Interaction between hydroxyethyl starch and propofol: computational and laboratorial study

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Background: Hydroxyethyl starch (HES) is one of the most used colloids for intravascular volume replacement during anesthesia. Aim: To investigate the existence of a chemical interaction between HES and the anesthetic propofol by in vitro propofol dosing, computational docking, and examination of a complex between propofol and HES by infrared (IR), ultraviolet (UV), and 1H and 13C nuclear magnetic resonance (NMR) spectroscopy. Methods: Ten samples with human plasma mixed with HES or lactated Ringers (n = 5 for each fluid) were prepared, and the propofol free fraction was quantified until 50 min, using gas chromatography-mass spectrometry. The docking study was performed between HES and propofol and compared with controls. The binding affinities between HES and the small molecules were evaluated by binding free energy approximation (ΔGb, kJ mol⁻¹). The IR, UV, and NMR spectra were measured for propofol, HES, and a mixture of both obtained by the kneading method. Results: Propofol concentrations were significantly lower in the HES samples than in the LR samples (p = .021). The spectroscopic characterization of propofol combined with HES revealed differences in spectra and docking studies reinforced a potential interaction between propofol and HES. Conclusions: Propofol and HES form a complex with different physical-bio-chemical behavior than the single drugs, which may be an important drug interaction. Further studies should evaluate its clinical effects.

Keywords: hydroxyethyl starch; propofol; magnetic resonance spectroscopy; infrared spectroscopy; ultraviolet spectroscopy

1. Introduction

Propofol (2,6-diisopropylphenol) is one of the most used general anesthetics worldwide. During general anesthesia, different fluids may be used for intravascular volume replacement. Currently, there is a debate of whether crystalloid or colloid fluids should be used, as colloids have faster and more prolonged action than crystalloids but have some drawbacks (Cohen, 2013). Hydroxyethyl starch (HES) 130/0.4 is one of the most used colloids due to its minimized adverse effects on coagulation (Mizzi et al., 2011) and improved tissue microcirculation (Hoffmann et al., 2002) when compared to other colloids.

The choice of crystalloids or colloids may result in different effects on propofol pharmacokinetics. Lower propofol free fraction was observed in samples containing HES when compared to a crystalloid in a previous laboratory study (Dawidowicz & Kalitynski, 2005). A study in experimental pigs also showed significantly lower propofol plasma concentrations in animals that received HES 130/0.4 following severe bleeding than those that received the crystalloid Lactated Ringer’s (LR) under the same conditions (Silva et al., 2012).

The chemical structure of both drugs (Figure 1) allows us to speculate of a possible interaction between propofol and HES, similar to that previously observed between propofol and cyclodextrins (Wallentine, Shimode, Egan, & Pace, 2011) or between HES and other small molecules such as hexanol and hexanal which form inclusion complexes with starch (Jouquand, Ducruet, & Le Bail, 2006). This possibility has been also suggested after two episodes of propofol anesthesia in human patients, in which the administration of a bolus...
of HES seemed to stabilize bispectral index of the electroencephalogram (Ferreira, Santos, Nunes, Amorim, & Antunes, 2005).

The existence of such interaction may have important clinical implications as HES is often administered to patients receiving propofol anesthesia and could alter the pharmacokinetics and pharmacodynamics of one or both drugs.

We hypothesized that chemical interactions could occur between propofol and HES, causing a decrease in propofol free fraction. This interaction was studied in three phases: (i) an *in vitro* assay was performed to compare the plasma concentrations of propofol mixed with a crystalloid or HES 130/0.4; (ii) putative interactions between these two drugs were analyzed by computational docking studies using HES as macromolecule; and (iii) a complex of propofol and HES obtained by the kneading method was examined in the solid state by infrared (IR), ultraviolet (UV), and ¹H and ¹³C nuclear magnetic resonance (NMR) spectroscopy.

