Turkish Journal of Thoracic and Cardiovascular Surgery 2019;27(2):185-191
http://dx.doi.org/doi: 10.5606/tgkdc.dergisi.2019.17091

Original Article / Özgün Makale

The cytotoxic properties and apoptotic potential of N-butyl and 2-octyl cyanoacrylates used in surgical treatment of chronic venous insufficiency

Kronik venöz yetmezliğin cerrahi tedavisinde kullanılan N-butil ve 2-oktil siyanoakrilatların sitotoksik özellikleri ve apoptotik potansiyelleri

Ahmet Barış Durukan[1], Esin Akbay[2], Ahmet Ünlü[1], Ayşe Özdemir[3], Mehmet Ali Onur[2]

Institution where the research was done:
Department of Biology, Hacettepe University Faculty of Science, Ankara, Turkey

Author Affiliations:
[1]Department of Cardiovascular Surgery, Medical Park Uşak Hospital, Uşak, Turkey
[2]Department of Biology, Hacettepe University Faculty of Science, Ankara, Turkey
[3]Department of Medical Biochemistry, Uşak University Faculty of Medicine, Uşak, Turkey

ABSTRACT

Background: This study aims to investigate the cytotoxic effects and apoptotic potential of N-butyl cyanoacrylate and 2-octyl cyanoacrylate used in surgical treatment of chronic venous insufficiency.

Methods: N-butyl cyanoacrylate and 2-octyl cyanoacrylate were cultured in cell-culture using human umbilical endothelial cell-line. Cytotoxicity and viability were assessed at 24 and 72 hours with lactate dehydrogenase and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays, respectively. Apoptotic potential was documented at 24 and 72 hours with relative caspase-3 activity.

Results: The mean cytotoxicity at 24 and 72 hours were: N-butyl cyanoacrylate with an area of dot/line: 37.0±3.9%/29.3±2.7% and 46.4±1.6%/45.1±7.1%, 2-octyl cyanoacrylate with an area of dot/line: 39.0±7.0%/37.3±4.6% and 47.0±2.3%/40.7±7.5%. Cytotoxicity increased by time in each group ($p<0.05$). The mean viability at 24 and 72 hours were: N-butyl cyanoacrylate with an area of dot/line: 53.4±7.7%/72.0±5.7% and 35.7±1.9%/37.8±3.7%, 2-octyl cyanoacrylate with an area of dot/line: 54.3±4.4%/73.5±19.9% and 33.6±2.8%/30.7±4.5%. The mean viability decreased by time in each group ($p<0.05$). The mean relative caspase-3 activity at 24 and 72 hours were: control group: 0.084±0.006 and 0.065±0.002, N-butyl cyanoacrylate with an area of dot/line: 0.940±0.037/0.924±0.053 and 0.999±0.072/1.056±0.015, 2-octyl cyanoacrylate with an area of dot/line: 0.900±0.044/0.928±0.018 and 0.989±0.084/0.999±0.072. The mean relative caspase-3 activity was higher than control group in each group at each time interval ($p<0.05$) and activity increased by time in N-butyl cyanoacrylate line and in 2-octyl cyanoacrylate line groups ($p<0.05$).

Conclusion: Our findings indicate that N-butyl cyanoacrylate and 2-octyl cyanoacrylate cause cytotoxicity in cell-culture media. We may also postulate that they induce apoptosis in cell-culture media.

Keywords: Apoptosis, caspase-3, cell culture techniques, cyanoacrylates.

ÖZ

Amaç: Bu çalışmada kronik venöz yetmezliğin cerrahi tedavisinde kullanılan N-butil siyanoakrilat ve 2-oktil siyanoakrilatın sitotoksik etkileri ve apoptotik potansiyelleri araştırıldı.

Çalışma planı: N-butil siyanoakrilat ve 2-oktil siyanoakrilat insan umbilikal endotelyal hücre hattı kullanılarak hücre kültüründe kültüre edildi. Sitotoksisite ve viabilite 24. ve 72. saatlerde sırasıyla laktat dehidrogenaz ve 3-(4,5-dimetiltiyazol-2-il)-2,5-difeniltetrazolium bromid çalışmaları ile değerlendirildi. Apoptotik potansiyel kaspaz-3 aktivitesi ile 24. ve 72. saatlerde belgelendi.

