Human mesenchymal stem cells as a novel platform for simultaneous evaluation of cytotoxicity and genotoxicity of pharmaceuticals

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Abstract

The in vitro micronucleus test is a well-known test for the screening of genotoxic compounds. However until now, most studies have been performed on either human peripheral lymphocytes or established cancer cell lines. This study provides human mesenchymal stem cells as an alternative to the conventional micronucleus test. We grew umbilical cord mesenchymal stem cells (UC-MSCs) on coverslips eliminating the cumbersome technique involving hypotonic treatment, fixation and preparing smears required for suspension culture (lymphocytes). The background frequency of nuclear blebs and micronuclei in UC-MSCs was found to be 7±5, in lymphocytes 16±3.5 and 9±3 and that for A549 cell line was 65±5 and 15±5 per 1000 cells, respectively, suggesting differences in the repair mechanism of normal and cancer cell lines. We inspected the cytotoxic and genotoxic effects of two known mutagens, mitomycin-C and hydrogen peroxide (H₂O₂), on UC-MSCs, lymphocytes and A549 cells. Treatment with mitomycin-C and H₂O₂ demonstrated drastic differences in the degree of cytotoxicity and genotoxicity suggesting a constitutional difference between normal and cancer cells. In addition we tested two solvents, dimethyl sulfoxide (DMSO) and ethanol, and two drugs, metformin and rapamycin. DMSO above 1% was found to be cytotoxic and genotoxic, whereas ethanol at same concentration was neither cytotoxic nor genotoxic indicating the minimal non-toxic level of the solvents. This study thus offers UC-MSCs as a better substitute to peripheral lymphocytes and cancer cell lines for high throughput screening of compounds and reducing the animal studies.

Introduction

The presence of nuclear blebs (NBs) and micronuclei (MNs) within cells is an indicator of genetic instability and predictable of risk for various cancers and degenerative diseases (1–4). Scoring MN is a well-known tool for genotoxicity testing. The cytokinesis block micronucleus assay (CBMN) using peripheral lymphocytes has been used as the most efficient approach to scoring MN. However, it suffers from various drawbacks such as requiring phytohaemagglutinin (PHA) stimulation for growing lymphocytes for one cell cycle division in order to avoid false negative results due to cytotoxicity, and culture conditions along with selection of proper donors (5). Therefore there is a need to look for alternative methodologies to study genotoxicity employing normal diploid cell cultures of human origin. Other sources for the in vitro micronucleus test (IVMT) are rodent cell lines (CHO, V79, CHL and L5178Y), which are already validated for genotoxicity testing; however these cell lines are not of human origin (5). To circumvent use of animal cell lines, several human origin cell lines such as TK6 lymphoblastoid cell line (6) and HepG2 cells (7,8) are used for genotoxicity studies. Moreover most of these cell lines are usually deficient in p53, DNA repair or apoptotic genes, which can lead to higher MN and hence most of the results have not been confirmed by in vivo studies (9).
Recently human mesenchymal stem cells (hMSCs) have been isolated from various postnatal and perinatal tissues such as bone marrow, adipose tissue, dental pulp, placenta, umbilical cord and amniotic membrane (10–14). Isolation of MSCs from the perinatal tissue is more advantageous due to easy availability, isolation with abundant cell supply and less ethical issues. These cells represent the normal cell type. In this study, we examined the potential of UC-MSCs for simultaneous assessment of cytotoxicity and genotoxicity of known mutagen (mitomycin-C and H2O2) and commonly used drug solvents (dimethyl sulfoxide (DMSO) and ethanol). Our studies project a platform for an easy, rapid, reliable and cost-effective screening of toxic drugs compounds.

Materials and methods

Cell culture

Human umbilical cords (n = 5) and blood were collected with the informed consent of the patients who were aged between 26 and 32 years. We followed guidelines of Institutional Ethics Committee (IEC) Manipal Hospital, Bangalore, India, for collecting the samples (UC-MSCs and blood). We followed the methodology previously reported by our group (12) for isolating UC-MSCs. Briefly, arteries and veins were removed from the cord tissue and washed with PBS (pH 7.2). Cord was cut into 10-cm length and processed for enzymatic digestion by using 1 mg/ml collagenase and dispase (7:1) for 30 min and subsequently adding 0.25% trypsin for 5 min. After neutralisation, the MSCs were centrifuged at 1800rpm and supernatant was discarded. Pellet was suspended in minimum essential medium (alpha MEM) (Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS). The above steps were repeated thrice. Finally, the plated cells were moved to a 37°C CO2 incubator (Thermo Scientific Inc., OH, USA). Human lung carcinoma cell line (A549) was purchased from National Centre of Cell Science Pune. The cells were cultured in alpha MEM, supplemented with 10% FBS (HyClone) and maintained in a 37°C CO2 incubator (Thermo Scientific Inc.).