2. Materials and methods

2.1. *In vitro* assay

Healthy plasma samples, surplus from blood donation, were obtained from the Portuguese blood bank from the Clinical Hematology Service of Centro Hospitalar do Porto/Hospital de Santo António, Porto. The assay consisted in the preparation of two sets of samples: (a) human plasma, mixed with pure propofol (Sigma-Aldrich, Sintra, Portugal) and HES 130/0.4 (Voluven, Fresenius Kabi, Portugal) and (b) human plasma, mixed with propofol and LR (BBraun, Queluz de Baixo, Portugal).

The mixtures were performed in 14 ml tubes with 5 ml plasma, and 5 ml of HES or LR. Five replicates of each solution were used. After mixing all the tubes and warming them at 37°C for 5 min, 200 μg of propofol was added to each tube to produce a final concentration of 20 μg/ml. The tubes were vortexed and the sampling started. Samples of 5 ml were extracted from each tube right after the tubes were vortexed (T₀), and 10, 20, 30 and 50 min after. The addition of propofol and the sampling were performed always in the same tube order to keep the time intervals constant, but the initial order chosen for the addition of propofol was random.

Propofol free fraction was quantified by GC-MS as previously described (Guitton et al., 1995). Propofol (2,6-diisopropylphenol) and internal standard (thymol-2-isopropyl-5-methylphenol) were obtained by Sigma-Aldrich (Portugal). Calibration curves were prepared in human plasma. Each analyte prepared from the stock solutions was added to the blank plasma. The final concentrations of the standard solutions were: 0, .5, 1.0, 2.0, 4.0, 5.0, 10.0, 20.0, and 50.0 μg/ml.

2.2. Computational docking studies

2.2.1. HES model construction

As the 3D structure of HES is not directly available in online databases, a 10-monomer model of that polymer was built by the conversion of the primary sequence into a 3D molecular model by linking together preconstructed 3D molecular templates of monosaccharides in the manner specified by the sequence. The obtained structure was minimized using semi-empirical Polak–Ribiere conjugate gradient method (RMS < .1 kcal Å⁻¹ mol⁻¹) (Zhang, Zhou, & Li, 2006) and further refined by molecular dynamics simulation for 1.0 ps with 1.0 fs time intervals at 300 K in vacuo (Hyperchem 7.5) (Froimowitz, 1993).

2.2.2. Computational docking studies on HES

AutoDock Vina (Trott & Olson, 2010) was used to perform the docking simulations, considering HES as macromolecule/target, and propofol and several known controls previously described as interacting non-covalently with starch derivatives (1-hexanol, hexanal, deferoxamine, cyclohexanol, 2-, 3-, 4-methylcyclohexanone, 1865
2.3. Spectroscopic analysis of the complex of propofol and HES

2.3.1. IR spectroscopy

IR spectra were measured at room temperature on a Spectrum RXI Perkin Elmer FTIR spectrophotometer (Perkin Elmer, UK) in potassium bromide (KBr) disks (cm\(^{-1}\)). The absorption spectra were obtained in the wave number range of 4000–600 cm\(^{-1}\). Baseline correction, normalization, and peak positions were determined for all spectra by Spectrum software v.5.3.1.

2.3.1.1. Propofol. Pure propofol (1 μl) (Sigma-Aldrich, Sintra, Portugal) (0.962 mg; 0.005 mmol) was placed between two KBr disks, each prepared with 100 mg of micronized KBr powder and compressed into disks at a force of 10 kN for 2 min using a manual tablet presser (PerkinElmer, Norwalk, USA).

IR \(v_{\text{max}}\): 3567, 3035, 2963, 2871, 2370, 1598, 1458, 1385, 1309, 1258, 1201, 1171, 1148, 1109, 1045, 929, 823, 791, and 747 cm\(^{-1}\).

2.3.1.2. HES. Pure HES (1 mg) (Sigma-Aldrich, Sintra, Portugal) was accurately weighed and gently mixed with 150 mg of micronized KBr powder and compressed into a KBr disk, as performed for propofol.

