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Toxicity assessment of extracts from infusion sets in cEND brain endothelial cells

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ABSTRACT

In vitro safety assessment of disposable medical devices, including infusion sets, is usually performed using L-929 mouse keratinocytes. However, cells of different origin (endothelial, lymphoid and myeloid cells) are also exposed to infusion sets' extractables during their clinical use. We studied whether the cEND mouse brain endothelial cells can be suitable for in vitro safety assessment of infusion sets. We analyzed infusion sets from different manufacturers that varied in design and storage time. cEND cells were incubated with extracts of individual parts of the infusion sets (tube, cup, latex), and relative toxicities were analyzed using MTT test, DCFH-DA-based analysis of reactive oxygen species formation, apoptosis and cell cycle analyses. We identified a pattern of yellowing of the infusion sets upon storage and revealed that it originated from the latex part. Extracts of the individual parts of the infusion sets, primarily of the latex, were toxic to the cEND cells leading to induction of apoptosis and cell death. We conclude that infusion sets release extractables that can be toxic to the endothelial cells of the patients that receive infusion. We suggest to use cEND cells for in vitro safety assessment of infusion sets and other medical devices that release extractables to the bloodstream.

1. Introduction

Quality and safety assurance of medical devices is a complex topic. Some medical devices have been studied comprehensively, producing information which was implemented by regulatory agencies, leading to well-defined specifications and testing methods. Unfortunately, this is not the case with most disposable medical devices (DMDs). These items are composed of different polymers (with or without metal parts) and are generally considered inert and harmless, but may occasionally harm both the patient and the medical personnel. The common incidents include functional impairment (e.g., in valves), structural defects and damage (e.g., fractured syringes, obstructed needles), impaired sterility, release of particles or/and of chemical components (extractables) (Anonymous, 1998, 2002; Danielson, 1992; Kurian et al., 2000; Rabinow and Roseman, 2000). To ensure the safety of the DMDs, mechanical, functional, and bio-toxicity tests are used (Anonymous, 2009a,b) that require use of specialized equipment and availability of trained personnel.

In our previous studies we analyzed the effect of storage time on the safety of DMDs (infusion sets, syringes, Foley catheters, etc.) (Schumacher et al., 2007). To this end, we applied analysis of their physical (mechanical analysis, color measurement, etc.) and chemical (analytical assessment of extractables) properties. We revealed substantial deterioration in quality of multiple critical parameters in the analyzed items. Specifically, during storage of infusion sets, color changes (yellowing) were found, indicating that degradation of the plastic polymer components was taking place. Analytical investigation revealed that yellowing was accompanied by release of the potentially toxic chemicals (diethylhexylphthalate, butylated hydroxytoluene, and others) and particles into the extraction solution (Schumacher et al., 2007).

Unfortunately, the composition and the manufacturing process of the DMDs are usually not disclosed by their manufacturers, and it cannot be easily revealed using the existing analytical techniques (e.g., by liquid chromatography or gas chromatography with mass-spectrum detection (Schumacher et al., 2007)). It is generally known that polyvinyl chloride (PVC) is the main component in the infusion sets and IV bags. PVC is brittle and hard, and in most cases plasticizers are added to make the polymer more flexible (Anonymous, 2002). Di(2-ethylhexyl)phthalate (DEHP), a phthalic ester-containing plasticizer, is frequently incorporated into PVC-made parts of medical devices (including infusion sets) (Jaeger and Rubin, 1970). DEHP is not bound chemically to the PVC and can

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be released/extracted from the polymer under certain conditions (Lay and Miller, 1987). It has been reported that DEHP is hepatotoxic, carcinogenic, teratogenic and mutagenic (Ganning et al., 1984; Hill et al., 2003; Kluwe et al., 1982). Therefore, the FDA warns that patients treated frequently with PVC-containing DMDs, including the infusion sets, may be exposed to high doses of DEHP, and thus can be exposed to health risks associated with DEHP use (Anonymous, 2002).

Similarly, toxicity can evolve from exposure to the components contained in the latex rubber, such as proteins (that might cause Type I IgE-mediated immediate allergic reactions) or chemical additives (e.g., carbamates, thiurams, mercaptobenzo-thiazole, and other chemicals that might cause Type IV T-cell mediated delayed hypersensitivity reactions) (Brehler et al., 2002; Curtis and Klykken, 2008). These materials can be released from the latex-containing DMDs, such as the infusion sets from some manufacturers, during their clinical use, but the extent of their release and their relative toxicity is not clear.