Lymphocytes were isolated using Lymphoprep™ (AXIS-SHIELD) according to the manufacturer's instructions. Briefly, blood was diluted with an equal volume of 0.9% NaCl and carefully layered over Lymphoprep™ in a 50-ml falcon tube and centrifuged at 800g for 30 min at room temperature (RT). After centrifugation a distinct band of mononuclear cells formed at the interface, which was removed with a pipette. Finally, the harvested fraction was diluted by the tryphan blue method instead of MTT. After 48 h treatment period, viability was calculated as percentage by exposure to drugs? For adipogenesis, osteogenesis and chondrogenesis, UC-MSCs were fed with respective differentiation media containing metformin (0.5, 1.0 and 2.5 mM) and rapamycin (500 pM, 500nM and 1 μM) for 21 days and stained as stated above.

Proliferation rate determination

Cytotoxicity was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (12). Fifty hundred cells/well were seeded into 96-well plates in 200 μl of growth medium in triplicate. After 2 days mitomycin-C (Sigma Aldrich), hydrogen peroxide, DMSO (Sigma Aldrich), ethanol, rapamycin (Sigma Aldrich) and metformin were added for 48h. After the treatment period, the supernatant was removed and 1 ml of final concentration MTT was added to each well and incubated for 3h at 37°C in a humidified incubator (Thermo Scientific Inc.). The reaction was terminated by removing MTT and adding 200 μl of DMSO to dissolve the formazan salt. The light absorbance was measured at 570nm. All the experiments were performed thrice in triplicate.

Viability was calculated in terms of percentage by the formula: 100 × OD of the treated sample/OD of the control sample. Control was assumed as 100% viable and cytotoxicity was calculated as Control – treated sample.

For population doublings, cells were seeded at inoculum density and harvested every 48 h. The population doubling time was calculated by the formula: TD = tln2/l(\ln N – lnNI). NI was the inoculum cell number; NH is the cell harvest number and t is the time of the culture (h). The experiment was performed thrice in triplicate. Mean and standard deviation were also calculated (16).

In vitro micronucleus test

The MSCs were grown on coverslips in four well and 24-well plates and after 2 days the medium was replaced with growth medium for another 48 h. After the treatment period, cells were fixed directly by adding 4% PFA for 30 min at RT or at 4°C for overnight to weeks. After fixation cells were treated with triton-X for permeabilisation for 1h at RT and stained with Giemsa (0.4%) for 30 min. MNs and NBs were analysed blind microscopically (bright field microscope with ×100 objective). The number of NBs and MNs per 1000 cells was recorded. All the experiments were performed thrice in triplicate. We followed the Organisation for Economic Co-operation and Development (OECD) guidelines 487 and all the parameters for

Immuno phenotyping analysis of UC-MSCs using flow cytometry

UC-MSCs from passages 5 were immunophenotypically analysed by flow cytometry for CD surface markers such as CD14, CD19, HLA-DR, CD90, CD105 and CD44 (12). MSCs were trypsinised and fixed in paraformaldehyde (PFA). For CD markers profile analysis, PFA was removed and MSCs were washed with PBS and incubated with mouse anti-human FITC/PE conjugated antibodies against CD14, CD19, HLA-DR, CD90, CD105 and CD44 for 1h on ice (all antibodies was purchased from Becton Dickinson, San Diego, CA, USA). Finally, the cells were identified using a flow cytometry laser 488 nm and analysed for 10 000 gated events using BD Cellquest Pro software.

Multipotent differentiation potential

MSCs were induced to differentiate into the trilineage by methodology published elsewhere and from our lab with UC-MSCs and placenta-derived mesenchymal stem cells (PD-MSCs) (12). The detailed methodology is described as below:

For adipogenesis, UC-MSCs were induced for 21 days with differentiation media containing dexamethasone 1 μM, IBMX 0.5 mM, insulin 1 μg/ml and indomethacin 100 μM (all reagents from Sigma Aldrich, St Louis, MO, USA). After 21 days, formed oil droplets were stained with Oil Red O to confirm adipogenesis (12).