IR \(v_{\text{max}}\): 3384, 2930, 2079, 1653, 1372, 1159, 1080, 1016, 930, 861, 765, and 710 cm\(^{-1}\).

2.3.1.3. Physical mixture of propofol and HES. Pure HES (1 mg) was accurately weighed and gently mixed with 200 mg of micronized KBr powder; and divided and compressed into two disks at a force of 10 kN for 2 min using a manual tablet presser (PerkinElmer, Norwalk, USA). Pure propofol (1 μl) (.962 mg; .005 mmol) was placed between the two HES/KBr disks immediately before the IR spectrum was recorded.

IR \(v_{\text{max}}\): 3567, 3071, 3036, 2961, 2871, 2367, 2081, 1910, 1853, 1654, 1590, 1440, 1384, 1363, 1341, 1309, 1258, 1201, 1170, 1149, 1109, 1044, 928, 886, 861, 823, 791, 747, 712, 670, and 617 cm\(^{-1}\).

2.3.1.4. Complexation of propofol with HES (kneading method). The complexation of propofol with HES was performed by adding water to the mixture of propofol and HES according to the kneading method (Yavuz, Bilensoy, Vural, & Sumnu, 2010). A homogenous paste was prepared by mixing 1 μl of propofol (.962 mg; .005 mmol), 1 mg of HES accurately weighed, and two drops of water in a vial. The paste was further kneaded for 30 min. The obtained mass was dried at 40 °C in an oven for 4 h.

IR \(v_{\text{max}}\): 3447, 2962, 2869, 2366, 1644, 1450, 1305, 1259, 1200, 1149, 1109, 1044, 1022, 929, 789, and 748 cm\(^{-1}\).

2.3.2. UV spectroscopy

The UV/visible (vis) spectra were recorded in ethanol using a pathlength of 1 cm, at room temperature on a Varian, CARY 1E spectrophotometer (Mulgrave, Vitoria, Australia) with \(\lambda_{\text{max}}\) in nm. The following ethanolic solutions were analyzed: .10 mg/ml HES, .01 mg/ml propofol, 1.00 mg/ml propofol in .10 mg/ml HES, .20 mg/ml propofol in .10 mg/ml HES, and .02 mg/ml propofol in .10 mg/ml HES. Qualitative analysis of spectra was made in the wavelengths range of 200–400 nm, satisfying or not satisfying the Lambert–Beer law.

2.3.2.1. Propofol

Propofol (.01 mg/ml) \(\lambda_{\text{max}}\) EtOH (nm): 203, 217, and 272 (ε resp. 344,300, 76,600, and 30,800)
2.3.2.2. Mixture of propofol and HES

Propofol (.01 mg/ml) λ 210 and 272 (ε resp. 171,000 and 24,100).

2.3.3. NMR spectroscopy

1H and 13C NMR spectra were taken in deuterium-dimethylsulfoxide (DMSO-d6) at room temperature, on a Bruker Avance 300 instrument (300.13 MHz for 1H and 75.47 MHz for 13C; Wissembourg, France). Chemical shifts (δ) are expressed in parts per million (ppm) values relative to tetramethylsilane (TMS) which was used as an internal reference. Coupling constants are reported in hertz (Hz).

2.3.3.1. Propofol.

1H NMR (DMSO-d6, 300.13 MHz) δ: 8.00 (1H, s, and OH), 6.96 (2H, d, J = 7.6, H-3, and H-5), 6.78 (1H, dd, J = 7.6, and H-4), 3.30 (1H, septet, J = 6.9, and H-1′), 1.15 (6H, s, 1″a-CH3), and 1.13 (6H, s, and 1″b-CH3).

13C NMR (DMSO-d6, 75.47 MHz) δ: see Table 4.

2.3.3.2. HES.

1H NMR (DMSO-d6, 300.13 MHz) δ: 5.52 (1H and s), 5.42 (1H and s), 5.10 (1H and s), 4.60 (1H and s), and 3.64–3.33 (11H and m).

