Original Article

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The Effect of Iodised Poly-3-Hydroxy Butyrate on DNA and BSA

İyotlu Poli-3-Hidroksi Butiratın DNA ve BSA Üzerine Etkisi

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ABSTRACT

Objective: An important criterion that radiopaque poly-3-hydroxy butyrate (PHB) derivatives synthesized and characterized by our study group do not interact with DNA and bovine albumin to be developed as an intravenous contrast agent for radiological imaging.

Methods: Interactions of radiopaque PHB derivatives between bovine serum albumin (BSA) and deoxyribonucleic acid (pUC19 DNA and polymerase chain reaction product) were investigated using absorption spectroscopy and agarose gel electrophoresis techniques.

Results: Absorption spectroscopy results showed that the radiopaque PHB derivatives did not show any interaction with BSA. Double-stranded annular DNA showed that the interaction between the double-stranded forms of linear DNA did not cause any damage.

Conclusion: It was determined that it did not interact with both BSA and various DNA forms.

Keywords: Albumin, DNA, iodinated poly-3-hydroxy butyrate, pUC19

ÖZ

Amaç: Çalışma grubumuz tarafından sentezi ve karakterizasyonu yapılan radyopak poly-3-hydroxy butyrate (PHB) türevlerinin, radyolojik görüntüleme kullanılacak, intravenöz kontrast madde olarak geliştirilebilmesi için, DNA ve sığır albumin ile etkileşim göstermemesi önemli bir kriterdir.

Yöntemler: Radyoopak PHB türevlerinin sığır serum albumin (BSA) ve deoksiribonükleik asit (pUC19 DNA'sı ve polimeraz zincir reaksiyonu ürünü) arasındaki etkileşimleri absorpsiyon spektroskopisi ve agaroz jel elektroforezi teknikleri kullanılarak incelenmiştir.

Bulgular: Absorpsiyon spektroskopisi sonuçları, radyoopak PHB türevlerinin BSA ile bir etkileşim göstermediği, absorpsiyon spektroskopisi ve agaroz jel elektroforezi sonuçları ise; çift iplikli halkasal DNA, çift iplikli lineer DNA formları arasın bir etkileşim ve hasara neden olmadığını göstermiştir.

Sonuç: Hem BSA hem de çeşitli DNA formları ile etkileşimi olmadığı tespit edilmiştir.

Anahtar Sözcükler: Albümin, DNA, iyotlu poli-3-hidroksibutirat, pUC19

Introduction

X-ray imaging using intravenous iodinated contrast agents, computed tomography, and fluoroscopy play important roles in medicine (1). The increased use of diagnostic radiological imaging has significantly increased radiation exposure and radiocontrast agent use in humans, and is associated with cancer risk. The chemotoxic and adverse effects of intravenous iodinated contrast agents, i.e., allergic reactions, kidney damage, and thyrotoxicosis are rare (2); however, they are well-known. In addition, intravenous iodinated contrast agents have shown to

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increase the risk of cancer following X-ray exposure by increasing deoxyribonucleic acid (DNA) damage after a certain dose. Since the late 1970s, studies showed that intravenous iodine contrast agent administration during X-ray imaging causes damage to blood cells (3). Results of other studies reported higher levels of DNA damage could also be explained by the potential effects of intravenous iodinated contrast agents. Most importantly, it is unclear whether the increase in DNA damage in blood cells triggers the risk of cancer if exposed to repeated doses with intravenous iodine contrast agents (4). This uncertainty adds to the risks of intravenous iodinated contrast media in addition to the risks of exposure to low-dose medical radiation, including risks from ongoing epidemiological studies (5).

To address this problem, a review of previously published studies investigating the effect of contrast agent on cell damage in diagnostic imaging was performed. Related articles, contrast radiotherapy, and studies investigating the dosimetric effects of elements with high atomic numbers in general were also collected (5). Parameters such as iodine concentration, time elapsed after exposure, and X-ray energy spectra vary between studies. In addition, intravenous iodinated contrast agents show low protein binding capacity (3-6).