During their use, infusion sets usually are in prolonged contact with the patient and can lead to his exposure to these chemicals and particles. Thus, assessment of potentially toxic consequences of this exposure that should be taken into account when stipulating the safety and the shelf-life of the infusion sets. Potential toxicity of infusion sets is usually assessed based on the guidelines specified by regulatory authorities, such as The United States Pharmacopoeia (USP) (Anonymous, 2009a,c) or ISO (Anonymous, 2009b). For instance, extracts from individual parts of the analyzed infusion sets are incubated with L-929 mouse fibroblast cells grown in monolayer in vitro and pass the test if they produce grade 2 (mild toxicity) or lower response. L-929 cells were selected for in vitro toxicity testing by the regulatory authorities based on their robust growth in culture under regular conditions (using the regular type of tissue culture media and common tissue culture equipment) as well as extensive experience of safety testing using this cell line. However, the L-929 cells may not be ideally suited for safety assessment of the infusion sets. This is because the cells that become exposed to the extractables from the infusion sets during their use are predominantly the cells that are lining the blood vessels (endothelial cells) and those present in the bloodstream (cells of the lymphoid and myeloid origin), and not fibroblasts (cells of the mesenchymal connective tissue).

Several research groups have assessed in vitro safety testing of DMDs using cell lines that are exposed to the leachables in vivo. For instance, human endothelial cells were used for safety assessment of polyetherurethanes (Bordenave et al., 1993), human urothelial cells from biopsies (primary cultures) were used for safety assessment of extracts from Foley (urinary) catheters (Pariente et al., 2000). In a similar fashion, human dendritic cells from human peripheral blood mononuclear cells (PBMCs) (Mueller et al., 2009) and THP-1 human monocytic cells (Pick et al., 2004) can be used to analyze the safety of the extractables that reach the bloodstream.

The objective of this study was to determine the suitability of the cEND brain endothelial cells for in vitro safety assessment of infusion sets and of DMDs in general. The cEND cells are murine immortalized cell line isolated at the laboratory of Prof. Carola Förster (University of Würzburg, Germany). cEND cells grow as monolayer in culture under regular conditions and have distinct morphology that can be affected by chemical treatment, facilitating toxicity assessment and grading. cEND cells are derived from the vascular endothelial cells that (along with the blood and bone marrow cells) are apparently exposed to the highest concentrations of potentially toxic extractables from the infusion sets during their use in vivo (exposure to extractables during medical procedures is usually assessed based on their content in the patient's blood (SCENIHR, 2008)). Moreover, cEND cells originate from the brain, the organ that can be more sensitive for toxic effects as compared to other organs in the patient's body. We used the cEND cells and the control epithelial cell line (the HeLa cells that are widely used in pre-clinical research) to analyze in vitro safety of extracts prepared from different parts of infusion sets. Infusion sets of different design stored for different periods of time, including the expired samples (i.e., stored beyond their expiration date), were studied using several experimental assays in order to reveal the potential toxicity of the samples and the relative sensitivity of different assays.

2. Materials and methods

2.1. Chemicals and materials

Phenol, sodium azide, tunicamycin, propidium iodide (PI), 7-AAD, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Triton X-100, hydrogen peroxide (H_2O_2), and 2′,7′-dichlorofluorescein diacetate (DCFH-DA) were obtained from Sigma–Aldrich Ltd (Rehovot, Israel). RNAse A was from Invitrogen. Analytical grade DMSO and ethanol were from Bio–Lab Ltd. (Jerusalem, Israel). Samples of the infusion sets that were used in this study were: IS1 (the oldest batch with big latex part; Migada Ltd., Lot M1248M8, expiry October 2003), IS2 (the old batch with small latex part; Amsino Ltd., Lot 081002A S007, expiry July 2007), IS3 (the new batch without latex part; Amsino Ltd., Lot 052508A, expiry April 2013). The samples of these batches were received from The Drug and Medical Equipment Research and Quality Control Laboratory, Israeli Defense Forces, within 3–4 months after their production, and were stored at room temperature prior to their analysis.

2.2. Color measurement

Two-centimeter length samples of the tube were cut out from the studied infusion sets. For the old and the oldest batches, the samples were collected from the parts that were adjacent to or more distant from the latex part. Samples of similar parts were also collected from the new batch (that does not contain the latex part).

The yellowness index (YE 313-98) of the collected samples was determined using a BYK Gardner Spectro-Guide 45/0 Gloss Portable Colorimeter (BYK-Chemie GmbH, Wesel, Germany) based on ASTM E313-00 standard practice for calculating yellowness and whiteness indices from instrumentally measured color coordinates.

2.3. Cells

cEND mouse brain capillary endothelium cells (Förster et al., 2005) and HeLa human cervical carcinoma cells were kept in DMEM supplemented with fetal calf serum (5% for HeLa cells and for propagation of cEND cells, and 1% for culturing & incubation of cEND cells with the studied samples), 4.5 g/L of D-glucose, 2 mM of L-Glutamine, 100 IU/mL of penicillin and 100 μg/mL of streptomycin (all from Biological Industries Ltd., Beit Haemek, Israel). The cells were maintained in an incubator at 37 °C in a humidified atmosphere with 5% CO_2.

