Generation of soluble antibodies against human tissue kallikrein 7 and the evaluation of their biopharmaceutical use with a poloxamer-based hydrogel drug delivery system

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May 5, 2020

Abstract

Human tissue kallikreins (KLKs) constitute a family of 15 serine proteases scattered in various tissues of the human body. They are implicated in several pathological disorders and physiological events, such as cancer, the formation of blood clot, and skin desquamation, among others, and therefore, it is believed that specific inhibitors of these enzymes may represent new therapeutic targets. KLK7 is an unusual serine protease that presents both trypsin-like and chymotrypsin-like specificity and seems to be upregulated in pathologies related to skin desquamation processes such as atopic dermatitis, psoriasis and Netherton syndrome, suggesting that this enzyme is a potential target for new therapeutic procedures. Thus, in this work, we describe the generation of recombinant IgG-like human antibodies (scFv-Fc) with high affinity for KLK7, which can be useful for detecting and neutralizing the activity of this enzyme in tissues where it appears to be upregulated. In addition, we have developed and characterized a poloxamer-based drug delivery system to encapsulate the anti-KLK7 antibodies considering the biopharmaceutical parameters of the antibodies.

1 INTRODUCTION

Proteases are enzymes essential to the survival of organisms, representing 2 to 4% of the whole proteome (Patel, 2017). Serine proteases represent approximately one-third of all proteases identified until 2009 (Cera & Di Cera, 2009); they are arranged in several clans and families; human tissue kallikreins (KLKs) are serine proteases in the PA clan (Patel, 2017). These enzymes are detected in many tissues and biological fluids at the protein and mRNA levels (Diamandis,*et al* ., 2000; Turk, 2006). They are in the biggest protease gene cluster in the human genome (Paliouras, Borgono, & Diamandis, 2007; Prassas *et al* ., 2015) and show important similarities in terms of their tertiary structures and protein and gene levels (Shaw & Diamandis, 2007). This family is composed of 15 genes*in tandem* in the long arm of chromosome 19q13.3-13.4, where a pseudogene can also be found (Avgeris, Mavridis, & Scorilas, 2012; Kryza *et al* ., 2016). KLKs can be detected in a wide variety of tissues, such as those of the skin, central nervous system, salivary glands and biological fluids, including serum and seminal plasma. The roles of many KLKs (KLK4 – KLK15) have not been completely elucidated, but studies suggest that they can play roles in processing peptide hormones within the pancreas (Komatsu et al., 2007) and the nervous system (Blaber *et al* ., 2005) in dissolving the blood coagula (Borgoño, Michael, & Diamandis, 2004), in maintaining epidermal homeostasis (Ekholm & Egelrud, 1999) and in tooth development (Wang*et al* ., 2009).

KLKs 5 and 7 participate in the process of epidermal homeostasis, particularly in skin desquamation (Wang *et al* ., 2009); these enzymes are responsible for the cleavage of corneodesmosomes, structures involved in cell cohesion (Lundwall & Brattsand, 2008). KLK7 is secreted directly into the intracellular space, where

it cleaves desmocollin 1 and corneodesmosin (Ishida-Yamamoto *et al* ., 2004). It has been shown that, in dermatologic pathologies such as atopic dermatitis (Voegeli *et al* ., 2011), psoriasis (Komatsu *et al* ., 2006) and Netherton syndrome (Komatsu *et al* ., 2008), KLK7 activity is higher than it is in normal skin. It is clear that dysregulation of KLK activity in the *stratum corneum* leads to pathological conditions such as atopic dermatitis (Igawa *et al* ., 2017; Komatsu *et al* ., 2006; Voegeli *et al* ., 2011), psoriasis, lichen planus, lichen sclerosus and poroketarosis (He*et al* ., 2017; Jonca *et al* ., 2011; Pampalakis & Sotiropoulou, 2007). In addition, a mutation in the SPINK5 gene, encoding the LEKTI inhibitor responsible for the inhibition of KLK5 and KLK7 activities in epidermis and other stratified epithelia, leads to Netherton syndrome (Chavanas *et al* ., 2000; Descargues *et al* ., 2005; Ishida-Yamamoto & Igawa, 2015). In this way, it has been noted that inhibition of KLK7 by exogenous molecules represents a new potential strategy for therapeutic treatment of these skin diseases.