Intravenous iodinated contrast agent may cause various types of DNA damage, including repeated DNA double helix breaks (6). DNA damage is normally repaired by the DNA repair system. However, genetic information may change, if errors are made in the repair of damaged DNA, thus leading to health effects such as cancer and vascular events (3). Therefore, it is important to examine the effects of increased intravenous iodinated contrast agent use, particularly the accurate quantification of the amount of DNA strand and double strand bonds that can be caused by intravenous iodinated contrast agent.

Here, we synthesized and characterized new radiopaque poly-3-hydroxy butyrate (PHB) derivatives previously reported by our study group (7). PHB is essential for *in vivo* medical applications to provide renewable, biodegradable, and biocompatible microbial polyester radiopacity. This study aimed to investigate the interaction of synthesized biodegradable and biocompatible polymers with DNA and plasma protein albumin. The synthesized polymers were named PHB-DEA-IB-6, PHB-DEA-IB-1, and PHB-DEA-IB-12 (7).

Methods

Preparation of Double-stranded DNA (pUC19)

pUC19 is a plasmid cloning vector. It is in the form of circular double-stranded DNA and has 2,686 base pairs. pUC19 is one of the most widely used vectors. In our laboratory, the pUC19 plasmid was isolated from *Escherichia coli* NEB5a, previously transformed with the plasmid by the Valipour group (8,9). The polymerase chain reaction (PCR) product double-stranded short DNA fragment was used by the Celik group, which was previously amplified from human genomic DNA. Figure 1 shows the sequence and schematized appearance of DNA in different forms according to the structure and size by agarose gel electrophoresis.

The pUC19 plasmid is in the form of supercoil. Using a factor that binds to the structure of a supercoil, the structural changes and agarose gel electrophoresis can be indicated by ultraviolet (UV) imaging. The treated polymer is nicked in the supercoil structure if it causes partial opening, which causes the supercoil structure to open completely, causing a linear form or fractures to create multiple images.

Measurement of pUC19 Plasmid DNA Using a UV-visible Spectrophotometer

The plasmid concentration of pUC19 was measured according to the Beer-Lambert Law, and the OD260 was measured after isolation. For samples containing a mixture of protein and nucleic acid for purity, the theoretical A260/A280 ratio is calculated using the following formula according to the Beer-Lambert Law (8,9):

$$A_{260} / A_{280} = \frac{(\varepsilon_{260,\,protein} \text{ X } (\%\text{Protein}) + \varepsilon_{260,\,DNA} \text{ X } (\%\text{ DNA})}{(\varepsilon_{280,\,protein} \text{ X } (\%\text{Protein}) + \varepsilon_{280,\,DNA} \text{ X } (\%\text{ DNA})}$$

Preparation of Radiopaque PHB Derivatives

For this, 1 mg of each of the radiopaque PHB-DEA-IB-6, PHB-DEA-IB-12, and PHB-DEA-3IB-1 polymers were ground to a powder. The powder was dissolved in a solvent system (chloroform, 1 mL). Samples were then allowed to stand for 4-5 h to completely dissolve. These solutions were filtered through Whatman filter paper, followed by microfiltration (0.2 umm). To verify the concentration of these polymers, the filtered solution was subjected to spectrophotometric analysis using the Beer-Lambert Law (8-10).