For osteogenesis, MSCs were fed for 21 days with induction media containing dexamethasone 10 mM, ascorbic acid 50 μg/ml, beta glycerol phosphate 10 mM (all reagents from Sigma Aldrich). After 3 weeks, calcium-phosphate mineral deposition was detected by von Kossa staining (12).

For chondrogenesis, UC-MSCs were induced using STEMPRO Chondrogenesis Differentiation Kit (Invitrogen) according to the manufacturer’s instructions. After 14–17 days of induction differentiated cells were stained with Alcian blue 8GX (Sigma) (16).

Is hMSCs differentiation compromised by exposure to drugs? For adipogenesis, osteogenesis and chondrogenesis, UC-MSCs were fed with respective differentiation media containing metformin (0.5, 1.0 and 2.5 mM) and rapamycin (500 pM, 500 nM and 1 μM) for 21 days and stained as stated above.

Is hMSCs differentiation compromised by exposure to drugs?
evaluating NBs and MNs were considered as reported by Fenech (17). Lymphocyte smear were made on glass sides and stained with Giemsa (0.4%) for 30 min. NBs and MNs were analysed as stated above. The experiment was performed thrice in triplicate.

Senescence assay (β-galactosidase staining)
UC-MSCs were cultured in 12-well plates and the senescence assay was done using Senescence β-Galactosidase Staining kit (Cell Signaling Technologies, Danvers, MA, USA) according to the manufacturer's instructions. Experiments were performed three times in triplicate.

Statistical analysis
Data are presented as mean ± standard error of the mean. Statistical comparisons were performed using the student's t-test and analysis of variance. *P <0.05 and ** P < 0.01 were considered statistically significant.

Results
Isolation and expansion of UC-MSCs
Our collagenase and dispase digestion method for MSCs isolation from 10-cm long human umbilical cord resulted in six confluent T25 flasks within 4–6 days of isolation. Morphologically these MSCs were plastic adherent and exhibited fibroblast appearance with abundant cytoplasm and large nuclei as shown in Figure 1 (C1 and C2). Further we characterised them for their immunophenotypical marker profile by flow cytometry for both mesenchymal and haematopoietic markers. UC-MSCs were immunopositive for CD90 (~98%), CD105 (~77.7%), CD44 (~81.45%) and immune-negative for HLA-DR (~99%) and haematopoietic markers CD19 (~99%) and CD14 (~99 %) as shown in Figure 1B. We further showed that these UC-MSCs could be differentiated towards adipogenic, osteogenic and chondrogenic lineage by specific staining as represented in Figure 1 (A1, A2, A3).

Treatment duration
The cell cycle time is usually variable among the different donors. According to the OECD guidelines, one cell cycle replication is necessary for defining the treatment duration of the test compound. The population doubling time was calculated for UC-MSCs and found to be 25.24±2.24 h for n = 3 samples and therefore 48 h treatment time was designated for UC-MSCs.

Assessment of the background frequency of NBs and MNs in UC-MSCs and A549 cell line
The major drawback usually associated with the most of the cell lines and primary cultures is the background MN frequency. We used A549 cell line as a positive control. We have evaluated the background frequency of NBs and MNs of UC-MSCs (Figure 1,
C1 and C2), lymphocytes (Figure 1, D1, D2 and D3) and A549 cell line (Figure 1, E1 and E2) per 1000 cells by the IVMT. We observed between 7 ± 5 NBs and MNs per 1000 cells (n = 5) of UC-MSCs (P < 0.001), 16 ± 3.5 NBs and 9 ± 3 MNs per 1000 cells (n = 3) of lymphocytes (P < 0.001) and 65 ± 5 NBs and 15 ± 5 MNs per 1000 cells of A549 cells in 48 h as shown in Figure 1F. Moreover, we also observed large number of abnormal nuclear shape in A549 cell line (Figure 1, E3), whereas UC-MSCs exhibited normal nuclear shape. Furthermore we checked the back ground NBs and MNs frequency of these UC-MSCs from passage 4 to 10 and found no increase or decrease in the number of NBs and MNs (data not shown).