Phage display is a technique first described by Smith in 1985 (Smith, 1985), and antibody phage display was developed in 1990–91 (Breitling *et al* ., 1991; McCafferty *et al* ., 1990). This technology enables the generation of human antibodies for research, diagnosis, therapy and *in vitro*selection processes independent of the restriction imposed by an*in vivo* immune response (Bradbury *et al* ., 2011; Schirrmann*et al* ., 2011). In 2016, six antibodies derived from phage display were EMA/FDA approved (Frenzel, Schirrmann, & Hust, 2016), and three years later, 12 antibodies generated by phage display were approved. In this work, we used the human naïve scFv phage display libraries HAL9/10 (Kügler *et al* ., 2015) to generate soluble recombinant antibodies capable of specifically inhibiting the proteolytic activity of KLK7.

Drug delivery systems are used to target cells, tissues and other targets for therapy with different kinds of compounds (Tibbitt, Dahlman, & Langer, 2016). Poloxamers (PLs) were first described in 1950 (Koffi*et al* ., 2006) as emulsifiers for pharmaceutical formulations. PLs are composed of ethylene oxide and propylene oxide units arranged in a triblock structure of the A-B-A type. They are found as registered brands (Pluronic®, Synperonic®, Tetronic®) and in many forms (Batrakova & Kabanov, 2008). Because of their amphipathic characteristics, they are able to encapsulate both hydrophobic and hydrophilic compounds, suggesting their value as drug delivery systems (Dumortier *et al* ., 2006); in addition, studies have shown that poloxamers enhance the pharmacodynamics and pharmacokinetics of the encapsulated agents (Akkari*et al* ., 2016; dos Santos *et al* ., 2015).

In this study, we report the use of a phage display to generate recombinant human antibodies against KLK7, which can inhibit the action of the target protease, and their incorporation into unique PL-based and binary hydrogels composed of PL407 and PL403, where they are characterized, in part, by considering the differences in their hydrophilic-lipophilic balance. All of these systems were characterized by their micelle interaction, rheological properties capacity for release *in vitro* , inhibition effectivity and cytotoxicity.

2 MATERIALS AND METHODS

2.1 Antibody generation

Antibodies against recombinant KLK7 were selected in scFv format from the human *naïve* antibody gene libraries HAL9/10 (Kügler *et al.* , 2015). Recombinant KLK7 was produced as described previously (De Souza *et al* ., 2013; Teixeira *et al.* , 2011). The selection and screening were performed as described before (Russo *et al* ., 2018). Recombinant KLK7 was immobilized on Costar high-binding microtiter plates (Sigma-Aldrich), and scFv phage from the HAL9/10 libraries was added to microtiter plates. Panning was performed at 37 °C. After three panning rounds, the soluble scFv antibodies were produced and screened for KLK7 binding by antigen ELISAs. The DNA of each binding candidate was isolated and sequenced. The unique scFv sequences were recloned into pCSE2.6-hIgG1-Fc-XP (Beer *et al* ., 2018) using NcoI/NotI (New England Biolabs Inc.) for production as an scFv-Fc antibody, which is an IgG-like antibody format. The production in HEK293-6E cells and subsequent protein A purification was performed as described (Jäger *etal* ., 2013). After the production and purification the IC_{50} values for the scFv-Fc antibodies were determined (Sebaugh, 2011).

scFv-Fc antibodies with lower IC_{50} values were selected to undergo an affinity maturation protocol to enhance

their specificity (Hust, Jonas, & Tomszak, 2018). After the affinity maturation, the new scFv antibodies were selected; these molecules were re-produced and screened for KLK7 binding by antigen ELISA as described above. The new candidates underwent the same process as the precursor antibodies, being re-cloned into pCSE2.6-hIgG1-Fc-XP for production and purified with protein A (Beer *et al* ., 2018; ·(Jäger et al., 2013). The IC_{50} values were determined for the new scFv-Fc antibodies.

2.1.1 Inhibitory assays of the scFv-Fc antibodies against KLK7

All enzymatic reactions were performed in 50 mM Tris-HCl buffer (pH = 7.5) in a Hitachi F2500 spectrofluorometer (wavelengths of excitation and emission = 320 and 420 nm, respectively). The scFv-Fc molecules were diluted to the appropriate concentration prior to the assays. These molecules were screened against KLK7 at an initial concentration of 10 μM to verify the degree to which they inhibit the targeted enzyme. The quartz cuvette was filled with 900 μL of the assay buffer, 0.2 μg of recombinant KLK7 and the selected scFv-Fc antibody and incubated for 5 minutes at 37 ºC. The reactions were started by the addition of the FRET substrate Abz-KLYSQ-EDDnp (GenOne Biotechnologies). Control assays were performed without the inhibitors (negative control). The IC_{50} values were independently determined by performing rate measurements for at least five concentrations of inhibitor, ranging from 0.1 nM to 1.0 μM. The values are presented as the mean ± SD of three individual experiments. The IC_{50} values were calculated by the GraFit 7 program.