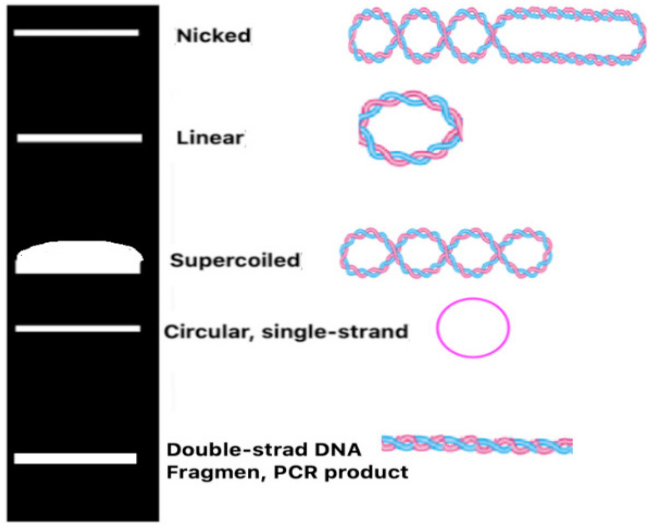

Figure 1. Band appearance and schematized DNA generated by agarose gel electrophoresis of the pUC19 plasmid and PCR product

PCR: Polymerase chain reaction

Preparation of Bovine Serum Albumin (BSA)

The absorbance value was measured on a spectrophotometer at a wavelength of 600 nm at varying concentrations of bovine serum albumin (BSA) with a stock solution of 2 mg/mL.

Preparation of BSA-radiopaque PHB Derivatives and pUC19-radiopaque PHB Derivatives

BSA and DNA solutions were prepared in a buffer solution of pH 7.4 prepared using 0.05 M Tris, 0.15 M sodium chloride, and hydrochloride. To prepare polymer solutions with a concentration between 0.01-0.10 μM, required amounts of the substance were taken from the stock solution and added to the solutions of BSA or pCU19 prepared at physiological pH [phosphate buffered saline (PBS)]. Then, the chloroform (the solvent of the PHB derivatives) was evaporated by leaving the tubes open for a while. Measurements were taken using samples prepared in this manner (BSA-radiopaque PHB derivatives and pCU19-radiopaque PHB derivatives).

Demonstration of the Interaction Between Radiopaque PHB Derivatives and pUC19 were Assessed by Agarose Gel Electrophoresis

Polymer solutions of different concentrations (20 μM, 2 μM, 0.2 μM, and 0.02 μM) and the pUC19 plasmid DNA prepared in order to demonstrate the effect of radiopaque PHB derivatives on pUC19 were incubated in PBS at 37 ºC for 50 min, then 1% agarose gel electrophoresis was carried out at 90 V for 60 min. PBS was used as the negative control, and the pUC19 plasmid without radiopaque PHB derivatives was used as the positive control.

Agarose Gel Electrophoresis Assessment of the Interaction Between Radiopaque PHB Derivatives and PCR Product DNA

A random PCR product obtained from different studies in the laboratory was incubated with different concentrations (20 μM, 2 μM, 0.2 μM, 0.02 μM) of radiopaque PHB derivatives at 37ºC for 50 minutes and carried out at 90 V for 40 minutes in 1% agarose gel electrophoresis. PBS was used as the negative control and the PCR product with and without chloroform and free of radiopaque PHB derivatives was used as the positive control.

Absorbance Spectra of Radiopaque PHB Derivatives with BSA

The absorption spectrum of BSA was found to have an absorption band with a maximum of 265 nm when examined. Changes in the BSA-containing medium after adding radiopaque PHB derivatives were monitored using the absorption spectra.

Results

Measurement and Calculation of the pUC19 Plasmid DNA Using UV-visible Spectrophotometry

UV-Visible spectrophotometry is an effective and easy-to-use method for evaluating interaction between DNA and molecules. It is known that DNA has the highest absorbance value at 260 nm with electron transitions due to chromophoric groups. Thus, molar absorptivity (ε) is at the level of 10^4 M^{-1} cm^{-1} and allows the measurement of DNA concentration. According to the Beer-Lambert Law, the concentration of pUC19 was 8 g/mL for optical density (OD) 260:0.008 after isolation. The A260/A280 and A260/A230 absorbance ratios are used to control the purity of DNA after isolation in various ways. The A260/A280 ratio was calculated according to the Beer-Lambert Law and the ratio of pUC19 plasmid DNA was found to be ~1.8.