Bulgular: Yirmi dört ve 72. saatlerdeki ortalama sitotoksisite şöyle idi: dot/line alanı olan N-butil siyanoakrilat: %37.0±3.9/%29.3±2.7 ve %46.4±1.6/%45.1±7.1, dot/line alanı olan 2-oktil siyanoakrilat: %39.0±7.0/%37.3±4.6 ve %47.0±2.3/%40.7±7.5. Her grupta sitotoksisite zamanla arttı ($p<0.05$). Yirmi dört ve 72. saatlerdeki ortalama viabilite şöyle idi: dot/line alanı olan N-butil siyanoakrilat: %53.4±7.7/%72.0±5.7% ve %35.7±1.9/%37.8±3.7, dot/line alanı olan 2-oktil siyanoakrilat: %54.3±4.4/%73.5±19.9% ve %33.6±2.8/%30.7±4.5. Her grupta viabilite zamanla azaldı ($p<0.05$). Yirmi dört ve 72. saatlerdeki ortalama relatif kaspaz-3 aktivitesi şöyle idi: kontrol grubu: 0.084±0.006 ve 0.065±0.002, dot/line alanı olan N-butil siyanoakrilat: 0.940±0.037/0.924±0.053 ve 0.999±0.072/1.056±0.015, dot/line alanı olan 2-oktil siyanoakrilat: 0.900±0.044/0.928±0.018 ve 0.989±0.084/0.999±0.072. Her zaman aralığında, her grupta ortalama kaspaz-3 aktivitesi kontrol grubundan daha yüksek idi ($p<0.05$) ve aktivite N-butil siyanoakrilat ve 2-oktil siyanoakrilat gruplarında zamanla arttı ($p<0.05$).

Sonuç: Bulgularımız, N-butil siyanoakrilat ve 2-oktil siyanoakrilatın hücre kültürü ortamında sitotoksisiteye neden olduğuna işaret etmektedir. Hücre kültürü ortamında apoptozu indükleyebilirler de söyleyebilir.

Anahtar sözcükler: Apoptoz, kaspaz-3, hücre kültürü teknikleri, siyanoakrilatlar.

Received: September 19, 2018 *Accepted:* February 06, 2019 *Published online:* April 24, 2019

Correspondence: Ahmet Barış Durukan, MD. Medical Park Uşak Hastanesi Kalp ve Damar Cerrahisi Bölümü, 64200 Uşak, Turkey.
Tel: +90 276 - 227 65 65 e-mail: barisdurukan@yahoo.com

Cite this article as:
Durukan AB, Akbay E, Ünlü A, Özdemir A, Onur MA. The cytotoxic properties and apoptotic potential of N-butyl and 2-octyl cyanoacrylates used in surgical treatment of chronic venous insufficiency. Turk Gogus Kalp Dama 2019;27(2):185-191

Chronic venous insufficiency (CVI) is a common disorder with reported incidences of up to 30% and is mostly due to superficial venous reflux. It decreases quality of life and requires multiple office visits.[1] The surgical treatment of CVI has changed considerably since the definition of high ligation and stripping by Myers in 1947. Endovenous thermal ablation (EVTA) techniques, mainly laser and radiofrequency have been defined in early 2000s and were recommended by guidelines as first-line treatment strategies. The treatment strategies have shifted to non-thermal methods, particularly cyanoacrylate (CA) ablation in 2010s with the advantages of no requirement for tumescent anesthesia and very low incidences of postoperative pain and discomfort. The reported mid-term results are comparable to first-line treatment strategies.[2]

However, the cellular effects of these CAs have not been documented in detail. The polymerization reaction induces a strong inflammatory reaction on vein wall followed by obliteration. This is followed by subacute vasculitis, a chronic granulomatous reaction and finally fibrosis.[2] *In vivo* studies demonstrated cellular reaction with loose fibrillar collagen network with macrophages and foreign body giant cells.[3] The cytotoxicity of CAs was documented previously in cell culture studies.[3-5]

In this study, we aimed to investigate the cytotoxic effects and apoptotic potential of N-butyl cyanoacrylate (NBCA) and 2-octyl cyanoacrylate (2-OCA) used in surgical treatment of CVI.

MATERIALS AND METHODS

This study was conducted at Department of Biology, Hacettepe University Faculty of Science between within May 2017. Human umbilical vein endothelial cell line was cultured in T/96-well culture plates (Greiner Bio-One, Frickenhausen, Germany) in Dulbecco's modified endothelial medium/Ham's F12 (Biochrom AG, Berlin, Germany) supplemented with 20% fetal bovine serum (Biochrom AG, Berlin, Germany), 100 µg/mL heparin, and 1% penicillin/streptomycin at 37°C in humidified atmosphere (5% carbon dioxide [CO_2] in air). Culture medium was refreshed every two days and sub-cultured till experimental protocol was applied. The reason why this cell line was employed was that it is an endothelial cell-line and CAs used in surgical treatment of CVI are in direct contact with and affect directly on endothelial cells. The study protocol was approved by the Hacettepe University Faculty of Science Ethics Committee.