2.4. Preparation of extracts and incubation with cells

Samples of individual parts of the analyzed batches were prepared according to the USP monograph <88> (Anonymous, 2009c) and extracts were prepared according to USP monograph <87> (Anonymous, 2009a) with minor modifications. In brief, individual parts of the infusion set (latex, tube and cup) were separated, weighed, cut into pieces (under sterile conditions), and samples of the individual parts were extracted into the cell growth medium (supplemented with 1% FCS for cEND cells, 5% FCS for HeLa cells) in

an incubator for 24 h at 37 °C in a humidified atmosphere with 5% CO_2. The cells were incubated for 24 h with the obtained extracts, with growth medium (negative control) or with growth medium containing 100 mM phenol, 40 mM sodium azide, or 24 μM tunicamycin (positive controls). Representative images of the cells were taken at the end of the incubation period using Nikon Eclipse TS100 fluorescent microscope equipped with Nikon DS Fi1 camera (Nikon, Japan).

2.5. MTT test

The cells were plated on 96-well tissue culture plate (40,000 cells per well). On the next day, the medium was replaced with 150 μL samples of the extracts or of the control solutions and the cells were returned to the incubator for 24 h. At the end of the incubation period, 5 μL of MTT stock solution (5 mg/mL MTT in PBS) were added and the cells were returned to the incubator for additional 3 h. Then, the medium was discarded, 200 μL DMSO were added to each well, and UV absorbance was measured at 570 nm with reference at 655 nm using Infinity 200 PRO plate reader (Tecan Ltd.). Percentages of decrease in the cell metabolic activity in the individual samples were calculated using the following formula: (UV absorbance of the sample/UV absorbance of the negative control) × 100%.

2.6. Apoptosis and cell cycle analysis

The cells were plated on 24-well tissue culture plate (250,000 cells per well). On the next day, the medium was replaced with 500 μL samples of the extracts or of the control solutions and the cells were returned to the incubator for 24 h. Then, the cells were washed with PBS, harvested using trypsin solution (Biological Industries Ltd., Beit Haemek, Israel), washed with PBS and counted.

For apoptosis analysis, 150,000 cells were transferred into an Eppendorf tube, resuspended in 250 μL buffer solution, incubated with 4 μg/mL 7-AAD for 5 min, and analyzed using FACSCalibur Flow Cytometer (Becton Dickinson Biosciences Ltd.) (Telford et al., 2004).

For cell cycle analysis, 150,000 cells were transferred into a 5-mL FACS tube, centrifuged, and the supernatant was discarded. The cells were resuspended in 4.0 mL fixation solution (ice-cold ethanol in DDW, 70:30, v/v) and incubated overnight at −20 °C. Then, the cells were washed with PBS, resuspended in 250 μL of 0.1% Triton X-100 solution, incubated with 100 μg/mL RNAse A and 40 μg/mL PI for 30 min at the room temperature, and analyzed using FACSCalibur Flow Cytometer.

2.7. Formation of the reactive oxygen species

Formation of the reactive oxygen species as a result of exposure of cEND cells to the studied extracts was analyzed using DCFH-DA-based test. This test is based on diffusion of DCFH-DA via biological membranes and its hydrolysis into DCFH by intracellular esterases. In presence of oxidizing agents (e.g., reactive oxygen species or/and H_2O_2), DCFH is oxidized to the fluorescent compound 2′,7′-dicholorofluorescein (DCF) that can be quantitatively measured using appropriate equipment (Rota et al., 1999).

The extracts from the individual parts of the analyzed batches were prepared as described in Section 2.4, but using medium without FCS. Control solutions of DCF-DA (200 μM) or/and H_2O_2 (2 mM) were prepared using the same serum-free medium. The cEND cells were plated on 96-well tissue culture plate (40,000 cells per well). On the next day, cells were washed with PBS and 150 μL samples of the extracts or of the control solutions were added to the wells and the plates were returned to the incubator. The fluorescence of the wells (at 485 nm excitation and 520 nm emission) was measured at several time-points (1.5, 2, and 2.5 h) using Infinity 200 PRO plate reader (Tecan Ltd., Switzerland).