13C NMR (DMSO-d6, 75.47 MHz) δ: 100.097 (C-1), 78.724 (C-2), 73.277 (C-3), 71.994 (C-4), 71.637 (C-5), and 60.471 (C-6).

2.3.3.3. Complexation of propofol with HES (kneading method).

1H NMR (DMSO-d6 and 300.13 MHz) δ: 6.96 (2H, d, J = 7.6, H-3, and H-5), 6.78 (1H, dd, J = 7.6, and H-4), 5.52 (10H and s), 5.43 (10H and s), 5.11 (10H and s), 4.60 (10H and s), 3.64–3.33 (m), 3.30 (1H, septet, J = 6.9, and H-1′), 1.15 (6H, s, and 1″a-CH3), and 1.13 (6H, s, and 1″b-CH3).

13C NMR (DMSO-d6, 74.47 MHz) δ: see Table 4.

2.4. Solubility measurements

Solubility measurements of propofol were carried out at a constant temperature (37 °C) using various HES aqueous solutions. .00, .01, .05, .10, and .20 mg/ml in 50.00 ml of deionized water. After vortexing, pure propofol (12 μl) was added to each tube containing the HES solution. The mixtures were vortexed for 5 min and then placed in a thermostatically controlled water bath shaker at 37 °C for 24 h. Then, an aliquot of aqueous phase of each mixture was transferred to a 5 ml syringe and filtered through a .45 μm membrane filter (Millipore, cellulose acetate). Next, about .5 ml of the clear filtrate was collected for GC-MS analysis as detailed in section 2.1. Additionally, a second aliquot of the aqueous phase of each mixture was also used for the GC-MS analysis without filtering. A new calibration curve was performed in water solutions with propofol concentrations ranging between .00 and 250.00 μg/ml.

2.5. Scanning electron microscopy

Three samples were analyzed: (1) pure HES (1 mg); (2) a mixture of HES (1 mg) and propofol (1 μl) according to the kneading method previously described in section 2.3.; and (3) a mixture of HES (1 mg) and propofol (1 μl) with two drops of water without kneading. The SEM exam was performed using a high resolution (Schottky) environmental scanning electron microscope with X-Ray microanalysis and electron backscattered diffraction analysis: Quanta 400 FEG ESEM / EDAX Genesis X4 M. Samples were coated with a Au/Pd thin film, by sputtering, using the SPI module sputter coater.

Table 1. Docking scores of propofol on HES.

| Pose nr | Docking score (kJ mol⁻¹) | RMSD lower bound | RMSD upper bound |
|---------|--------------------------|------------------|------------------|
| Pose 1  | -3.6                     | 0                | 0                |
| Pose 2  | -3.5                     | 4.574            | .132             |
| Pose 3  | -3.4                     | 11.851           | 10.907           |
| Pose 4  | -3.3                     | 4.039            | 2.238            |
| Pose 5  | -3.3                     | 18.841           | 16.897           |
| Pose 6  | -3.2                     | 19.044           | 16.951           |
| Pose 7  | -3.2                     | 4.374            | 2.94             |
| Pose 8  | -3.2                     | 4.773            | 2.049            |
| Pose 9  | -3.2                     | 4.784            | 1.331            |

RMSD = root mean square deviation; conformations with lower RMSD values are more similar to the best docked conformation.

*RMSD/upper bound matches each atom in one conformation with itself in the other conformation, ignoring any symmetry.

**RMSD/lower bound compares equivalent atoms (the closest atom of the same element type) between conformations.

Figure 2. Propofol concentrations (μg/ml) measured in the plasma in two groups of samples: LR and blood or plasma (gray line) and HES and blood or plasma (black line). The mean and standard deviation of the concentrations at the different sampling time points (T0–T50) are shown.
Samples were coated with Au/Pd for 90 sec and with a 15 mA current.