N-butyl cyanoacrylate (VenaBLOCK®, RD Global Research & Development, Ankara, Turkey) with a volume of 0.2 µL and 2-OCA (Veinoff®, RD Global Research & Development, Ankara, Turkey) of 1 µL were applied by pipette with an area of dot and a line at the bottom of the well-plate[6] followed by seeding of cell line at an initial density of 2×104 cells/well. The non-treated group served as the control group.

For assessment of cytotoxicity, at the end of designed incubation times (24 and 72 hours), lactate dehydrogenase (LDH) release was measured using the Pierce LDH cytotoxicity assay kit according to manufacturer's defined protocols (Pierce LDH cytotoxicity assay kit, Thermo Scientific, Waltham, MA, USA). The absorbance was measured at 490 nm using the scanning multi-well spectrophotometer (μQuant™, BioTek® Instruments Inc., Winooski, VT, USA). Each experiment was performed in six-well plate and repeated for six times.

Also, % of cytotoxicity was calculated according to manufacturer's protocols:

Cytotoxicity (%)= (Compound-treated LDH activity-Spontaneous LDH activity)/(Maximum LDH acitivity-Spontaneous LDH activivty) ×100

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine cytotoxicity. It is a standardized cellular viability test. It is a calorimetric method that assesses the ability of viable cells to form MTT formazan by the mitochondrial enzyme succinate dehydrogenase.[7]

Following treatment of cells with chemicals, well-plates were cultured at 37°C and 5% CO_2. For assessment of cellular viability, at the end of the designed incubation periods (24 and 72 hours), the culture medium was replaced with 200 μL medium containing 10% MTT and plates were incubated for four hours at 37°C. To dissolve the formazan crystals, the solution was removed and replaced with isopropanol alcohol containing 0.1 normolality hydrochloride. The resulting solution (crystal violet in color) was removed and transferred to another T/96 well plate. The absorbance was measured on a spectrophotometer microplate reader (μQuant™, BioTek® Instruments Inc., Winooski, VT, USA) at a wavelength of 570 nm. Each experiment was performed in six-well plate and repeated for six times.

Cellular Viability (%)= (Treated Group Absorbance)/(Control Group Absorbance) ×100

Caspase-3 activity was studied to assess apoptosis. Caspase-3 activity was determined using the

Table 1. Lactate dehydrogenase cytotoxicity assay for N-butyl cyanoacrylate and 2-octyl cyanoacrylate

	NBCA dot		NBCA line		2-OCA dot		2-OCA line	
Cytotoxicity (t)	Mean±SD	Min-Max	Mean±SD	Min-Max	Mean±SD	Min-Max	Mean±SD	Min-Max
24 hours	37.0±3.9	33.9-42.1	29.3±2.7	26.0-32.2	39.0±7.0	31.5-47.2	37.3±4.6	34.3-43.3
72 hours	46.4±1.6	44.4-47.9	45.1±7.1	39.3-54.4	47.0±2.3	44.2-49.4	40.7±7.5	34.3-53.8
*p**	0.026		0.027		0.026		0.285	

NBCA: N-butyl cyanoacrylate; 2-OCA: 2-octyl cyanoacrylate; SD: Standard deviation; * Wilcoxon signed-rank test; Min: Minimum; Max: Maximum.

Caspase-3/CPP32 colorimetric assay kit (BioVision Inc., CA, USA). After 24 and 72 hours of incubation with both cyanoacrylate agents, six samples of untreated (control) and treated cells (2×104 cells/well) were washed in cold phosphate-buffered saline, re-suspended in 50 μL cell lysis buffer, and incubated on ice for 10 min. Cell lysates were pelleted, followed by the transfer of supernatants to microcentrifuge tubes. 50 μL of 2x reaction buffer with 5 mmol/L dithiothreitol and 5 μL of 1 mmol/L DEVD-p-nitroanilide (pNA)-conjugated CPP32 substrate were added to each tube, followed by one hour incubation in a water bath at 37°C. A control reaction of treated cells without DEVD-pNA was included. Optical density for each specimen was determined at 405 nm using the spectrophotometer microplate reader (μQuant™, BioTek® Instruments Inc., Winooski, VT, USA). Activity was standardized to total protein concentration determined with Bradford assay protocol. Background reading was subtracted from readings of all samples before fold increase in caspase-3 activity was calculated. The results were expressed as relative caspase-3 activity. Each experiment was performed in six-well plate and repeated for six times.