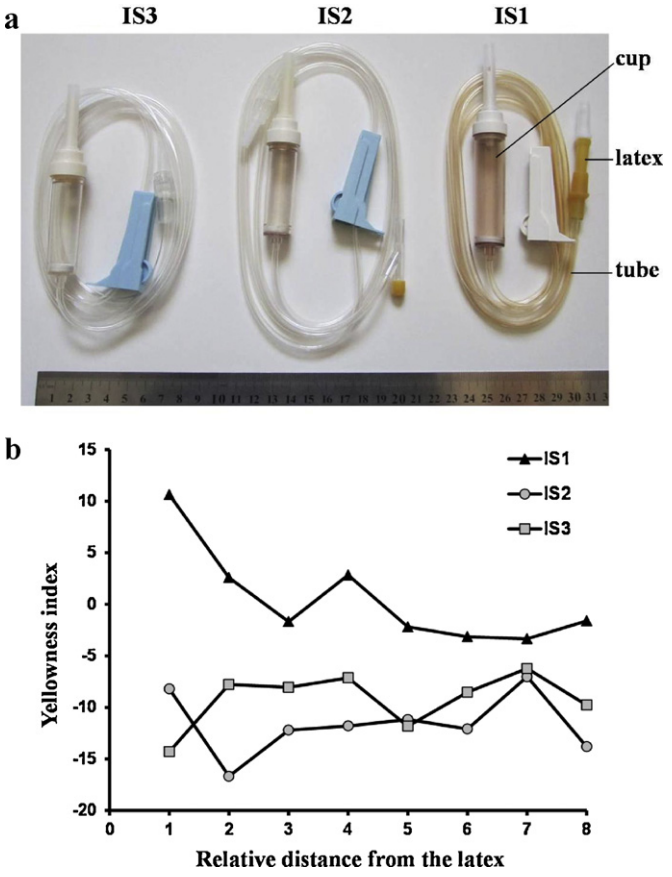

Fig. 1. The studied infusion sets: photograph of the individual samples (a) and the tube color measurement results (b).

2.8. Statistical analysis

The experiments were performed in triplicates, were repeated at least three times, and the outcomes of one representative data set are reported. The data are presented as mean ± standard deviation. Differences in the studied parameters between the individual treatments vs. the appropriate control were analyzed using the two-tailed Kruskal–Wallis ANOVA with Dunnett post-test using InStat 3.0 software (GraphPad Software Inc.). *P* value of less than 0.05 was considered significant.

3. Results

3.1. Appearance of the infusion sets

The studied infusion sets consisted of several parts, including the tube, the cup, and the latex (see Fig. 1a). The major difference between the samples was the size of the latex part that was the biggest in the IS1 sample, smaller in the IS2 sample, and absent in the IS3 sample. The IS1 sample exhibited significant yellowing, as compared to the other samples (see Fig. 1a). Quantitative analysis of the tube color revealed that extent of yellowing inversely correlated with the distance from the latex part (see Fig. 1b). Intensity of the yellowing inversely correlated with the size of the latex part and with storage time (data not shown). Moreover, yellowing intensity increased upon storage of the latex-containing infusion sets at the elevated temperature (37 °C, data not shown).

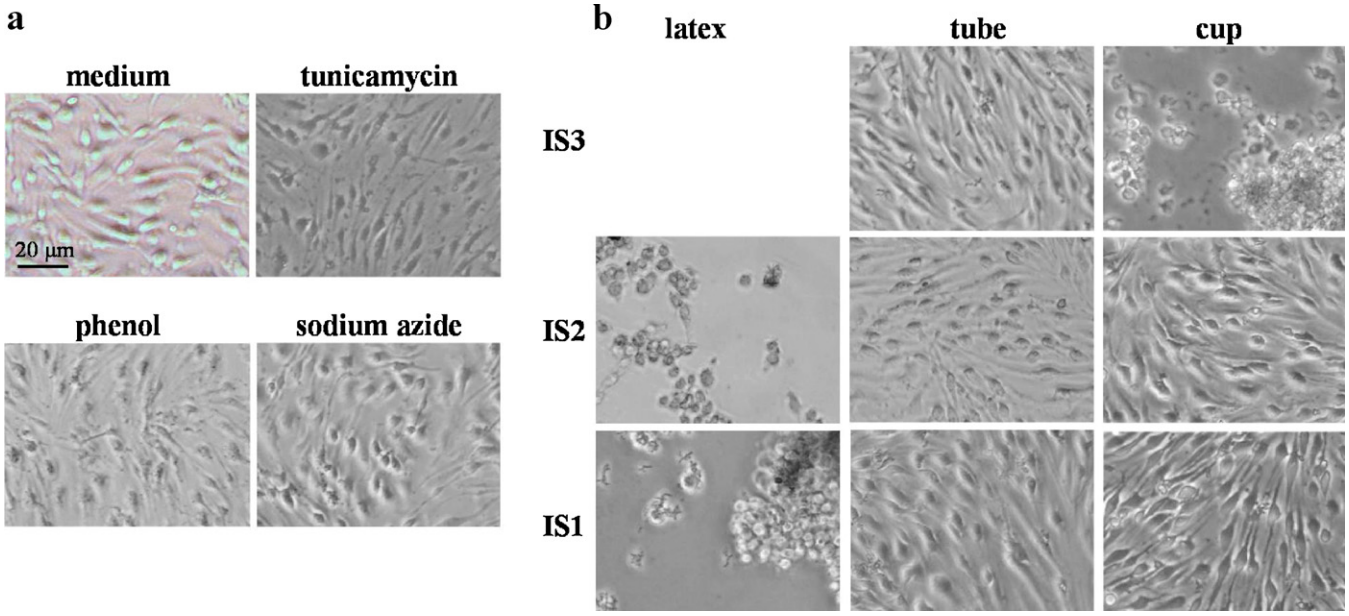

Fig. 2. The morphology of the cEND cells exposed to different treatments: control samples (a), and extracts of the individual parts of the studied samples (b).