Cytotoxic and genotoxic effect of mitomycin-C on UC-MSCs, lymphocytes and A549 cell line

We have checked the cytotoxic effect of various concentration of mitomycin-C on UC-MSCs, lymphocytes and A549 cell line as shown in Figure 2A. Cytotoxicity was directly proportional to the mitomycin-C concentration. Mitomycin-C treatment (0.1 µg/ml) resulted in 23.3, 31.84 and 36.7% cytotoxicity (P < 0.001) and 0.3 µg/ml mitomycin-C resulted in 25.9, 39.16 and 45.24% cytotoxicity (P < 0.001) in UC-MSCs, lymphocytes and A549 cells, respectively. We further noticed that mitomycin-C resulted in an increase in the number of NBs and MNs in a dose-dependent manner in UC-MSCs, lymphocytes and A549 cells, as shown in Figure 2B and C. Mitomycin-C (0.1 µg/ml) treatment resulted in ~4, 2 and 1.2-fold increases in the number of NBs (P < 0.001) and 4, 3 and 10 fold increases in the number of MNs (P < 0.001) in UC-MSCs, lymphocytes and A549 cells, respectively.

Cytotoxic and genotoxic effect of hydrogen peroxide on UC-MSCs, lymphocytes and A549

We observed that 146 µM H2O2 resulted in 33.4, 34.2 and 52.5% reduction and 292 µM H2O2 resulted in 49.267, 43.19 and 69.2% reduction in viability of UC-MSCs, lymphocytes and A549 cells (P < 0.001), respectively, as shown in Figure 2D. We analysed its genotoxic effect and observed that 29.2 µM H2O2 was non-genotoxic whereas 58.4 µM and 146 µM H2O2 were slight genotoxic to UC-MSCs, lymphocytes and A549, but the difference was not statistically significant. However 292 µM H2O2 showed statistically significant genotoxicity in UC-MSCs (P = 0.01) and lymphocytes (P < 0.0089) as evident by the increase in the number of NBs and MNs as shown in Figure 2E and F. H2O2 at 292 µM was highly cytotoxic to A549 cells so its genotoxic effect was not examined.

Cytotoxic and genotoxic effect of DMSO and ethanol on UC-MSCs, lymphocytes and A549 cells

We observed 2 and 3% DMSO to be highly cytotoxic leading to 42.25, 39.41 and 40.65%, and 63.63, 54.43 and 60.93% cell death in UC-MSCs, lymphocytes and A549 cells (P < 0.001), respectively, as shown in Figure 3A. DMSO at 0.5 and 1% does not cause genetic
damage as evidenced by the lower number of NBs and MNs as shown in Figure 3B and C. However DMSO above 1% showed slight genotoxicity in UC-MSCs ($P = 0.0018$) and lymphocytes ($P = 0.02$), but in A549 cells the difference was not statistically significant.

In addition to DMSO, we assessed the cytotoxic and genotoxic effects of ethanol on UC-MSCs. We observed 0.05% (8.565 mM) ethanol to be non-cytotoxic whereas 0.5% (85.65 mM) ethanol was ~8.7, 17.1 and 8.27% cytotoxic and 1% (171.3 mM) was 12.43, 24.62 and 11.45% cytotoxic to UC-MSCs, lymphocytes and A549 cells ($P < 0.001$), respectively. The 5% (856.5 mM) ethanol concentration was ~25, 40 and 20% cytotoxic to the UC-MSCs, lymphocytes and A549 cells ($P < 0.001$) as shown in Figure 3D. Furthermore we also observed slight decreases in the number of NBs and MNs with the above-mentioned ethanol concentrations with all the three different types of cells as compared to control as shown in Figure 3E and F, but differences were not statistically significant.

Cytotoxic and genotoxic effect of metformin and rapamycin on UC-MSCs, lymphocytes and A549 cells

We observed 1 mM metformin resulted in 17% cytotoxicity in UC-MSCs and lymphocytes and 37.4% in A549 cells and 2.5 mM metformin led to 22, 26 and 44.5% cytotoxicity in UC-MSCs, lymphocytes and A549 cells ($P < 0.001$), respectively, as shown in Figure 4A. Furthermore we also observed a gradual increase in the number of NBs in UC-MSCs ($P < 0.001$) and lymphocytes ($P = 0.028$) above 0.5 mM concentration of metformin. However in A549 cells no increase of NBs was observed. Moreover there was only a slight increase in the number of MNs with the increasing concentration of metformin with all the three different cells used in this study; however the difference was not very significant as shown in Figure 4B and C.