Relative Caspase-3 activity= (Treated group absorbance)/(Control group absorbance)

Statistical analysis

Statistical analyses were performed using IBM SPSS version 22.0 software (IBM Corp., Armonk, NY, USA). Continuous data were presented with mean ± standard deviation and minimum-maximum values. Wilcoxon signed-rank test was used to compare 24 hour and 72 hour values and Mann-Whitney U test was used to compare values at same time interval. Statistical significance was set as $p<0.05$.

RESULTS

The mean cytotoxicity of NBCA with an area of dot was 37.0±3.9% at 24 hours and 46.4±1.6% at 72 hours ($p<0.05$) whereas with an area of line 29.3±2.7% at 24 hours and 45.1±7.1% at 72 hours ($p<0.05$). For 2-OCA with an area of dot, mean cytotoxicity was 39.0±7.0% at 24 hours and 47.0±2.3% at 72 hours ($p<0.05$) whereas for 2-OCA with an area of line 37.3±4.6% at 24 hours and 40.7±7.5% at 72 hours ($p>0.05$) (Table 1 and Figure 1).

The viability determined by MTT revealed that mean viability at 24 hours was 53.4±7.7% at 24 hours and 35.7±1.9% at 72 hours for NBCA with an area of dot ($p<0.05$) whereas 72.0±5.7 at 24 hours and 37.8±3.7% at 72 hours for NBCA with an area of line ($p<0.05$). Mean viability for 2-OCA with an area of dot was 54.3±4.4% at 24 hours and 33.6±2.8% at 72 hours ($p<0.05$) whereas for 2-OCA with an area of line was 73.5±19.9% at 24 hours and 30.7±4.5% at 72 hours ($p<0.05$) (Table 2 and Figure 2).

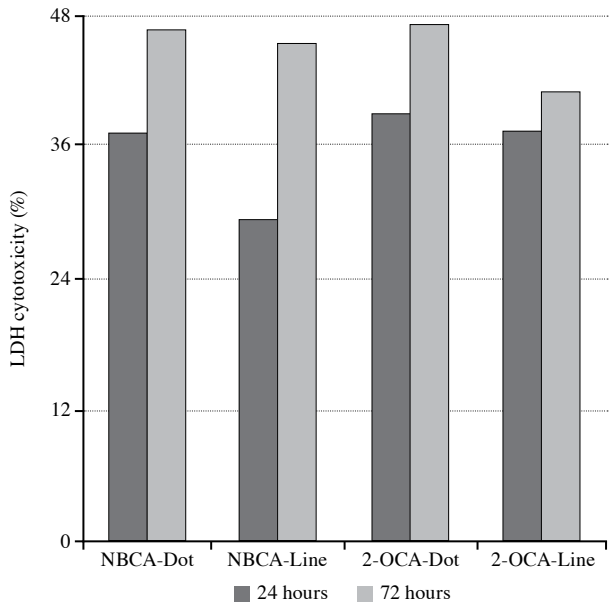

Figure 1. Lactate dehydrogenase cytotoxicity assay for NBCA and 2-octyl cyanoacrylate. Mean cytotoxicity of NBCA with an area of dot and line were increased at 24 and 72 hours ($p<0.05$ for both). For 2-2-octyl cyanoacrylate with an area of dot, mean cytotoxicity was increased at 24 hours and 72 hours ($p<0.05$) whereas for 2-octyl cyanoacrylate with an area of line, there was no statistically significant difference ($p>0.05$).
LDH: Lactate dehydrogenase; NBCA: N-butyl cyanoacrylate; 2-OCA: 2-octyl cyanoacrylate.

Table 2. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide viability assay for N-butyl cyanoacrylate and 2-octyl cyanoacrylate

	NBCA dot		NBCA line		2-OCA dot		2-OCA line	
Viability (t)	Mean±SD	Min-Max	Mean±SD	Min-Max	Mean±SD	Min-Max	Mean±SD	Min-Max
24 hours	53.4±7.7	47.6-69.0	72.0±5.7	65.0-79.3	54.3±4.4	49.2-61.1	73.5 ±19.9	53.9-99.2
72 hours	35.7±1.9	33.9-39.2	37.8±3.7	34.2-43.1	33.6±2.8	30.6-36.9	30.7±4.5	23.5-37.4
*p**	0.028		0.028		0.028		0.028	

NBCA: N-butyl cyanoacrylate; 2-OCA: 2-octyl cyanoacrylate; SD: Standard deviation; * Wilcoxon signed-rank test; Min: Minimum; Max: Maximum.