3.2. Relative sensitivity of cEND and HeLa cells to the treatments

Both cEND and HeLa cells are immortalized cell lines that are generally suitable for in vitro toxicity testing of infusion sets and other medical disposable items. cEND and HeLa cells have somewhat different morphology, but are similar in size and form adherent monolayer cultures in vitro (see Figs. 2 and 3).

We analyzed the relative toxicity of different apoptosis and cell death-inducing chemicals in cEND and HeLa cells. cEND cells are generally more sensitive to the exposure of toxic chemicals, as can be seen from more profound changes in their morphology and metabolic activity upon exposure to phenol and sodium azide (see Figs. 2–4). Tunicamycin treatment was highly toxic in both cell lines at the exposure conditions that were used in our experiments (24-h incubation with 24 μM tunicamycin).

3.3. In vitro toxicity of the extracts from the infusion sets

Viability of HeLa cells was little affected by the extracts of the analyzed batches. The morphology of HeLa cells was not affected by these treatments (see Fig. 3b), and no significant effects on the apoptosis markers and on the cell cycle were found (data not shown). Outcomes of the MTT test indicate that the metabolic activity of the HeLa cells was moderately inhibited (~15–20%) by the extracts of the latex parts and of the cup from the IS3 sample (see Fig. 4b).

On the other hand, cEND were affected to a higher extent by the applied treatments. Extracts that were moderately toxic in HeLa cells (the latex parts and the cup of IS3) induced major morphologic changes on the cultures of the cEND cells (see Fig. 2b). These outcomes were consistent with the data obtained in other tests, and the same samples indeed induced profound (~80–90%) decrease in

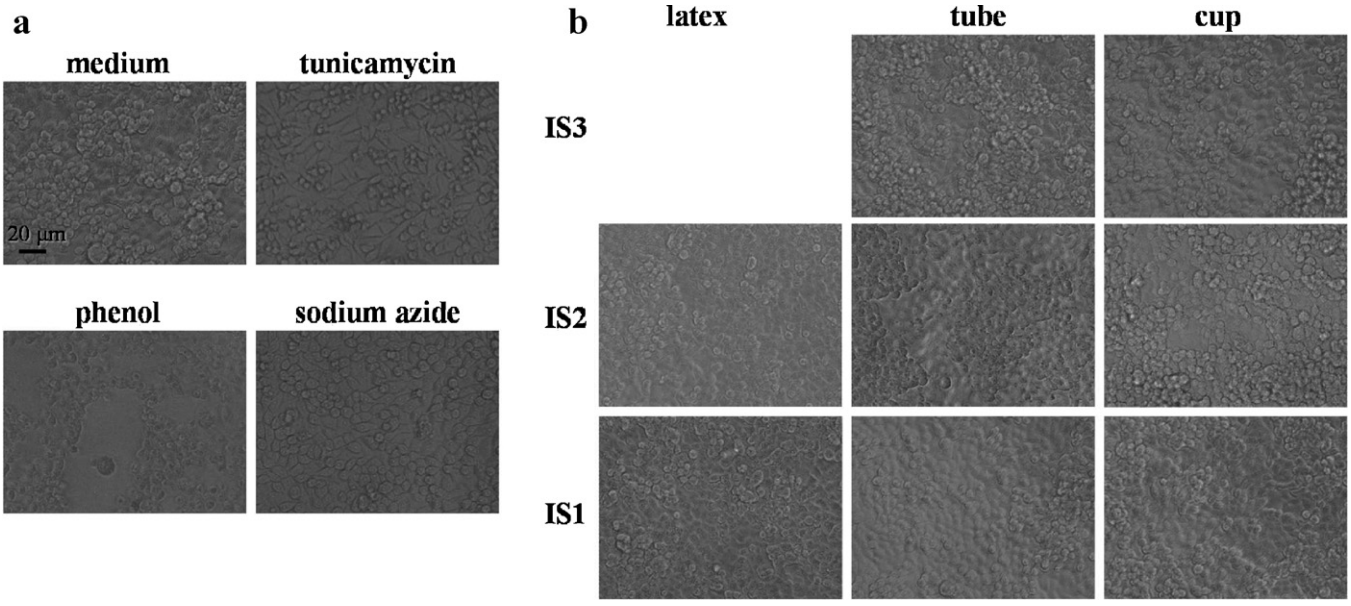

Fig. 3. The morphology of the HeLa cells exposed to different treatments: control samples (a), and the extracts of the individual parts of the studied samples (b).