We observed 500 nM and 1 µm rapamycin was 30–40% cytotoxic to UC-MSCs, lymphocytes and A549 cells ($P < 0.001$), respectively, as represented in Figure 4D. Furthermore there was no significant increase in the number of NBs and MNs in UC-MSCs and lymphocytes with the different concentrations of rapamycin (0.1, 0.5, 1 and 500 nM) whereas with A549 there was significant increase ($P < 0.001$) in the number of NBs and MNs as shown in Figure 4E and F.

Analysis of senescence in UC-MSCs after exposure to metformin and rapamycin

In order to understand whether a drug can induce senescence in UC-MSCs we performed β-galactosidase staining. We checked the different concentrations of rapamycin (0.5, 500 and 1000 nM) and metformin (0.5, 1.0 and 2.5 mM). We observed no morphological change in the UC-MSCs when treated with drugs continuously for 7 days (two passages) without any signs of senescence, as evidenced by β-galactosidase staining (data not shown).

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**Fig. 3.** Cytotoxic effect of DMSO and ethanol on UC-MSCs, lymphocytes and A549 cell line (A, D). Dose-dependent effect on the frequency of nuclear blebs induced by DMSO and ethanol on UC-MSCs, lymphocytes and A549 cell line (B, E). Dose-dependent effect of DMSO and ethanol on the generation of MN on UC-MSCs, lymphocytes and A549 cell line (C, F).
Effect of metformin and rapamycin on the differentiation potential of UC-MSCs

We observed rapamycin at (0.5, 500 and 1000 nM) led to decrease in adipogenesis as shown in Supplementary Figure 1, available at Mutagenesis Online (A1, A2, A3, A4, A5, A6 and A7) and chondrogenesis as represented in Supplementary Figure 1, available at Mutagenesis Online (C1, C5, C6 and C7). Furthermore metformin at 2.5 mM led to cell death in chondrogenesis while 0.5 and 1 mM has shown reduction in chondrogenesis as shown in Supplementary Figure 1, available at Mutagenesis Online (C1, C2, C3 and C4). Moreover osteogenesis was not affected by both the drugs; see Supplementary Figure 1, available at Mutagenesis Online (B1, B2, B3, B4, B5, B6 and B7).

Discussion

Hitherto various types of immortalised cell lines and primary cultures of lymphocytes have been used for cytotoxicity and genotoxicity testing. However isolation of lymphocytes is a tedious procedure accompanied by batch-to-batch variation. Even though the cell lines are easy to maintain, most of them are not of human origin and usually they show abnormal behaviour and phenotypes, which do not reflect the scenario of normal cells. Moreover difference in the protocols of hypotonic treatment, fixation and slide preparation can affect cell integrity and lead to variations in the results (18). Therefore in this study we tested hMSCs for in vitro cytotoxicity as well as genotoxicity evaluations in comparison with lymphocytes cultures and an immortalised cell line. Our MSCs isolation protocol resulted in abundant cell number as evident by the 6 confluent T25 flasks within 4–6 days of isolation from 10-cm long cord. Recently a modified cytokinesis block micronucleus (CBMN) assay was reported with hMSCs for evaluating genetic instability (19). We have simplified and improved the methodology over the traditional technique reported therein, making it more cost effective and suitable for high throughput screening of drugs and/or toxic agents. Advantage has been taken of the glass/plastic adherent nature of MSCs to make coverslip culture suitable for the evaluation of NBs and MNs eliminating the need for trypsinisation, centrifugation, hypotonic treatment, fixative treatment and making smears on slides, which are time consuming, tedious processes and require personal training. Thus the technical simplicity of our model will permit large-scale screening programmes required for assessing cytotoxicity and genotoxicity of pharmaceuticals.

The major drawback usually associated with the most cell lines and primary cultures is the background MN frequency. Lymphocytes and A549 cells are recommended by OECD guidelines and frequently used as an in vitro model for determining genotoxicity (20–22). We observed UC-MSCs had ~1–10 times fewer NBs and equal or three times fewer MNs compared with lymphocytes. Recently we reported fewer NBs and MNs in UC-MSCs compared with PD-MSCs from