Measurement and Calculation of Radiopaque PHB Derivatives Using UV-visible Spectrophotometry

Spectrophotometry measurements of radiopaque PHB derivatives were performed according to their molecular weight (0.001 μM) and dissolved in 1 mL of chloroform, then measured at wavelengths of 200-800 nm using UV-visible spectrophotometry, and 230 nm according to the resulting absorbance-wavelength graph (Figure 2). It was determined to be the preferred wavelength for these measurements.

According to the Beer-Lambert Law, the intensity (1) of light passing through a solution decreases with respect to the inlet (10). Extinction, a measure of OD, is directly proportional to the concentration (c) dissolved in the solution. As long as the average solute dimensions remain constant, a linear relationship is present between the optical density values and the solute density at lower limits of the OD. Absorbance was measured to calculate the extinction coefficient according to the equation "A = εcℓ," where "ε" is the extinction coefficient, "c" is the concentration of the solution, and "ℓ" is the path length. The value of "ℓ" is 1 cm since 1 ml cuvettes were used. "c" is the amount of polymer present in a volume of 1 mL (mL/cm^{-1} mol^{-1}). According to this equation, the calculation of the extinction coefficient of radiopaque PHB derivatives is shown in Table 1. The PHB-DEA-IB-6, PHB-DEA-IB-1, and PHB-DEA-IB-12 polymers were measured by preparing 1.0×10-3 M stock solutions in chloroform. In the continuation of the study, PHB-DEA-IB-6 is P1, PHB-DEA-IB-1 is P2, and PHB-DEA-IB-12 is P3.

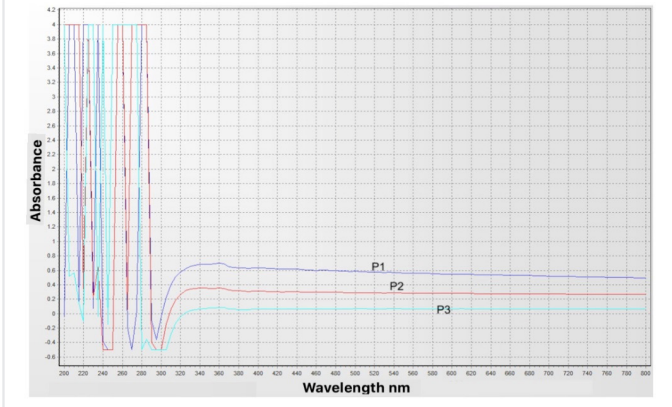

Figure 2. Measurement of P1, P2, and P3 at 200-800 nm with UV-visible spectrophotometry

UV: Ultraviolet

After the determination of the extinction coefficient, it was possible to calculate the absorbance-dependent concentration of radiopaque PHB derivatives without spectrophotometric measurements.

Measurement of BSA by UV-visible Spectrophotometry

BSA was used as the model protein. Albumin is the plasma protein with the highest concentration that will encounter radiopaque PHB derivatives delivered intravenously. BSA was used because of its availability and ease of operational standards. Structurally, human serum albumin (HSA) and BSA is very similar, as HSA contains only one tryptophan residue instead of two in BSA.

Absorbance values were measured by UV-visible spectrophotometry at 600 nm with varying concentrations of BSA from a stock solution of 2 mg/mL (Figure 3).

pUC19 Measurement of Radiopaque PHB Derivatives by UV-visible Spectrophotometry

Changes in the environment such as pH and ionic pressure create changes in molar absorptivity. Consequently, interactions between polymers and DNA result in an aggregating interaction between the aromatic chromophore and the DNA base pair. These interactions occur as hypochromic and bathochromic effects. The decrease in absorbance with increasing DNA concentration is hypochromism, and the increase in absorbance with increasing DNA concentration is hyperchromism. If DNA is treated with denaturing agents, the double helix is separated from the hydrogen bonds holding it together, and a single chain form arises and various bases can remain free. By decreasing the interaction between bases, the UV absorbance of the DNA increases. The extinction coefficient is calculated using the UV-visible spectrophotometry. The Beer-Lambert Law states that constant molar absorption depends on the concentration of dissolved substances in solution at a specific wavelength.