2.2 Physicochemical characterization of the drug delivery system

2.2.1 Micelle and hydrogel preparation

Poloxamers (PLs) were acquired from Sigma-Aldrich. For micellar solution preparation, 3% (m/v) PL 407, alone or in a binary system with PL 403, was solubilized in ultrapure water and kept at 4 ºC while stirred magnetically (150 rpm) until a transparent solution was obtained (Schmolka, 1972). In the same manner, for hydrogel preparation, two formulations were standardized: formulation 1 (F1), composed of 30% PL407 and formulation 2 (F2), 2% PL403 and 28% PL407 PL were dispersed in deionized water until they were complete dissolved.(Akkari *et al* ., 2016) The antibodies were dispersed into the formulations at a final concentration of 0.1% (m/v). The formulations were stored at 4 ºC until use.

2.2.2 Micellar hydrodynamic diameter: antibody-micelle interaction

The micellar hydrodynamic diameter and the size average distribution were determined using a Zetasizer ZS particle analyzer (Malvern Instruments Ltd.) at a fixed angle of 173º and temperatures of 25 ºC and 32.5 ºC to simulate the micelle behavior at room temperature and physiological skin temperature. All samples were analyzed before and after antibody incorporation. All measurements were repeated at least three times for each sample.

Rheological analysis

Rheological analysis was carried out on a Kinexus rotational rheometer (Malvern Instruments Ltd.), using cone-plate geometry (40 mm diameter size). For the sol-gel transition temperature (Tsol-gel) determination, measurements were performed at a temperature that ranged from 10 to 50 ºC using a sample volume of 1 mL, a gap between the plates of 1 mm, a frequency of 1 Hz and shear stress of 2 Pa. Additionally, a frequency sweep analysis ranging from 0.1 to 10 Hz (at 32.5 °C) was performed. All measurements were used to determine the parameters related to the elastic modulus (G´), the viscous modulus (G“) and viscosity (η). RSpace for Kinexus software was used for the data analysis.

2.3 *In vitro* release assay

In vitro release assays were performed to evaluate the amount of antibody released from the PL-based hydrogel formulations that are effectively available to inhibit recombinant KLK7. To perform this experiment, a glass mold was connected to the bottom of a 40-mL beaker and maintained at 32.5 ºC, as previously described (Akkari *et al* ., 2016). One milliliter of each formulation was inserted into the glass mold, and as soon as they turned into gel, the beaker was filled with 40 mL of 0.9% saline buffer. A magnetic stirrer was placed inside the beaker, and the system was maintained at 32.5 ºC for 24 hours under magnetic stirring

(350 rpm). The samples were collected at different time intervals (30 minutes and 1, 2, 4, 6, 8 and 24 hours), and the same volume that was removed was replaced after each collection.

The procedure for quantifying the antibodies is presented in the supplementary material.

2.4 Inhibition assay

The inhibition assay was designed to evaluate whether the percentage of antibody released would have a significant effect on inhibiting recombinant KLK7 *in vitro* .

The assay was performed using the FRET substrate Abz-KLYSQ-EDDnp (GenOne Biotechnologies). Recombinant KLK7 (5 ng/uL) diluted in phosphate-buffered saline (pH = 7.4) was added to Greiner CELLSTAR® black polystyrene 96 flat wells (Greiner Bio-One). Each well was filled with 100 µL of assay buffer (50 mM Tris, pH = 7.5). Recombinant KLK7 and the assay buffer were incubated for 2 minutes at 37 ºC prior to the addition of the substrate. The substrate (1 mg/mL) was added to the wells. The total read time was 15 minutes at 37 ºC under agitation. The positive control was only KLK7; to determine the residual activity of KLK7 against the antibodies, 100 µL of the aliquots taken at 6, 8 and 24 hours was added prior to the addition of the substrate. All readings were carried out with a Hitachi F 2500 fluorometer (Hitachi Science & Technology); the excitation and emission slits were adjusted to 5 nm and wavelengths were set to 380 and 460 nm. All reads were performed in triplicate.