The results for relative caspase-3 activity defined as the ratio between compound treated to control activity were designated in Table 3 and Figure 3. Mean caspase-3 activity for control group was 0.084±0.006 at 24 hours and 0.065±0.002 at 72 hours ($p<0.05$). For NBCA with an area of dot, mean relative activity was 0.940±0.037 at 24 hours and 0.999±0.072 at 72 hours ($p>0.05$) whereas for NBCA with an area of line was 0.924±0.053 and 1.056±0.015 at 24 and 72 hours, respectively ($p<0.05$). The mean relative caspase-3 activity was 0.900±0.044 at 24 hours and 0.989±0.084 at 72 hours for 2-OCA with an area of dot ($p>0.05$) whereas for 2-OCA with an area of line, it was 0.928±0.018 at 24 hours and 0.999±0.072 at 72 hours ($p<0.05$). When relative apoptotic activity of the compound treated groups was compared with control, it was observed that activity was higher for all groups at 24 and 72 hours ($p<0.05$ for each group).

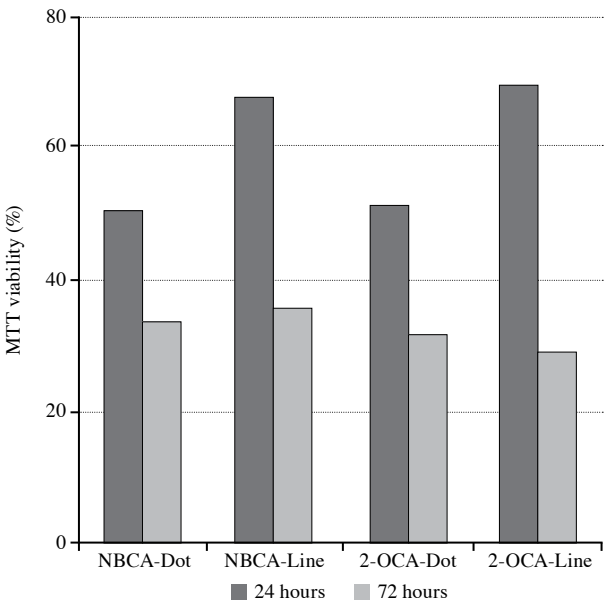

Figure 2. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra zolium bromide viability assay for NBCA and 2-OCA. Viability determined by MTT was decreased at 24 and 72 hours for both NBCA with an area of dot and line ($p<0.05$ for both). Viability for 2-OCA with an area of dot and for line were also decreased ($p<0.05$ for both).

NBCA: N-butyl cyanoacrylate; 2-OCA: 2-octyl cyanoacrylate; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

DISCUSSION

Polycyanoacrylates have been used for surgical treatment of CVI for five years and promising results comparable to first-line therapies, namely EVTA, have been documented.[1,2] Yet, still very less is known about the precise biochemical mechanisms on cellular level. Our study aimed to document the cellular effects by means of cytotoxicity-viability and also to find out whether these CAs induced apoptosis at cell-culture media.

Cyanoacrylates are used mainly as medical adhesives and drug delivery vehicles in medicine. The most commonly employed CAs for treatment of CVI are NBCA and 2-OCA.[2] The chemical structure consists of CH_2=C (CN)CO_2R, R as the organic molecular group as butyl (C_4H_9) or octyl (C_8H_{17}).[3] The monomers rapidly polymerize with contact to weak nucleophiles like water and become a stable water-resistant polymer. The side chains of CA esters determine the mechanical and thermal properties as well as biocompatibility, viscoelasticity and most importantly biocompatibility. Polymer of butyl-CA forms a strong and instantaneous, but brittle bond whereas octyl-CA forms a relatively flexible adhesive bond, while having a longer polymerization time compared to its butyl ester.[4]

An ideal CA should be simply applied, biocompatible, biodegradable with minimal tissue toxicity, without foreign body/inflammatory response and be cost-effective.[3,8] Numerous previous studies have revealed simple applicability, biocompatibility, minimal toxicity and to an extent, cost-effectiveness of CAs used in surgical treatment of CVI.[1,2] In this

Table 3. Caspase-3 apoptosis activity for N-butyl cyanoacrylate and 2-octyl cyanoacrylate

