baixa biodisponibilidade de fármacos, principalmente de macromoléculas (COURSEY et al., 2015; REIMONDEZ-TROITÍÑO et al., 2015). Contudo, com o avanço da nanotecnologia, surge a possibilidade de incorporação de fármacos peptídicos em sistemas de liberação nanoparticulados, aumentando sua biodisponibilidade e diminuindo a frequência de instilação destas formulações.

Neste contexto, os lipossomas são uma estratégia conveniente para a liberação de fármacos na região anterior ocular. Lipossomas são vesículas pequenas, de formato esférico, variando de 30 nm até alguns micrômetros, formadas, em sua maioria, pela associação de fosfolípidos biocompatíveis e colesterol (ALLEN; CULLIS, 2013). Quando hidratados em soluções aquosas, os fosfolípidos formam estruturas vesiculares que consistem em uma ou mais bicamadas lipídicas fechadas em torno de um núcleo aquoso, semelhantes às membranas celulares (AKBARZADEH et al., 2013). Por serem sistemas formados por fosfolípidos

biocompatíveis e biodegradáveis, podem ocorrer interações entre as vesículas lipossomais e os lipídeos presentes na membrana celular epitelial e na camada lipídica do filme lacrimal. Essas interações podem levar a um aumento do tempo de residência da formulação na superfície da córnea, possibilitando a liberação direcionada da INS para os receptores aí presentes, aumentando sua biodisponibilidade local (NIU et al., 2011; HU et al., 2013; HOFAUER et al., 2013; CUI et al., 2015; ELNAGGAR, 2015). Do mesmo modo, a formulação lipídica pode favorecer a lubrificação da superfície ocular, formando uma camada de proteção contra a evaporação do fluido lacrimal (SOUZA et. al., 2013; FANGUEIRO et. al., 2014; SEVERINO, et. al., 2014; FANGUEIRO et. al., 2015).

Logo, uma formulação que seja capaz de associar nanodispersões lipossomais à INS demonstra ser uma estratégia inovadora e promissora para a terapia da DOS, podendo trazer além do alívio aos sintomas, restauração da homeostase da superfície ocular, aumentando, sobretudo a qualidade de vida dos pacientes.

7 CONCLUSÕES

Diante dos resultados obtidos e discutidos no presente trabalho, pode-se concluir que os lipossomas desenvolvidos para encapsulação da INS, obtidos a partir da associação de PC e NaC, são sistemas promissores para o tratamento da DOS, uma vez que apresentaram características físico-químicas e estabilidade física adequadas para a administração ocular. Os estudos de segurança, citotoxicidade, irritação ocular e potencial inflamatório em cultura de células de epitélio de córnea humana também se mostraram promissores, à medida que os lipossomas apresentaram baixo potencial irritante em membrana CAM, não causaram morte celular e nem potencializaram o processo inflamatório instalado em um modelo *in vitro* de inflamação da superfície ocular mediada por LPS. A INS demonstrou não estimular a expressão de MMP-9 neste modelo, sugerindo um papel importante no tratamento da DOS, enquanto que o aumento na IL-6 pode trazer benefícios para o processo de cicatrização de feridas de córnea. Os estudos sugerem que esses lipossomas podem vir a ser testados *in vivo* para avaliação de seu potencial clínico para tratamento da DOS e auxílio no tratamento de feridas de córnea, sendo uma alternativa promissora às terapias existentes hoje.

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