2.5 Cytotoxicity analysis

The Vero cell lineage, established from the kidney cells of the African green monkey (*Cercopithecus aethiops*) (CCIAL 057, Adolfo Lutz Institute, São Paulo), was used for the *in vitro* experiments. The Vero cells were cultured in Ham F-10 medium (Sigma-Aldrich), with fetal bovine serum (10%; Sigma-Aldrich) and 100 µg/mL penicillin/streptomycin, and the culture was maintained at 37 °C with 5% CO_2. Assays were performed in triplicate, according to ISO10993-5 (2009).

For this assay, the antibodies were diluted in Ham F-10 medium (Sigma-Aldrich) with concentrations ranging from 2 mg/mL to 0.0625 mg/mL.

For the MTT assay, the Vero cells were seeded in 96-well plates (Corning) at a density of 1.5 x 10^6 cells/well. After 24 hours, the culture medium was removed and substituted with 100 µL of the diluted antibody solution. The plate was incubated for 24 hours, and then, the cell morphology was analyzed. The analysis was performed with phase contrast in an inverted microscope (Axiovert A1 Axioscope, Zeiss).

After the morphologic analysis, 100 µl of 10% MTT solution (5 mg/ml, Sigma-Aldrich) in phosphate-buffered saline was added to each well, and the cells were incubated for 2 hours at 37 ºC. After removing the MTT solution, 50 µL of dimethyl sulfoxide (Synth) was added to each well for 10 minutes, and the absorbance was measured with an automated spectrophotometric microtiter plate reader (SpectraMax M5) using a 570 nm filter.

2.6 Statistical analysis

Each experiment was carried out in triplicate unless otherwise specified. All results are presented as the mean ± standard deviation (SD). The experimental data were analyzed using GraphPad Prism software, version 6.0, and Microsoft Excel. For comparisons among more than two groups, ANOVA (one-way and two-way) and the post hoc Tukey test. To determine the significance between two groups, Student's t-tests were performed. Statistical significance was set at a p-value [?] 0.05.

3 RESULTS AND DISCUSSION

3.1 Antibody generation

After the panning procedures using the HAL9/10 libraries, the scFv antibodies were produced in XL1-Blue MRF' *E. coli,* and ELISA assays were performed to select the soluble antibody fragments that bind to KLK7 (figure 1). Seventeen antibody clones were selected with interaction values higher than 0.1. These clones

were sequenced, and it was found that many sequences were repeated or truncated, resulting in 9 unique functional scFv sequences against KLK7.

All nine selected scFv antibodies were produced in the scFv-Fc format and tested against recombinant KLK7 to determine the IC_{50} value. LUP-14G10 showed a powerful inhibitory response against recombinant KLK7, with a potency of 2.3 nM (figure 2).

To select the strong inhibitors, the clone with the lowest IC_{50} (LUP-14G10) was subjected to an affinity maturation process, which involves replacing its light chain fragment with a library of light chain fragments to generate a new LUP-14G10-based library. This new library was submitted to the panning procedures using KLK7 as the antigen. After the off-rate panning procedure was completed, four new scFv antibodies against KLK7 (LUP-37A10, LUP-37B10, LUP-37C11 and LUP-37D11) were selected with higher affinity for the target protease than that exhibited by LUP-14G10. These new scFv antibodies were produced in mammalian cells in the scFv-Fc format and purified with a protein A column. After production, they were also assessed for their inhibitory potential towards recombinant KLK7 (figure 3). Antibody LUP-37B10 displayed an IC_{50} value above that observed for the original antibody (6.2 nM); for that reason, we did not use this antibody in the other experiments.

Many molecules are capable of inhibiting KLK7, as described in the literature (Arama *et al.,* 2015; Freitas *et al* ., 2012; Jendrny & Beck-Sickinger, 2016; Oliveira *et al* ., 2014). Molecules reported by Oliveira *et al* . and Freitas *et al* ., based on isomannide, showed the highest IC_{50} values, ranging from 10.2 to $>$ 1000 μM (Oliveira *et al* ., 2014) and 13.5 to 205.2 μM (Freitas *et al* ., 2012), respectively. A more recent study by Jendrny and coworkers found compounds able to inhibit KLK7 at very low values (0.6 – 1.1 μM) based on a sunflower trypsin inhibitor (Jendrny & Beck-Sickinger, 2016). Although there are many other molecules described for inhibiting KLK7 with different inhibition constant values, ranging from medium nanomolar up to high micromolar, there are no reports about the use of the diverse universe of recombinant antibody fragments as inhibitors for KLK7. In this work, we present at least five new recombinant antibodies with the capacity to inhibit the target protease with an inhibition constant in the very low nanomolar range, which may be a new, powerful way to generate inhibitors for these family of proteases.