2.6. Statistical analysis
Two-way repeated measures ANOVA was used to compare the propofol concentrations obtained in the in vitro study for the two sets of tubes and throughout the sampling times. Paired sample t test was used to compare the propofol concentrations between filtered and not filtered samples in the solubility studies. All the statistical analysis was performed using Graphpad Prism (Version 5, GraphPad Software Inc, San Diego, California).

3. Results
In the in vitro experiment, the propofol concentrations ranged between .15 and .30 µg/ml, which reflects high protein binding by the drug (around 99%). Propofol concentrations were significantly lower in the HES samples than in the LR samples ($p = .021$) and there were significant differences in the propofol concentrations throughout time ($p = .014$) (Figure 2).

Docking simulations using HES as macromolecule were performed to analyze putative interactions between HES and propofol. The free energies predicted for the propofol–HES complexes are negative, which suggests favorable interactions between the starch derivative and the anesthetic compound (Table 1). Propofol is predicted to bind HES through the establishment of hydrogen...
interactions with hydroxyl or ether groups on the glucose monomers (Figure 3). The acetyl groups were not predicted as being involved in the binding.

In order to validate the docking study, several compounds previously described as interacting with starch or starch derivatives were also docked to the HES model (Table 2). Most of the compounds present higher free energies scores than propofol (Table 2, entries 3-13). Moreover, the docking onto HES model led to good enrichment metrics (Huang, Kalyanaraman, Irwin, & Jacobson, 2006) (Supplementary data, Figure S1) and, therefore, HES model is able to perfectly distinguish the known HES ligands. These results accentuate the probability of propofol to bind glucose polymers. No significant structural changes were observed in silico for HES and propofol after complexation as only minor deviations of HES chains were observed in order to better accommodate propofol.

During this study, it was also hypothesized that the interactions of propofol with HES could change their chemical properties. The preparation of the propofol–HES solid complex was performed by the kneading method which uses a small amount of water to facilitate the complexation of propofol in HES during energetic kneading. The structure of propofol and HES complex was established by IR, UV, and NMR techniques. The

Figure 4. IR spectra of (A) propofol; (B) HES; (C) physical mixture of propofol and HES; and (D) complex of propofol and HES.

Figure 5. UV spectra of propofol, HES, and the ethanolic mixture of propofol and HES (A) satisfying the Lambert–Beer law and (B) outside the linear range. Lambert–Beer law (Figure 4(A)). Outside the linear range (Figure 4(B)), no significant differences were observed.

interactions with hydroxyl or ether groups on the glucose monomers (Figure 3). The acetyl groups were not predicted as being involved in the binding.
addition of water to the mixture of propofol and HES, allied with energetic kneading (Yavuz et al., 2010), allowed the formation of a jelly, probably by the driving force created by the dislodgement of the water molecules present in the polymer by the hydrophobic compound, propofol. The IR spectra of propofol, HES, physical mixture (compounds gently mixed), and of the complex (kneading method) are shown in Figure 4.

The IR spectra of propofol (Figure 4 – spectrum A) indicated the presence of a wide band at 3568 cm\(^{-1}\) and another at 1653 cm\(^{-1}\) which respectively belong to the stretch and in-plane vibrations of O–H, as well as, bands corresponding to the CH and CH\(_3\) stretching (2870 cm\(^{-1}\)) and bending (1458 cm\(^{-1}\)) and to C=C aromatic bonds (fingerprint region). The IR spectra of HES (Figure 4 – spectrum B) present a wide band at 3384 cm\(^{-1}\) and another at 1653 cm\(^{-1}\) which belong to the stretch and in-plane vibrations of O–H, respectively, bands at 2930 cm\(^{-1}\) corresponding to C–H stretching and a strong and complex band at 1159, 1080, and 1015 cm\(^{-1}\) due to C–O bond stretching. No significant alterations were observed in the IR spectra of the physical mixture, obtained by the gentle contact of propofol with HES (Figure 4 – spectrum C), indicating the presence of typical bands of propofol and of HES. In contrast, IR spectra of the HES–propofol complex obtained by the kneading method (Figure 4 – spectrum D) showed several modifications in the peaks intensity and shape; pronounced shift variations were observed in the bands which belong to the O–H stretching (3448 cm\(^{-1}\)) and to O–H in-plane vibrations (1644 cm\(^{-1}\)) and in the fingerprint region, indicating that interactions...
between propofol and HES have taken place, and probably involve their hydroxyl groups.