Fig. 4. Cytotoxic effect of rapamycin and metformin on UC-MSCs, lymphocytes and A549 cell line (A, D). Dose-dependent effect on the frequency of nuclear blebs induced by rapamycin and metformin on UC-MSCs, lymphocytes and A549 cell line (B, E). Dose-dependent effect of rapamycin and metformin on the generation of MN on UC-MSCs, lymphocytes and A549 cell line (C, F).
the same patient justifying the age (12). There are several reports which state age-related increases in the MN frequency in peripheral lymphocytes (23,24), which can result in batch to batch variation due to donor selection. Furthermore we observed UC-MSCs had ~5–30 times (P > 0.001) fewer NBs and equal or three times fewer MNs whereas lymphocytes had 3–6 times fewer NBs than A549 cells. Consequently high background frequency and abnormal nuclear shape in A549 cell line than that of UC-MSCs and lymphocytes point toward differences in the repair mechanism of normal and cancer cell lines. Recently we have also reported higher numbers of NBs and MNs in HeLa cells compared to UC-MSCs, again indicating the difference in normal and cancer cells (12). Furthermore our data also demonstrate the genetic stability of UC-MSCs till higher passages. Thus easy availability, isolation, abundant cell number and their genetic stability suggest UC-MSCs as a better candidate for genotoxicity screening.

In order to gain more insight for using UC-MSCs for toxicology testing we compared the cytotoxicity and genotoxicity of known mutagens (mitomycin-C and hydrogen peroxide) on UC-MSCs, lymphocytes and A549 cells. The population doubling time for UC-MSCs was found to be 25.24 ± 2.24h therefore 48h treatment time was defined for toxicology testing. We assessed the cytotoxicity by MTT and genotoxicity by the in-vitro micronucleus test. Mitomycin-C is a well-known potent DNA crosslinker acting as a clastogen to result in double strand breaks and has been used as a positive control for various genotoxicity studies (25–27). We observed mitomycin-C (0.3 µg/ml) resulted in 20–30 and 10–15% more cytotoxicity in A549 cells and lymphocytes compared with UC-MSCs. Likewise H₂O₂ has also been used as the genotoxic agent in some validation studies (26, 27). We observed 146 µM and 292 µM H₂O₂ caused ~20 and 30% higher cytotoxicity, respectively, in A549 cells compared with UC-MSCs, while there was no significant difference observed between the UC-MSCs and lymphocytes on cytotoxicity of H₂O₂. Therefore our cytotoxicity results revealed higher toxicity in A549 cells than in UC-MSCs and lymphocytes at same concentration of the test substance (mitomycin-C and H₂O₂). Earlier reports have also stated a higher cytotoxicity caused by mitomycin-C in tumour cells than in normal cells (28). This feature clearly advocates the use of normal cells for cytotoxicity testing instead of cancer cell lines. We further checked the genotoxic effect of mitomycin-C on UC-MSCs and A549 cells. Dose-dependent increases in the number of NBs and MNs were observed with all the three different cells used in this study. However UC-MSCs and lymphocytes exhibited higher numbers of NBs and lower numbers of MNs than A549 cells. It has been reported earlier that NBs and MNs have different mechanistic origins (29). This clearly suggests the difference in the action of mitomycin-C in causing genetic damage in different cells signifying the variation in the repair mechanism of normal and cancer cell lines. Earlier reports by Genies et al. (30) also stated the difference in the response of various cell lines towards benzo[α]pyrene genotoxicity. Thus the selection of cells for cytotoxicity and genotoxicity evaluation is of utmost importance. Therefore our data support the choice of normal diploid cell culture of human origin for correct prediction of mutagenicity of test compounds.

In addition we also tested the two widely used drug solvents, DMSO and ethanol. For genotoxicity testing of many drugs DMSO is the most commonly used solvent. We observed that DMSO altered the morphlogy of UC-MSCs and resulted in elongation of the cells at concentrations above 1% (data not shown) and it was highly cytotoxic, ~65% (P < 0.001), at 3% concentration. Earlier reports on epithelial cell line SW620 also stated morphological changes such as elongation, enlargement and formation of process-like structure upon DMSO treatment (31). In addition we also observed DMSO at 3% was ~47 and 61% cytotoxic to lymphocytes and A549 cells (P < 0.001), respectively. Moreover DMSO above 1% was also slight genotoxic but at ≤ 0.5 %, it was non-cytotoxic as well as non-genotoxic in all the three different cells used in this study. In addition DMSO has also been known to have many therapeutics applications in the treatment of various diseases and disorders such as arthritis and connective tissue injuries, brain edema, amyloidosis, interstitial cystitis and schizophrenia (32,33). Therefore from a therapeutic point of view our data alert for the treatments that require higher percentage doses of DMSO. In addition for using it as a drug solvent our data supports use of concentrations ≤ 0.5–1%, which has been found to be non-cytotoxic as well as non-genotoxic.