	Control		NBCA dot		NBCA line		2-OCA dot		2-OCA line	
Relative Caspase-3 Activity (t)	Mean±SD	Min-Max	Mean±SD	Min-Max	Mean±SD	Min-Max	Mean±SD	Min-Max	Mean±SD	Min-Max
24 hours	0.084±0.006	0.076-0.089	0.940±0.037	0.916-0.988	0.924±0.053	0.869-0.988	0.900±0.044	0.845-0.940	0.928±0.018	0.916-0.952
p^* (vs. control)			0.002		0.002		0.002		0.002	
72 hours	0.065±0.002	0.063-0.068	0.999±0.072	0.907-1.061	1.056±0.015	1.046-1.076	0.989±0.084	0.907-1.092	0.999±0.072	0.938-1.092
p^* (vs. control)			0.002		0.002		0.002		0.002	
p^{**} (24 h vs. 72 h)	0.026		0.113		0.026		0.113		0.026	

NBCA: N-butyl cyanoacrylate; 2-OCA: 2-octyl cyanoacrylate; SD: Standard deviation; * Wilcoxon signed-rank test; ** Mann-Whitney U test; Min: Minimum; Max: Maximum.

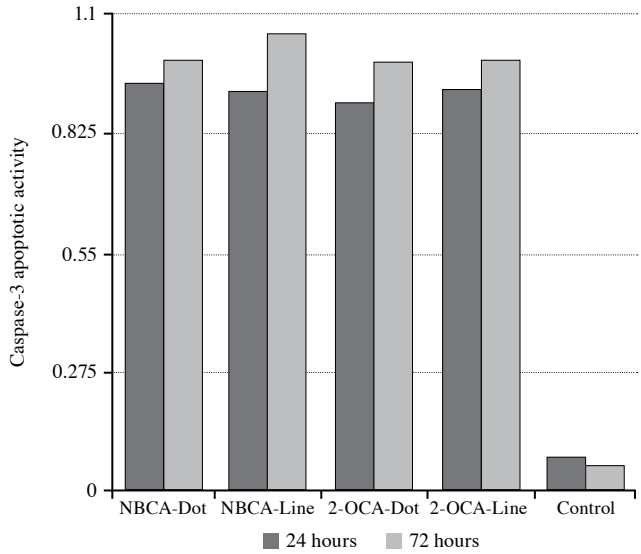

Figure 3. Caspase-3 apoptosis activity for NBCA and 2-OCA. For NBCA with an area of dot, there was no statistically significant different mean relative activity at 24 and 72 hours ($p>0.05$) whereas for N-butyl cyanoacrylate with an area of line, mean apoptotic activity was increased at 24 and 72 hours ($p<0.05$). Mean relative caspase-3 activity was not statistically significantly different at 24 and 72 hours for 2-OCA with an area of dot ($p>0.05$) whereas for 2-OCA with an area of line, it was increased at 24 and 72 hours ($p<0.05$). When relative apoptotic activity of compound treated groups was compared with control, it was higher for all groups at 24 and 72 hours ($p<0.05$ for each group).
NBCA: N-butyl cyanoacrylate; 2-OCA: 2-octyl cyanoacrylate.

study, the main aims were to document toxicity quantitatively and also apoptotic potential of CAs rather than clinical characteristics.

It was documented previously that hydrolytic degradation of CAs gives rise to formaldehyde formation and finally depolymerization. The cyanoacrylate toxicity in cell culture has been reported to be proportionate to the rate of formaldehyde release.[4] Moreover, Mizrahi et al.[9] stated that formaldehyde release is not the only determinant of cytotoxicity, proving that degradation products such as poly (cyanoacrylic acid) and corresponding hydrophobic alcohols also attribute to toxicity. In contrary, in a study on biocompatibility of poly OCA, Lee et al.[5] reported no significant cellular toxicity or deoxyribonucleic acid damage. To decrease cytotoxicity of CAs, longer side of chains has been added as in NBCA and OCA and resulted in better biocompatibility both *in vivo* and *in vitro*.[5,8] This is because the shorter side chains are degraded faster and lead to higher degradation products causing cytotoxic and inflammatory reactions