The UV spectra of propofol, HES, and the ethanolic mixture of propofol and HES are depicted in Figure 5. A saturated ethanolic solution of HES (.1 mg/ml) does not absorb in the UV region (bottom line). The UV/vis spectrum of propofol showed three absorption maxima at 203, 217, and 272 nm (middle line). The propofol in an ethanolic solution of HES revealed a slightly different spectrum (top line). The propofol at 203 nm maximum was shifted to 210 nm (hyperchromic and bathochromic effects) and the absorption maximum at 217 nm disappeared. These correspond to the most significant alterations achieved satisfying the Lambert–Beer law (Figure 5(A)). Outside the linear range (Figure 5(B)), no significant differences were observed.

Solubility of HES sample (10 mg) in DMSO-d6 (5 ml) is notably affected by the presence of propofol as observed in Figure 6 with the solid complex revealing higher solubility when compared to HES alone. The complex formation of propofol with HES was also confirmed by 1H and 13C NMR spectroscopy. The 1H NMR (Figure 7(A, B)) and 13C spectra of propofol and HES in DMSO-d6 were fully consistent to those described with other solvents (Heins, Kulicke, Käuper, & Thielking, 1998; Momot, Kuchel, Chapman, Deo, & Whittaker, 2003; Trapani et al., 1998). The 1H NMR spectra of the complex (Figure 7) were essentially a combination of the spectra of the components except that the signal at δH 8.00 ppm (Figure 7 gray area) corresponding to the proton of the hydroxyl group of propofol was much

| HES (mg/ml) | Propofol (µg/ml) |
|------------|------------------|
| .00        | 153.2 ± 17.7     |
| .01        | 123.9 ± 17.1     |
| .05        | 123.6 ± 17.5     |
| .10        | 143.7 ± 9.7      |
| .50        | 147.5 ± 2.2      |

Table 4. Solubility of propofol in different concentrations of HES in aqueous solution.
broader. Additionally, downfield shifts signal of protons of the glucose units of HES of .01 ppm was indicative that aromatic molecules are located close to the protons for which a shift is observed.

It is well known that $^{13}$C NMR chemical shifts are sensitive probes of molecular environments and can be used to derive information on complexation (Dinis-Oliveira et al., 2008; Matsuda et al., 1993; Trapani et al., 1998). The chemical shifts for the carbons of propofol both in the absence and presence of HES are summarized in Table 3 as $\delta$ free and $\delta$ complex, respectively. It is possible to observe a significant downfield shift for aromatic carbons of propofol in the presence of HES.

The solubility of propofol in water decreased with the addition of HES (Table 4). The concentrations of propofol were significantly lower in the samples that were filtered than in those that were not filtered ($p = .03$).

SEM revealed that when propofol was added to HES, the shape of the subsequently dried granules was almost similar but the HES granules were aggregated to each other, and these samples showed a continuous matrix texture being possible to observe a film-like structure covering the HES granules and connecting them (Figure 8).

4. Discussion
In this study, we investigated the possibility of a chemical interaction between propofol and HES, in which propofol would bind to the HES molecule decreasing its available fraction in the plasma. This hypothesis was tested by performing laboratory experiments (in vitro assay) with propofol quantification over time, docking studies, and the structure characterization of complexes through IR, UV, and NMR spectroscopy.