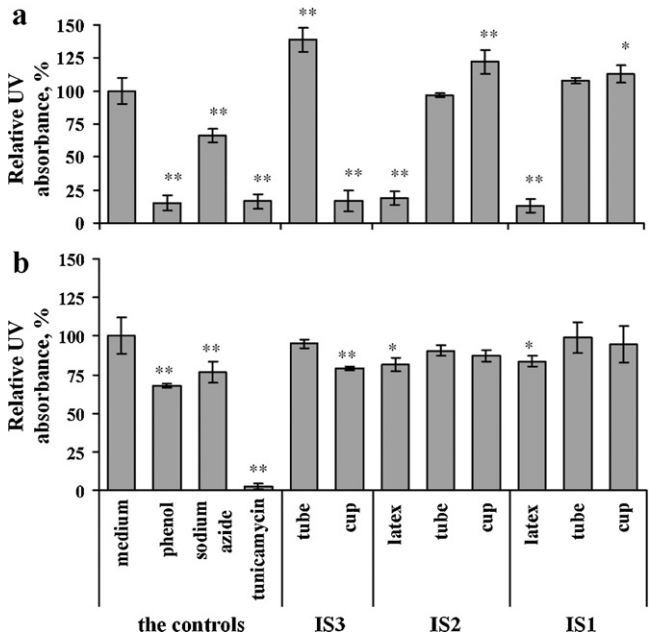

Fig. 4. The outcomes of the cell viability analysis using the MTT test: cEND cells (a) and HeLa cells (b) incubated with the control samples and the extracts of the individual parts of the studied samples. Statistical analysis was performed using two-tailed Kruskal–Wallis ANOVA with Dunnett post-test (vs. the 'medium' control group, $^{*}P<0.05$, $^{**}P<0.01$).

the metabolic activity of cEND cells measured by the MTT test (see Fig. 4b). Profound death of the cEND cells following these treatments precluded quantitative analysis of the cell apoptosis and the cell cycle changes in the above-mentioned samples (see Fig. 5).

For the cEND cells, the outcomes of the apoptosis testing were generally consistent with the outcomes of the MTT test, and inhibition of the cells' metabolic activity correlated with increased apoptosis (see Figs. 4a and 5a). In some of the studied samples (the IS3 tube, IS2 and IS1 cups) the outcomes of the MTT test were higher as compared to the negative control sample (medium only), and for the cup of IS1 the extent of apoptosis did not correlate with the metabolic activity (see Figs. 4a and 5a). The reason for these discrepancies is not clear, but apparently all these samples, except of the IS1 cup, exerted limited toxicity on the cEND cells.

Cell cycle analysis did not reveal significant effects, except those that induced profound cell death and precluded quantitative analysis of the cell cycle changes (e.g., IS1 latex, see Fig. 5b). On the other hand, extracts from all the analyzed parts of the infusion sets induced prominent formation of the reactive oxygen species, as can be seen from the outcomes of the DCFH-DA-based assay (see Fig. 5c that presents the data for the 2-h incubation time-point; similar data were obtained for the 1.5 and 2.5 h time-points). The statistical analysis revealed no significant differences between the experimental groups and the positive control (cells incubated with DCFH-DA and H_2O_2). A trend for reduced formation of the reactive oxygen species in the latex samples (albeit it did not lead to statistically significant differences at the analyzed early time points) apparently stems from reduced metabolic activity of the cells due to toxicity of these samples (see Fig. 4b).

Overall, the data obtained in the cEND cells indicate that some treatments (including some of the studied extracts) are highly toxic and induce significant decrease in the cells' metabolic activity and cell death, but apparently have no effect on the cell cycle. The MTT test and the light microscopy were the most informative parameters for in vitro assessment of toxicity of the studied extracts, and the analysis of the apoptosis and of the cell cycle were less informative and resulted in partial outcomes only.

4. Discussion

4.1. Choice of the cell line for in vitro toxicity analysis of infusion sets

In this study we used cEND murine brain endothelial cells to analyze the in vitro toxicity of extracts prepared from infusion sets. We reasoned that these cells may be more appropriate for toxicity assessment of infusion sets, since vascular endothelial cells in the cannulated vein can be exposed to higher amounts of extractables during the use of infusion sets in patients, as compared to other cell types, (including the cells in the bloodstream that are apparently exposed to diluted extractables).

Generally, there are several types of vascular endothelial cells that can be used for in vitro safety assessment of infusion sets. Primary (non-immortalized) cells can be isolated from large blood vessels (such as human umbilical vein endothelial cells, HUVEC) (Baudin et al., 2007) or from the brain of mice (Wu et al., 2003), rats (Lu et al., 2005), cows (Cecchelli et al., 1999), and other animals. Several lines of immortalized vascular endothelial cells are also available, including cEND (Förster et al., 2005) and b.End3 (Montesano et al., 1990) mouse brain endothelial cells, RBE4 rat brain microvessel endothelial cells (Roux et al., 1994), human brain microvascular endothelial cells (HBMEC) (Greiffenberg et al., 1998), etc.