and inhibit the healing process. Increasing side chains also improve elastic properties, decrease adhesive strength, and improve tissue healing.[3] In animal studies, a non-upregulated inflammatory response was documented with OCA which is poorly reactive to host tissue supported by interleukin-6 and tumor necrosis factor-alpha levels. This is the rationale why NBCA and 2-OCA are widely preferred in CVI. *In vivo* tests have revealed low absorption of CAs leading to macrophage and polymorphonuclear cell invasion contributing to tissue damage.[8] With extensive experience on cytotoxicity, Montanaro et al.[10] established a reasonable accepted threshold of 70% for viability to identify toxicity. In this study, we did not document formaldehyde release, since the relationship with cytotoxicity has well been documented; however, we studied the degree of cytotoxicity and also cellular viability. The viability results of our study revealed that for NBCA with an area of dot at 24 hours and 2-OCA with an area of dot at 24 hours and for all groups at 72 hours, the toxicity was defined since mean viabilities were lower than 70%. However, for NBCA with an area of line at 24 hours and for 2-OCA with an area of line at 24 hours, the mean viabilities were over 70%. The cytotoxicity assay showed that for NBCA, the mean quantitative cytotoxicity was 30%-40% and about 45% at 24 and 72 hours, respectively. For 2-OCA, the mean cytotoxicity was approximately 38% at 24 hours and 40%-47% at 72 hours. The mean increase in cytotoxicity by time in each group was statistically significant. Based on these findings, we may say that for both NBCA and 2-OCA, quantitative cytotoxicity was documented in cell-culture media. However, based on results of caspase-3 activity, we may suggest that the cell death is partly by apoptosis, and not completely due to pure cytotoxic effects. Therefore, we may assume that the healing process has a benign course and is more physiologic compared to EVTA techniques that cause heat-induced necrosis.

It is very well-known that CA polymerization reactions are in general exothermic.[4,11] For comparison, we know that laser ablation produces 700°C of heat and radiofrequency ablation produces over 120°C of heat at the tip of the catheter.[2] Polymerization of acrylic acid (basic monomer of CA), in other words, transition from liquid to solid state produces 15 to 18.5°C, and these values were reported nearly seven decades ago.[12] Therefore, it is clearly understood that the heat produced by CA polymerization reaction is far beyond thermal ablation techniques. It is the heat that produces tissue necrosis in EVTA. Based on these findings, we may at least hypothesize that EVTA produces much more necrosis than CA ablation. Moreover, in this study, we have shown that CAs induce apoptosis in cell-culture.

The issue of depolymerization is also a popular discussion subject for CAs. To add on this subject, one should first of all know the chemical depolymerization process in detail. The entire polymer is depolymerized, in other words, decomposed at melting T° (Tm), also known as the depolymerization T°. For NBCA, Tm is 240°C and for 2-OCA, it is 237°C.[11] Therefore, knowing that cytotoxicity is determined mostly by formaldehyde release during depolymerization, we may hypothesize that in body T°, complete degradation and formaldehyde release are not possible. The metabolic process of CAs used in CVI is primarily based on chronic granulomatous reaction and fibrosis, rather than complete polymerization.[2]

There have been speculations on apoptotic potential of CAs; however, no well-documented studies were published. Bellón et al.[13] compared the apoptotic response of NBCA and N-hexyl CA used as adhesives in tissue level and reported about 30% of apoptosis by Terminal deoxynucleotidyl transferase (TdT) 2´-Deoxyuridine, 5´-Triphosphate (dUTP) Nick-End Labeling (TUNEL) technique without statistically significant difference. In our study, we have used relative caspase-3 activity to document apoptotic potential of NBCA and 2-OCA. Caspases, also known as cysteine-aspartic proteases, are known to have important roles in cell death and inflammation for two decades. Caspases 2, 3, 7, 8, 9 and 10 are called apoptotic caspases. Intrinsic apoptosis initiated by a death-inducing stimulus causes activation of p53 and pro-apoptotic Bcl-2 proteins which soon leads to mitochondrial outer membrane permeabilization and activation of a death-inducing platform called the apoptosome. Then, caspase-9 is cleaved and activates other effector caspases like caspase-3.[14] Therefore, determination of caspase-3 activity is a determinant for apoptotic activity. Based on relative caspase-3 activity, we may say that NBCA and 2-OCA induces apoptosis at 24 hours and 72 hours when compared to control group and the results are statistically significant. We also believe that the results are also valuable regarding the cell-line used, because cell-line consists of human endothelial cells. In surgical treatment of CVI, both CA agents are directly in contact with and exert their effect directly on endothelial cells.