The in vitro assay revealed lower propofol concentrations in the samples with HES than with LR, which suggests that HES may have a decreasing effect on the free fraction of propofol. In a previous study, lower propofol concentrations were also observed in samples containing HES, when compared to a crystalloid (Dawidowicz & Kalitynski, 2005). In that study, the authors suggested that increased propofol binding in carbohydrate infusion fluids could result from the formation of glycoproteins and glycoprotein-like macromolecules and that the free propofol fraction in plasma was altered by HES (Dawidowicz & Kalitynski, 2005) which was also observed in the present study.

Docking studies revealed negative predicted free energy for HES–propofol complexes which favors their potential ligand–target interaction. Docking simulations suggested that propofol may interact with HES.

A way of evaluating docking results is to assess how well annotated ligands, placed within a large database of other presumed non-binding “decoy” molecules, are retrieved. Thus, the success of a docking study is evaluated by its ability to rank a small number of known active compounds in the top scores when screening a database with a much higher number of decoy molecules (Huang et al., 2006). The database’s decoys should share the physical properties of the annotated ligands (Table S1), so that enrichment is not simply a separation of trivial physical features. But, the decoys should be chemically distinct from the ligands because they are predicated as non-binders (Huang et al., 2006). The results investigated show a remarkable enrichment for docking results (Figure S1). In fact, the docking onto HES model led to good enrichment metrics. Therefore, the HES model is able to perfectly distinguish the known HES ligands. From the overall results, hydrogen interactions were predicted to be established between the hydroxyl group of propofol and the hydroxyl groups of the glucose units of HES.

This finding was then confirmed by several spectroscopic techniques which reinforce a hydrogen interaction between propofol hydroxyl group and HES; both docking and spectroscopic studies such as NMR indicated that interactions between propofol and HES involved the hydroxyl groups. We therefore reasoned that molecular dynamics simulations and docking combined with spectroscopic techniques might be the approaches of choice to further clarify the mode of anesthetics binding to starch derivatives.

Starch is known to form inclusion complexes with small molecules such as aroma compounds (Jouquand et al., 2006). Propofol is an aromatic compound – a phenol substituted with two isopropyl groups in each of the positions adjacent to the hydroxyl group (Figure 1). Its aromatic ring and isopropyl side groups are highly lipophilic which results in a highly lipophilic molecule (Trapani, Altopane, Liso, Sanna, & Biggio, 2000; Tsuchiya, 2001). This property has made the development of adequate vehicles for propofol a challenge and several different formulations have been investigated, starting from lipid emulsions and then alternate emulsion formulations, as well as non-emulsion formulations based on cyclodextrins which are starch derivatives, for example, propofol–hydroxypropyl-β-cyclodextrin (Trapani et al., 1998), sulfobutylether β-cyclodextrin (Captisol®; CyDex Inc., Lenexa, KS), or micelle formulations such as poloxamers (Morey et al., 2006). In a general sense, cyclodextrins can be used to increase solubility and dissolution rate, and/or increase the stability of drugs (Tiwari, Tiwari, & Rai, 2010). In the present study, the solubility of propofol was not increased by HES, suggesting that the complex formed by the two drugs may have limited water solubility. Additionally, the concentrations of propofol were significantly lower in the samples that were filtered than in those that were not filtered ($p = .03$),
which reinforces the existence of an association between propofol and HES.

The analysis of the interactions between small molecules and starch derivatives has been intensively described in the food industry, concerning the retention and release of aroma compounds from food matrices (Tietz, Buettner, & Conde-Petit, 2008). The formation of helical inclusion complexes is an example of specific binding of compounds such as aroma compounds (alcohols, aldehydes, terpenes, and lactones) and lipids to starch (Conde-Petit, Escher, & Nuessli, 2006). It has been described that the binding strength is higher for long linear molecules compared to bulky molecules like terpenes, but the opposite is true for the binding capacity (Arvisenet, Le Bail, Voilley, & Cayot, 2002). Some compounds may be irreversibly linked by the cryotexture pointing to the formation of supramolecular complexes (Terenina et al., 2002).