In this study, interactions between radiopaque PHB derivatives and pUC19 DNA were investigated using a UV-visible spectrophotometry. The absorbance properties of radiopaque PHB derivatives were determined both in non-DNA medium and in DNA containing medium in buffer solution (pH: 7.4). Radiopaque PHB derivatives were dissolved in chloroform to obtain a concentration of ~40 µM. Radiopaque PHB derivatives at 222 nm for P1 and 225 nm for P2 and P3 were kept constant, and 2 µL (10 times) of pUC19 DNA was added and incubated for 5 min with a 5 min incubation time. Figure 4 shows the absorption spectrum of media with radiopaque PHB derivatives and pUC19 DNA.

Considering Figure 4, no significant reduction or increase was found in the intensity of the absorption band of polymers at 222 and 225 nm with the addition of DNA. No significant difference was found in the measured values obtained by the addition of pUC19 DNA, thus the mean of the measurement results were plotted (Figure 4). The absorption spectra in the buffer medium clearly show no interaction between the radiopaque PHB derivatives and pUC19 DNA.

Demonstration of an Interaction Between Radiopaque PHB Derivatives and pUC19 by Agarose Gel Electrophoresis

Polymer solutions of different concentrations (20 µM and 2 µM) and pUC19 plasmid DNA prepared in order to demonstrate the effect of radiopaque PHB derivatives on pUC19 were incubated in PBS at 37 °C for 50 min, and subjected to agarose gel electrophoresis for 60 min at 90 V (Figure 5). After incubation with the P1, P2, and P3 polymers at 20 µM and 2 µM, c, no distinctive break in the structure of the super-stranded DNA sample was detected when the control group (c) containing chloroform was compared to the group (d) without chloroform. It was seen as a smear because no opening was found in the supercoil structure.

Table 1. Calculation of extinction coefficients of radiopaque PHB derivatives

	Absorbance (mm)	Concentration (µM)	Extinction coefficient (ε) mL/cm-1 mol-1
P1	0.535	0.001	535
P2	0.276	0.001	276
P3	0.066	0.001	66

PHB: Poly-3-hydroxy butyrate

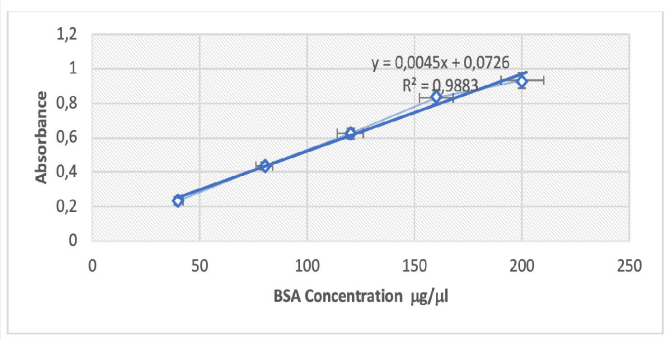

Figure 3. Concentration-dependent absorbance of BSA at 600 nm

BSA: Bovine serum albumin

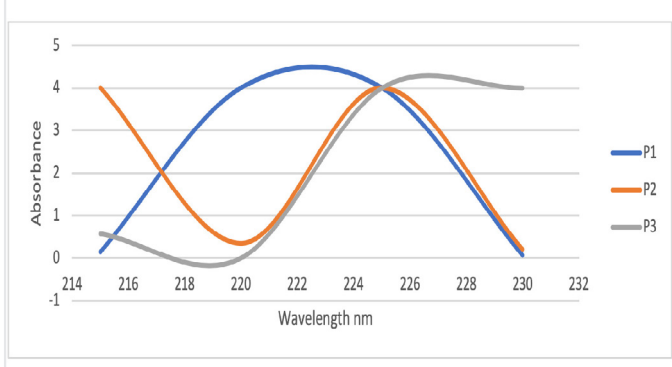

Figure 4. Average spectra of radiopaque PHB derivatives obtained by increasing the amount of pUC19 DNA