Furthermore we observed ethanol was less cytotoxic to UC-MSCs, lymphocytes and A549 cell line than DMSO. Ethanol at 5% (856.5 mM) was ~25, 40 and 20% cytotoxic whereas 3% DMSO showed ~65, 47 and 61% cytotoxicity (P < 0.001). Earlier reports by Pal et al. (34) showed that 20 and 50 mM ethanol decreased viability and affected early and late stage markers during human embryonic stem cell (hESC) differentiation. Moreover 20 mM ethanol equates to a blood alcohol concentration (0.1%) which is usually observed in people after casual drinking. Consumption of alcohol can lead to adverse health effect such as liver damage, abnormal foetal development (fetal alcohol syndrome) during pregnancy. Various studies have reported the effect of ethanol on the inhibition of proliferation, differentiation and induction of apoptosis (35–39). However our data indicate that UC-MSCs are highly resistant to ethanol cytotoxicity and suggest that UC-MSCs can also be used in the treatment of patients with liver damage. Several groups have already conducted studies on toxicology of ethanol; however there are controversial reports about the toxic action of ethanol and very few studies are on the genotoxicity of ethanol (40). Our genotoxicity analysis has revealed a non-genotoxic effect of ethanol verified in UC-MSCs, lymphocytes and A549 cells, advocating safety in the use of ethanol as a drug solvent.

We further tested two widely used drugs, metformin and rapamycin. Metformin is an insulin sensitisier and first line of drug choice in the treatment of type 2 diabetes (41,42). It also has a wide range of applications in the treatment of metabolic abnormalities associated with HIV disease, regulating an ovulation and sustains pregnancy in polycystic ovary syndrome patients (43–50). Moreover metformin has also received a lot of attention for its anti-tumorigenic role (51–53). Our data indicate that metformin is ~20% more cytotoxic to A549 cells than UC-MSCs and lymphocytes. A recent report by Lee et al. (52) has also shown the anti-cancer effect of metformin (0.5–10 mM) against MCF-7 and MDA-MB-231 human breast cancer cells. Therefore our data also support the therapeutic potential of metformin for anti-cancer therapy. However there are controversial reports regarding the genotoxicity of metformin. We observed that metformin does not cause MN formation in UC-MSCs, lymphocytes and A549 cells; however, it increases NBs in UC-MSCs and lymphocytes at 1.0 and 2.5 mM but not in A549 cells. NBs are also known as the cancer biomarker thus for using metformin as anti-tumorigenic agent our data suggest the use of concentrations below 1 mM.

Rapamycin was discovered more than 40 years ago and possesses anti-fungal and immunosuppressive properties; thus it’s widely used during transplantation to reduce organ rejection (54). Moreover it also possesses anti-tumour properties, as has been widely described in vitro and in vivo (55–59). However we observed that rapamycin at and above 500 mM was 30–40% cytotoxic to UC-MSCs,
lymphocytes and A549 cells. Moreover rapamycin above 0.5 nM was slightly genotoxic to UC-MSCs and lymphocytes however the difference was not very significant, whereas in A549 cells rapamycin treatment led to increase in the number of NBs and MNs above 0.5 nM. Earlier reports have also reported chromosome malsegregation induced by rapamycin in Chinese hamster cells (60). Therefore our data indicate that rapamycin below 1 μM is non genotoxic to normal cells supporting its use for therapeutic purposes and the choice of normal human cells for genotoxicity studies. However it is noteworthy that both the drugs do not induce senescence whereas they affect lineage-specific differential potential. An earlier study has also reported the inhibitory effect of rapamycin on adipogenic (61). Several reports have demonstrated the effect of drugs on the differential potential of MSCs (61–63). Hence detailed studies are needed on the effect of these drugs on MSCs function. Therefore, our data and previous reports demonstrate the choice of drugs for different lineages is critical for therapeutic clinical applications.

Conclusion

In conclusion we demonstrate for the first time the use of a non-diploid UC-MSCs culture as a platform for simultaneous testing cytotoxicity and genotoxicity of pharmaceuticals as a suitable alternative to peripheral lymphocytes and cancer cell lines. Our investigation if put into practice would improve the in vitro toxicity screening platform leading to a decrease in attrition of drugs in clinical trials.

Supplementary data

Supplementary Figure 1 is available at Mutagenesis Online.

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