As the aim is the generation of inhibitors for human tissue kallikreins to be used as archetypes for the future development of new therapeutic agents for the pathologies in which KLKs appear to be related, we also decided to investigate the possibility of encapsulating these scFv-Fc antibodies against KLK7 in a poloxamer-based system of drug delivery.

3.2 Physicochemical characterization of the drug delivery system

3.2.1 Micellar hydrodynamic diameter

In table 1, the data for the hydrodynamic diameters (nm) and average distribution patterns (%) are available. These data refer to the DLS technique used to observe the effects of temperature, micelle composition and drug incorporation that is based on micellar hydrodynamic diameter and size distribution parameters.

Regarding the micelle population for both formulations, at 25 ºC, a population of micelles with diameters $<$ 5 nm was found in all compounds (4.52 ± 0.55 nm with intensity = 5.2% and 4.73 ± 0.23 nm, with intensity = 3,1% for F1 and F2, respectively). At 32.5 ºC, no other populations were found.

For both formulations, the increase in temperature promoted a decrease in the micellar diameter, which was being more pronounced for LUP-37D11 in formulation F1 (25.61%) and for LUP-37A10 in formulation F2 (32.53%). Reductions in the micellar hydrodynamic diameter were directly related to the increase in temperature, which was promoted by the dehydration of the propylene oxide (PPO) units in the micellar core (Freitas Mariano *et al* ., 2019; Zhang, Lam, & Tan, 2005). The increase in temperature also influenced the hydrophobicity of the micellar core, favoring the formation of colloidal systems with low polydispersity (Freitas Mariano *et al* ., 2019;·(Su, Wang, & Liu, 2002). Similar results were reported on the influence of temperature on PL407 micelles only (Akkari*et al* ., 2016; dos Santos *et al* ., 2015; Freitas Mariano*et al* .,

2019; Nascimento *et al* ., 2018) There was one report in which PL407 was combined with PL403, and it confirmed the results shown in this work.(Freitas Mariano *et al* ., 2019)

3.2.2 Rheological analysis

Rheological analysis was performed for all formulations based on the different micelle compositions and the presence or absence of antibodies, and it was possible to determine the elastic (G´) and viscous (G´´) moduli, viscosity and temperature where the more pronounced viscosity variation was observed and was considered the sol-gel temperature (Tsol-gel). Table 2 provides all the information from the rheological analysis.

For topical delivery, the formulations of the gel should be at skin and body temperature (32 – 36 ºC) while existing as a solution at room temperature (Ban *et al* ., 2017). All formulations meet this criteria; the highest gelation temperature being 20.04 ºC. The Tsol-gel (ºC) did not show significant changes independent of the addition of scFv-Fc antibodies to the PL-based formulations. The results support reports in the literature, where high PL407 concentrations indicated Tsol-gel formation between 25 and 33 ºC (Dorraj & Moghimi, 2015; Freitas Mariano *et al* ., 2019).

The behavior of the hydrogels was temperature-dependent, as observed for more gels with the more pronounced viscosity values at 32.5 ºC compared to those of the gels at 25 ºC. However, the G´ values were lower in the binary formulation (F2), showing that even a small amount of PL403 could change the rheological parameters. The differences between G´ and G´´ in the binary system could be attributed to the physicochemical properties of PL403 in relation to those of PL407. PL403 had a lower HLB value (8) compared to that of PL407 (22) (Freitas Mariano *et al* ., 2019).

The results obtained agree with those of other studies: a hydrogel that is applied on the skin should be in a solid state. For topical formulations, the hydrogel should exhibit sufficient viscosity (6000 – 7000 cps) (Freitas Mariano *et al* ., 2019;ˈBan*et al.* , 2017;ˈPillai & Panchagnula, 2003). For all the formulations, the viscosity increased with increasing temperature, and the elastic modulus was greater than the viscous modulus.

3.2.3 *In vitro* liberation and inhibition assays

The three selected antibodies were analyzed for liberation from encapsulation, and the results are shown for the two proposed formulations in figure 4.

It is possible to observe that at least ˜20% of the antibodies in both formulations were liberated from the micelles, as they were detected by the indirect ELISA assay. In general, formulation F2, the more hydrophobic formulation, showed the capacity to interact with the antibodies in a stronger way than F1, explaining the release profile observed. After 24 hours, the concentrations of the antibodies were ˜1.10 nM for LUP-37A10 in both formulations, ˜0.70 and ˜1.12 nM for LUP-37C11 in formulations F1 and F2, respectively, and ˜0.91 and 1.30 for LUP-37D11 in formulations F1 and F2, respectively.