However, many of these cell types are not suitable for in vitro safety assessment of infusion sets which requires stable cell line with easy, consistent and rapid growth and propagation. In addition, a robust assay requires cells with certain sensitivity (not too high and not too low) to the toxic compounds and distinct morphology that is altered due to exposure to these compounds. Therefore, the majority of the above-mentioned cell lines, including all primary and some of the immortalized cell lines are probably not suitable for safety assessment of the infusion sets. In our preliminary experiments, robustness of the assay was low for HBMEC cells that require plate coating and growth supplements for their culturing, and for the b.End3 cells that were characterized by excessive sensitivity to all the types of studied extracts (data not shown). HeLa cells, that were used as a control cell line in this study (a widely used epithelial cell line that does not originate from the vascular endothelium), are characterized by robust growth but are not sensitive enough to toxic treatments (see Figs. 3 and 4b).

4.2. In vitro toxicity analysis of infusion sets using cEND cells

As opposed to the above-mentioned cell lines, cEND cells appear to be highly suitable for toxicity testing of the infusion sets and of the DMDs in general. This is because cEND cells grow rapidly and consistently on regular (uncoated) plates in a regular growth medium (without special growth supplements) and in regular conditions. These cells grow in monolayer and have a characteristic morphology that changes substantially following exposure to toxic chemicals (see Fig. 2). Moreover, the growth of the cEND cells is moderately sensitive to the presence of toxic compounds, and therefore allows differentiation between the effects of non-toxic vs. moderately toxic vs. highly toxic samples (see Fig. 4a).

In this study we used cEND cells to determine the relative toxicity of the extracts prepared from the individual parts of infusion sets. We studied samples of infusion sets from different manufacturers that varied in design and storage time. To gain more detailed insights into safety of the studied samples, we applied both qualitative (morphology changes, as specified by the regulatory guidelines) and quantitative (MTT test, formation of reactive oxygen species, apoptosis and cell cycle assays) tests. Based on the experimental outcomes obtained in this study, both changes in the cEND cells' morphology and the MTT test are suitable for