PHB: Poly-3-hydroxy butyrate

Agarose Gel Electrophoresis Image of Interaction of Radiopaque PHB Derivatives with the PCR DNA Product

The product obtained by CR is in the form of double-stranded short-chain linear DNA. Stability is poor compared to the circular and supercoil DNA forms. PCR products were treated with different concentrations (0.2 µM and 0.02 µM) of radiopaque PHB derivatives by incubating them together at 37 ºC for 50 min, then subjected to 1% agarose gel electrophoresis for 40 min at 90 V (Figure 5). After incubation with the P1, P2, and P3 polymers at 0.2 µM and 0.02 µM, comparison of the control group (a) containing chloroform and (b) without chloroform, the DNA sample was disrupted when the P1 and P3 polymers were used at 0.2 µM, which prevented band formation in the gel. No differences in DNA structure were detected in two concentrations of the P2 polymer. In addition, chloroform had an effect on DNA.

Investigation of the Binding Balance Between Radiopaque PHB Derivatives and BSA

BSA is a widely studied model protein due to its similar properties to HSA, medical importance, easy availability, low cost, ease of purification procedure, and stability. Many drugs are known to specifically bind to serum albumin. The effectiveness of drugs depends on their ability to bind to BSA. Many studies have been reported in important research areas (such as clinical medicine, life sciences, and chemistry) to clarify the structural properties of drugs after binding to BSA and determine the remedial efficacy of drugs. The absorption spectrum of BSA at physiological pH is presented in Figure 6.

In the absorption spectrum of BSA examination, an absorption band with a maximum of 265 nm was observed. Changes to the BSA medium following the addition of polymers were monitored by the absorption spectra. No significant changes were observed in the absorption spectra of BSA after the addition of radiopaque PHB derivatives. This is important in showing the interaction and binding between BSA and radiopaque PHB derivatives. Figure 7 shows the absorption spectrum of BSA in the presence of 10 µM polymer concentrations.

As shown in Figure 7, no significant increase was found in the intensity of the absorption band at 265 nm of the BSA with the addition of polymers. In addition, no significant increase or decrease in the absorption band at 265 nm of BSA as the polymer concentration increased.

Discussion

In this study, interactions between radiopaque PHB derivatives and BSA and DNA (pUC19 DNA and PCR products) were investigated using absorption spectroscopy and agarose gel electrophoresis.

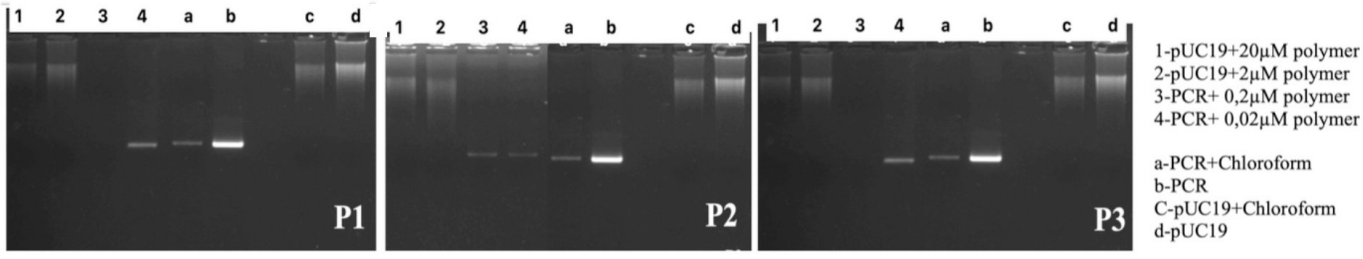

Figure 5. Effect of radiopaque PHB derivatives on pUC19 and PCR product
PHB: Poly-3-hydroxy butyrate, PCR: Polymerase chain reaction

Figure 6. Absorption spectrum of BSA at physiological pH
BSA: Bovine serum albumin