We assessed the residual inhibitory activity of the antibodies released from the micelles after 6, 8 and 24 hours. The results are shown in figure 5. For LUP-37A10, 1.10 nM of antibody was able to inhibit approximately 90% of the recombinant KLK7 activity in formulation F1, while in formulation F2, the antibody inhibited ˜60% of the enzyme activity. For LUP-37C11, in formulation F1, 0.7 nM of antibody inhibited more than 95% of the KLK7 activity. In formulation F2, 1.12 nM of this antibody inhibited ˜80% of the KLK7 activity. For the LUP-37D11 formulation, F1 might have changed the antibody's kinetics; that is, ˜1.10 nM inhibited no less than 20% of the KLK7 activity, while in F2, ˜1.30 nM inhibited more than 85% of the enzyme activity.

3.2.4 Morphology and MTT assays

After all these tests, we also decided to perform a cytotoxicity test to investigate the reliability of these antibodies for use in potential*in vivo* therapy. Vero cells were used for cytotoxicity assays, because they are the standard cell used in international studies with biomaterials, and they have fibroblast-like features (Lombello, Malmonge, & Wada, 2000; Nascimento *et al* ., 2017). Vero cells presented a morphological pattern very similar to the growth pattern of the cells on the culture plate, forming a confluent monolayer of

cells with predominantly elongated morphology, thereby indicating no cytotoxicity induced by the antibodies.(Nascimento et al., 2017) It was also observed, through the images obtained by light microscopy, that the core presented with one or more nucleoli and vacuoles and had proliferated to a high cell density.

The results of the MTT assay for cell viability confirmed the noncytotoxic characterization of the antibodies. The principle of the method was to reduce a yellow biomaterial, salt tetrazolium MTT, by mitochondrial enzymes, resulting in a blue/purple product called formazan, which may be quantified by spectrophotometry. Thus, this reaction occurs only in living cells and is activated in the mitochondria, thereby serving as a versatile and quantitative method for assessing cell viability (Mosmann, 1983; Shen *et al* ., 2010).

Figure 6 and 7 how the results of the cytotoxicity and morphology assays, respectively. Figure 6 shows that the antibodies did not show cytotoxic activity in the Vero cells. The cell viability remained greater than 80% for all the antibodies tested in all formulations. Figure 7 presents the morphological effects of the antibodies on the Vero cells. It was possible to observe that the cells had conserved their fibroblast-like features.

The cytotoxicity induced by the scFv-type antibodies is mostly studied in multiple types of cancer, specifically for tumor targeting (Hutt*et al* ., 2018; Klement *et al* ., 2017; Marty & Schwendener, 2005; Singh *et al* ., 2007; Sokolowska-Wedzina *et al.* , 2017); in these cases, it is interesting that antibodies display certain cytotoxic effects towards the cancer cells. In our case, however, the antibodies should be capable of only inhibiting the enzyme without inducing cytotoxicity. Little is known about the effects of the scFv-Fc antibodies alone; however, Hutt *et al* . were able to demonstrate cytotoxic activity of scFv-Fc-scTRAIL fragments, which promoted cell apoptosis (Hutt *et al* ., 2018), and Sokolowska-Wedzina *et al* . were able to conjugate a scFv-Fc antibody with a cytotoxic drug for delivery to lung cancer cells (Sokolowska-Wedzina *et al* ., 2017).

4. CONCLUSIONS

Using the HAL9/10 libraries, we were able to select antibodies with high affinity for KLK7, and they also showed a high capacity to inhibit the proteolytic activity of this targeted enzyme. These recombinant antibodies were incorporated in a drug delivery system that was generated from poloxamers, and we demonstrated the viability of the system to encapsulate and deliver the antibodies, showing satisfactory pharmacological parameters for LUP-37A10 and LUP-37C11 in formulation F1 and LUP-37D11 in formulation F2. Our work has combined phage display and drug delivery methodologies to generate new molecules and formulations with potential for use in therapies for the pathologies in which KLK7 appears to be involved.

5. ACKNOWLEDGMENTS

The authors are grateful to the staff of Central Multiusuário of Universidade Federal do ABC for technical assistance.

The authors declare that they have no conflict of interest.

6. FUNDING

FAPESP process 2016/21441-3, CAPES and UFABC.

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