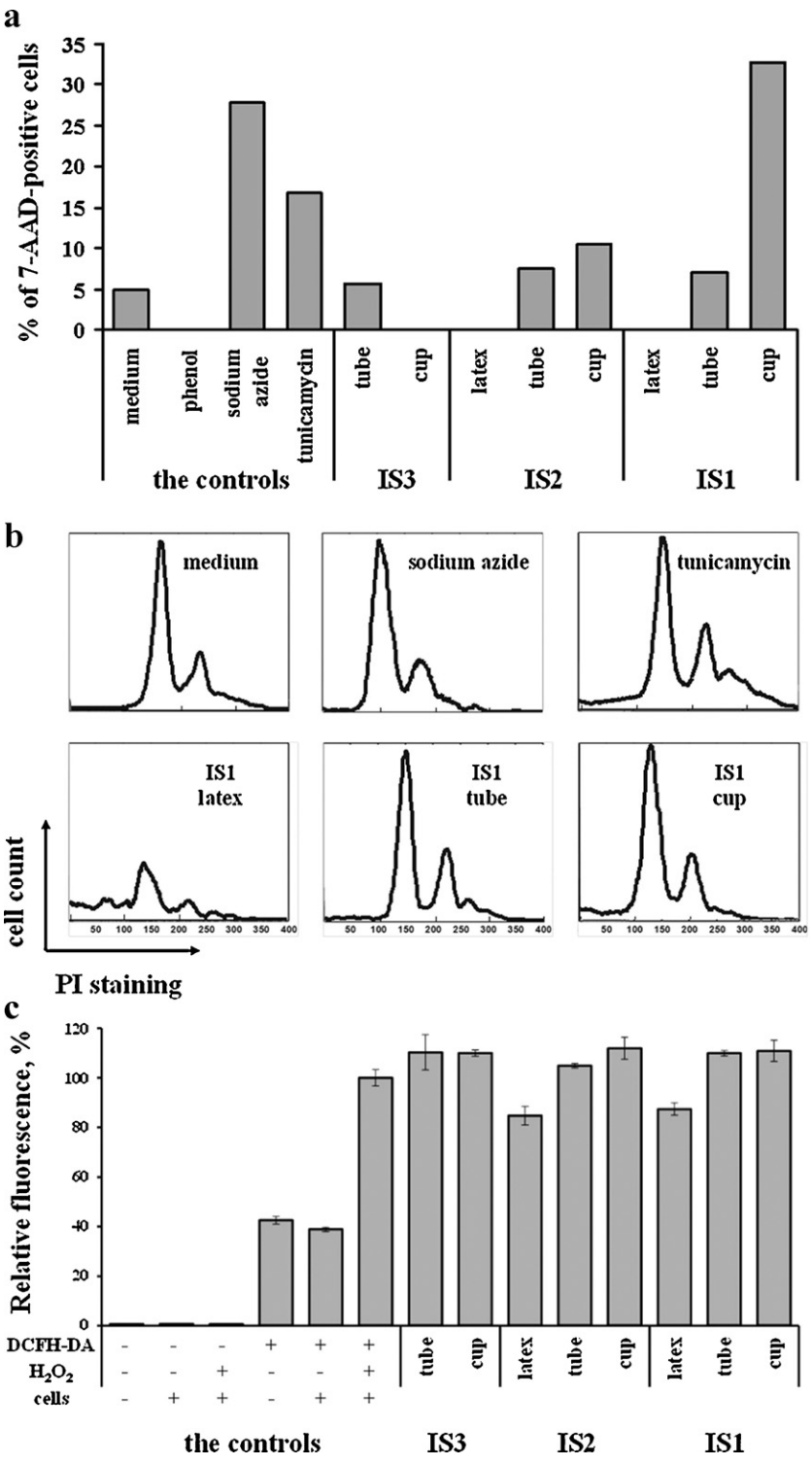

Fig. 5. Toxic effects of the control samples and the extracts of the individual parts of the studied samples on the cEND cells: the outcomes of FACS analysis of the cell apoptosis (a) the changes in the cell cycle (b), and formation of the reactive oxygen species (c). Statistical analysis of the reactive oxygen species formation (two-tailed Kruskal–Wallis ANOVA with Dunnett post-test) revealed no significant differences between the experimental groups and the positive control (cells incubated with DCFH-DA and H_2O_2).

in vitro toxicity evaluation of the infusion sets. Robustness of the apoptosis and the cell cycle assays was not sufficient for analysis of the studied samples and the use of these assays for the safety assessment is not recommended. Outcomes of the DCFH-DA test indicated that all the studied extracts efficiently induced formation of reactive oxygen species already at the early time points (~2 h) that did not induced cell toxicity in majority of the studied samples at the later time points (24 h, all the samples except the latex parts). Therefore, formation of reactive oxygen species does not appear to be the driving force for the cell toxicity and is not recommended for the safety assessment of the studied samples.

4.3. Potential toxicity of the studied infusion sets

In vitro toxicity analysis of samples in cEND cells indicates that the latex part is the most toxic part of the studied infusion sets (see

Figs. 2 and 4a). This conclusion is supported also by the outcomes of MTT test in HeLa cells (see Fig. 4b). Therefore, extracts from the latex are toxic to the cells in vitro and can potentially produce toxic effects during the use of the infusion sets in patients. On the other hand, yellowing of the tube and the cup during storage of the infusion sets (due to the leachables originating from the latex part) is not associated with increased toxicity, apparently due to limited toxicity of these leachables, or their low extractability to the medium during the applied sample preparation procedure. Thus, increased safety of the infusion sets can be attained by reducing the size or entirely eliminating the latex part, although this reduces the convenience of bolus drug dosing to the patient that receives infusion via the infusion set. Indeed, these changes in the design of the infusion sets have been made by several manufacturers (see Fig. 1), and some of the infusion sets that are currently in use in emergency medicine and in hospital settings are lacking the latex part.

Age of the studied samples (e.g., the parts from the IS1 and IS2 produced by the same manufacturer) had no major effect on their relative toxicity (see Fig. 4), and does not appear to be a major factor that determines the sample toxicity and the infusion sets shelf-life. This conclusion should be verified in future studies by analyzing the safety of the samples (latex and non-latex parts) from higher number of batches stored for different periods of time, and in forced degradation conditions. In addition to the latex, other parts of the infusion sets can also be toxic, even in the infusion sets that are far from approaching the end of their shelf-life (e.g., the IS3 cup, see Figs. 2, 4a and 5a). We plan to perform extensive in vitro toxicity characterization of infusion sets produced by different manufacturers using cEND cells in order to reveal their relative safety. Subsequently, we plan to perform analytical analysis of the extracts of the toxic parts (Schumacher et al., 2007) in order to identify and quantitate the toxic compounds and to set the limits of their content in the infusion sets.

5. Conclusions

We conclude that the studied infusion sets can release extractables that can be toxic to the endothelial cells of patients who receive infusion. cEND mouse brain endothelial cells appear to be appropriate for in vitro analysis of infusion sets toxicity and we suggest to use them to analyze the safety and stability of infusion sets produced by different manufacturers and their deterioration with storage. cEND cells can be also applied for testing of additional disposable items that release potentially toxic compounds into the bloodstream, such as Venflon cannulas, winged scalp vein sets, etc. In general, we suggest to perform in vitro toxicity analysis of disposable medical devices using suitable immortalized cell lines from the cells that can be exposed to the extractables during the clinical use of the specific device. For instance, assess the safety of infusion sets using immortalized cell lines from the vascular endothelium (such as cEND), lymphoid and myeloid cells; assess the safety of the Foley (urinary) catheters using urinary epithelial cells, etc.

Conflict of interest statement

The authors have declared no conflict of interest.

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