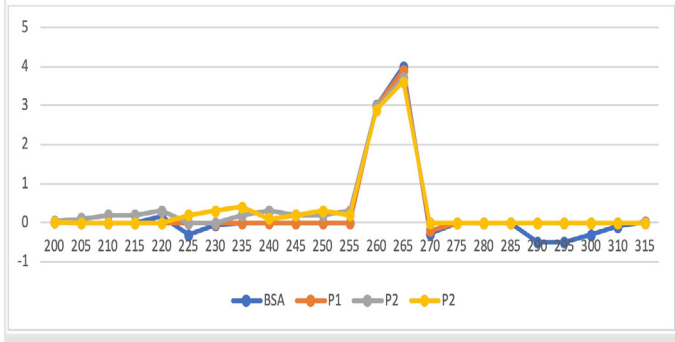

Figure 7. Absorption spectrum of 10 µM BSA concentration in the presence of radiopaque PHB derivatives
BSA: Bovine serum albumin, PHB: Poly-3-hydroxy butyrate

The absorbance properties of BSA were determined in PBS with a pH of 7.4. In the absorption spectrum of BSA examination, an absorption band with a maximum of 265 nm was found. Changes in the absorption spectra of BSA with the addition of radiopaque PHB derivatives are very important in terms of showing the interaction and binding between BSA and polymers. Changes to BSA medium with the addition of radiopaque PHB derivatives were monitored using the absorption spectrum. No significant increase or decrease in the intensity of the absorption band at 265 nm of BSA with the addition of polymers.

The spectral properties of pCU19 DNA were determined in buffer solution at pH 7.4. In the absorption spectrum of DNA examination, an absorption band with a maximum of 260 nm was observed. No significant reduction or increase was found in the intensity of absorption bands of the radiopaque PHB derivatives at 222 and 225 nm with the addition of DNA. This stability in absorbance intensity is indicative that the radiopaque PHB derivatives do not interact with DNA helix.

The pUC19 plasmid DNA and PCR DNA were incubated with iodinated poly-3-hydroxybutyrate and its derivatives, and the interaction of DNA with agarose gel electrophoresis was compared with the positive control, showing no denaturation both at the supercoiled form of plasmid DNA and the double-stranded DNA form of the PCR product. This also coincides with UV-visible spectrophotometry results.

Commercially available intravenous iodinated contrast agents are known as not binding to proteins. Similarly, iodinated poly-3-hydroxy butyrate and its derivatives, which are designed as intravenous iodinated contrast agents, are found to not interact with BSA (2). This information is important for the prediction that the possible interaction of iodinated poly-3-hydroxybutyrate and its derivatives with the intravenous administration of blood plasma proteins is low. At the same time, the lack of interaction with different DNA forms is an advantage over existing commercial products. Thus, it paves the way for intravenous administration *in vivo*.

Conclusion

Adverse reactions to intravenous iodinated contrast media are common; however, severe reactions are rare. The most severe acute reactions and contrast-induced nephropathy occur with the use of contrast-enhanced substances with high osmolarity ionic monomeric intravenous iodine. The best way to treat all adverse reactions is to prevent them from occurring. Identifying patients at risk for side effects and following preventive measures or developing an alternative intravenous iodine contrast agent may help to reduce the incidence of adverse effects. In addition, in situations requiring the use of intravenous iodinated contrast agents, addressing the adverse consequences and potential risks, benefits, and alternatives associated with this class of drug is necessary. For this purpose, interactions of iodized poly-3-hydroxy butyrate and its derivatives with DNA and BSA has been shown. It was found that it does not interact with DNA, unlike currently available contrast agents. No substance interferes with or with the structure of DNA, thus cells in contact with the radiopaque material have no effect on the genomic material. The lack of interaction with DNA forms is advantageous over existing commercial products.

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Ethics

Ethics Committee Approval: The study does not require ethics committee approval.

Peer-review: Externally peer reviewed.

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