The hypothesis of a chemical interaction between HES and anesthetics has been previously suggested after the observation of two episodes of anesthesia in human patients, in which the administration of a bolus of HES 130/0.4 prior to remifentanil bolus injection in patients under propofol- and remifentanil-based anesthesia prevented the expected haemodynamic responses and resulted in a higher stability in the values of the bispectral index (Ferreira et al., 2005). Subsequently, in a study in pigs, volume replacement with HES caused the propofol plasma concentrations to decrease when compared to LR (Silva et al., 2012). However, it is not possible from those studies to conclude whether an interaction between propofol and HES could actually contribute to the results. Further clinical studies should investigate this possibility.

The effect of HES, LR, and mannitol on propofol concentrations was compared in human patients (Adachi et al., 2000). Mannitol caused the highest decrease in the propofol plasma concentrations, followed by HES. However, these decreases were attributed to the higher increase in the intravascular volume caused by these fluids than LR, with consequent propofol dilution. The results in our study suggest that these changes in propofol concentrations may also be due to chemical interactions rather than just changes in the intravascular volume.

HES 130/0.4 is the most recent HES which was created with a lower molecular weight and lower degree of substitution to overcome the side effects of previous heavier starches (Dart, Mutter, Ruth, & Taback, 2010). However, in June 2013 (EMA, 2013), the marketing authorizations for all HES products were suspended in the UK by the European Medicines Agency. The debate regarding the benefits and risks of colloids has lasted for a long time due to the contradictory data published in the subject but three large randomized controlled trials (Brunkhorst et al., 2008; Myburgh et al., 2012; Perner et al., 2012) were the basis for the recent decision of the European Medicines Agency. The use of HES was associated with higher rates of acute kidney failure or dialysis in three studies (Brunkhorst et al., 2008; Myburgh et al., 2012; Perner et al., 2012) and to an increased 90-day mortality rate in one study (Perner et al., 2012). Also, a meta-analysis showed increased incidence of kidney failure associated with the use of HES compared to crystalloids (Zarychanski et al., 2013). A Cochrane review of the use of colloids or crystalloids in critically ill patients also concluded that HES might increase the risk of death and that there is no benefit in colloids over crystalloids in survival rates (Perel, Roberts, & Ker, 2013). Based on these data, the European Medicines Agency concluded that the increased risk of renal dysfunction and mortality outweighs the benefits of HES administration. The Food and Drug Administration has also concluded that HES solutions should not be used in critically ill adult patients, including patients with sepsis and those admitted to the ICU, and warranted a Boxed Warning to include the risk of mortality and severe renal injury (FDA, 2013).

However, the European Medicines Agency decision was not well accepted by practitioners who point out several limitations of the studies that were the basis for the decision (Chappell & Jacob, 2013). Furthermore, because relatively small volumes of colloid are used in the surgical patient, normally to treat haemorrhage, it may not be valid to generalize to the surgical patient the results of studies undertaken in critically ill patients (Nolan & Mythen, 2013).

The existence of an interaction between HES and propofol, as shown in the present study, may enrich the discussion regarding the use of colloids in certain patient groups. Propofol should be used with care in hypovolemic patients due to its cardiorespiratory depressant effects (Bryson, Fulton, & Faulds, 1995), which can exacerbate the dynamic compromise effects caused by hypovolemia. However, anesthesiologists sometimes encounter unexpected high-volume blood loss associated with surgical procedures during propofol anesthesia, which can be faced by the administration of fluids (el-Beheiry, Kim, Milne, & Seegobin, 1995). In these situations, it is important to take in consideration possible interferences of HES in propofol pharmacokinetics and pharmacodynamics.

The results from our study may yield important contributions for the improvement of the propofol pharmacokinetic tricompartmental models, and its intercompartmental constants in situations where colloids are being administered. Additionally, in clinical situations where the administration of intravenous colloids and propofol are required, such as during general anesthesia,
the clinician should be aware of the propofol–HES interaction.

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Supplementary material

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