The main limitation of this study is that it is a cell-culture study, which is *in vitro*. Despite the fact that cell-culture studies are very widely employed for toxicity and viability, we strongly believe that

apoptotic potential of NBCA and 2-OCA should also be documented *in vivo.*

In conclusion, N-butyl cyanoacrylate and 2-octyl cyanoacrylate, which are the most common agents employed for surgical treatment of chronic venous insufficiency, cause cytotoxicity. We may also postulate that N-butyl cyanoacrylate and 2-octyl cyanoacrylate induce apoptosis in cell-culture media. To our knowledge, this is the first study to document the cytotoxic characteristics and apoptotic potential of the most commonly employed cyanoacrylates in surgical treatment of chronic venous insufficiency. Compared to endovenous thermal ablation methods that cause heat-induced necrosis; cyanoacrylates induce apoptosis; therefore, a more physiological and benign healing process. Since the cell-culture is employed in human endothelial cells, the results are more reliable regarding these agents that mainly act on and are in direct contact with endothelial cells.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding

The authors received no financial support for the research and/or authorship of this article.

REFERENCES

1. Vos CG, Ünlü Ç, Bosma J, van Vlijmen CJ, de Nie AJ, Schreve MA. A systematic review and meta-analysis of two novel techniques of nonthermal endovenous ablation of the great saphenous vein. J Vasc Surg Venous Lymphat Disord 2017;5:880-96.
2. Durukan AB. What is popular may no longer be popular: Cyanoacrylate and other alternatives in surgical treatment of chronic venous insufficiency. Turk Gogu Kalp Dama 2017;25:159-63.
3. Pascual G, Sotomayor S, Rodríguez M, Pérez-Köhler B, Kühnhardt A, Fernández-Gutiérrez M, et al. Cytotoxicity of Cyanoacrylate-Based Tissue Adhesives and Short-Term Preclinical In Vivo Biocompatibility in Abdominal Hernia Repair. PLoS One 2016;11:e0157920.
4. Barkan Y, Levinman M, Veprinsky-Zuzuliya I, Tsach T, Merqioul E, Blum G, et al. Comparative evaluation of polycyanoacrylates. Acta Biomater 2017;48:390-400.
5. Lee YJ, Jung GB, Choi S, Lee G, Kim JH, Son HS, et al. Biocompatibility of a novel cyanoacrylate based tissue adhesive: cytotoxicity and biochemical property evaluation. PLoS One 2013;8:79761.
6. Kang R, Li H, Lysdahl H, Quang Svend Le D, Chen M, Xie L, et al. Cyanoacrylate medical glue application in intervertebral disc annulus defect repair: Mechanical and biocompatible evaluation. J Biomed Mater Res B Appl Biomater 2017;105:14-20.
7. Durukan AB, Erdem B, Durukan E, Sevim H, Karaduman T, Gurbuz HA, et al. May toxicity of amiodarone be prevented by antioxidants? A cell-culture study. J Cardiothorac Surg 2012;7:61.
8. Lee YJ, Son HS, Jung GB, Kim JH, Choi S, Lee GJ, et al. Enhanced biocompatibility and wound healing properties of biodegradable polymer-modified allyl 2-cyanoacrylate tissue adhesive. Mater Sci Eng C Mater Biol Appl 2015;51:43-50.
9. Mizrahi B, Stefanescu CF, Yang C, Lawlor MW, Ko D, Langer R, et al. Elasticity and safety of alkoxyethyl cyanoacrylate tissue adhesives. Acta Biomater 2011;7:3150-7.
10. Montanaro L, Arciola CR, Cenni E, Ciapetti G, Savioli F, Filippini F, et al. Cytotoxicity, blood compatibility and antimicrobial activity of two cyanoacrylate glues for surgical use. Biomaterials 2001;22:59-66.
11. Szanka I, Szanka A, Kennedy JP. Rubbery wound closure adhesives. II. initiators for and initiation of 2-octyl cyanoacrylate polymerization, J Polym Sci, Part A: Polym Chem 2015;53:1652-9.
12. Roberts DE. Heats of Polymerization. A Summary of Published Values and Their Relation to Structure. U. S. Department of Commerce National Bureau of Standards, Part of the Journal of Research of the National Bureau of Standards. Research Paper RP2073 1950;44:221-32.
13. Bellón JM, Fernández-Gutiérrez M, Rodríguez M, Sotomayor S, Pérez-Köhler B, Kuhnhardt A, et al. Bioassay of cyanoacrylate tissue adhesives used for intraperitoneal mesh fixation. J Biomed Mater Res B Appl Biomater 2017;105:312-9.
14. Shalini S, Dorstyn L, Dawar S, Kumar S. Old, new and emerging functions of caspases. Cell Death Differ 2015;22:526-39.