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Research article

DMSO MODULATES THE PATHWAY OF APOPTOSIS TRIGGERING

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Abstract: We demonstrate here that distribution of caspase-9 influences the pathway of apoptosis triggering, since caspase-9 is activated efficiently only when it is distributed solely in the cytosol. Caspase-9 moves to the nuclei in a response to cell stress during isolation of primary hepatocytes; this is called preapoptotic cell stress response. The dimethyl sulfoxide (DMSO) treatment cannot prevent the migration of caspase-9 into the nuclei when it is added to primary hepatocytes immediately after isolation; however, it can trigger redistribution of caspase-9 from the nuclei into the cytosol when added 1 day post-isolation. This redistribution is temporary, since caspase-9 returns to the nuclei within 48 hours of DMSO treatment. Thereafter, some caspase-9 is retained in the nuclei of DMSO-treated hepatocytes for longer than in the nuclei of untreated hepatocytes. By measuring caspase activities, we demonstrate that the addition of DMSO to cell culture medium can temporarily normalize the susceptibility of hepatocytes for apoptosis triggering through the intrinsic pathway. DMSO contributes also to the prolonged pathway inactivation, i.e., by extending preapoptotic cell stress response. We propose that DMSO extends the survival of primary hepatocytes by modulating preapoptotic cell stress response, which could be exploited for extending the lifespan of other primary cell cultures.

Key words: Apoptosis, Preapoptotic cell stress response, DMSO, Hepatocytes, Caspase-9, Intrinsic pathway

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Abbreviations used: CHO – Chinese hamster ovary; DMSO – dimethyl sulfoxide; FCS – fetal calf serum; MTT – 2-(4,5-dimethyl-2-thiazolyl)-3,5-diphenyl-2H-tetrazolium bromide; STS – staurosporine

INTRODUCTION

Human hepatocytes are the best model for studying metabolism and drug effects *in vitro*. There have been attempts to use hepatocytes as substitutes to orthotopic liver transplantation to increase the availability of donor material [1]. For example, hepatocyte transplantations were used as a treatment for acute liver failure [2-4] and for some metabolic diseases in adults and children, such as urea cycle defects [5, 6], glycogen storage disease types 1a and 1b [7, 8], and Crigler-Najjar syndrome type 1 [9, 10]. Nevertheless, the metabolism of isolated hepatocytes somewhat differs to that within tissue. The best known changes are in the expression of cytochrome P450, which declines progressively during the first few days in culture [reviewed in 11]. Also, there are significant perturbations of genes encoding for antioxidant enzymes, heat shock proteins, nitric oxide synthase and methionine adenosyltransferase. Albumin secretion rapidly decreases after 2 days in culture to almost zero after 10 days [12]. Clearly, cell isolation and culturing have a profound effect on hepatocytes' viability and metabolism.

Apoptosis is a mechanism for controlling cell number in hepatic tissue [13]. There are several molecular pathways for triggering apoptosis; the central two involve the proteases caspases [14]. These pathways are (1) the extrinsic pathway, which originates from the cell surface, and involves the activation of caspase-8 and (2) the intrinsic or mitochondrial pathway originating from mitochondria through the activation of caspase-9. The aforementioned initiator caspases activate execution caspases, such as caspase-3. All caspases are synthesized as inactive zymogens and are activated through proteolytic processing [14]. There are two main pathways for caspase-9 activation: within the apoptosome, a large protein complex, which consists of caspase-9, cytochrome c, and Apaf-1, or by proteolytic cleavage by a previously activated caspase, which involves its dimerization. Staurosporine (STS) is a broad spectrum inhibitor of protein kinases. It is used extensively to promote intracellular stress-induced apoptosis through the mitochondrial pathway [15]. In some melanoma cell lines, STS additionally induces apoptosis via a caspase-independent pathway [16].

The stress from hepatocytes' isolation can lead to a preapoptotic cell stress response [17]. This process is characterized by reversible changes in distribution of some proapoptotic proteins (e.g. caspase-9, Bax) and by reduced ability to trigger apoptosis by caspase-9 through the intrinsic pathway [17]. The changes in protein distribution during preapoptotic cell stress response appear similar to those at the beginning of apoptosis, however, the isolated hepatocytes are not apoptotic, since the changes in intracellular protein distribution are reversible; the cells have a normal morphology and function and mitochondria are energized. Apoptosis can be triggered in the presence of the additional signal, but it is not initiated through the activation of caspase-9 [17]. In hepatocytes,

preapoptotic cell stress response may prevent unnecessary apoptosis caused by mild stress; therefore, it may extend the survival of isolated hepatocytes.

The addition of dimethyl sulfoxide (DMSO) into culture medium also improves the cell viability of isolated hepatocytes. DMSO is a polar molecule, which readily permeates cell membranes. In cell biology, it is commonly used as a solvent for chemicals designated for the treatment of cells in culture [18], for cryopreservation of many cell types [19, 20], and for improving the viability of primary cells in cultures, especially primary hepatocytes (see below). Many different effects of DMSO on cells were reported, such as restoration of contact inhibition, positive and negative effects on apoptosis triggering, changes in protein synthesis, etc. These changes depended on the cell type. The induction of growth arrest in G1 phase and restoration of contact inhibition was observed in Chinese hamster ovary (CHO) cells [21] and human lymphoid pre-T cells RPMI-8402 [22]; however, in the latter study it increased apoptosis by about 30% after 72 hours incubation with DMSO. No such effect was observed in pre-B cell line KM-3, which was examined in the same study. In contrast, exposure of CHO cells to DMSO reduced both cell density-dependent apoptosis and necrosis, and resulted in increased Bcl-2 expression in CHO cells [21]. The addition of 2% DMSO to HepG2 cells induced heme synthesis and decelerated cell growth [23]. DMSO (0.5–2%) was used to attenuate the loss of differentiation of cultured primary hepatocytes and to reduce degeneration, therefore, to extend their morphological and biochemical features for weeks to months [12, 18, 24, 25]. DMSO-treated hepatocytes in culture for 40 days retained the ability to express albumin RNA at 45% of the level of normal liver. The expression levels of five other liver-specific genes (α1-antitrypsin, ligandin, phenylalanine hydroxylase, phosphoenolpyruvate carboxykinase, and transferrin) ranged from 21% to 72% of the normal liver levels [26]. Albumin secretion decreased more slowly in the presence of DMSO [12]. The objectives of this work were to determine the role of DMSO on triggering apoptosis and preapoptotic cell stress response.

MATERIALS AND METHODS

All basic chemicals and materials were purchased from Sigma (Taufkirchen, Germany) and Merck (Darmstadt, Germany).

Cell cultures of primary rat hepatocytes and liver slices

Primary hepatocytes were isolated from adult male rats (Wistar, Hannover, 180-280 g) by reverse two-step collagenase perfusion as described previously [17, 27]. Isolated hepatocytes were placed on collagen type 1 coated coverslips or 96-well microtiter plates (~2 x 10^5 cells/cm^2), incubated for 4 hours to permit adhesion in William's medium E with 10% fetal calf serum (FCS), penicillin and streptomycin (50 U/ml, each) and insulin (0.1 U/ml) in a humidified atmosphere with 95% air and 5% CO_2 at 37°C. The cultures were then further incubated overnight in a similar medium that contained 1 μM hydrocortisone hemisuccinate

instead of FCS and incubated for the indicated periods. The medium was changed every 24 hours. Each experiment was performed on the cells from at least three independent isolations.

The cells were treated with or without DMSO for different time periods. When required, apoptosis was induced using 1 μM STS (Sigma, Missouri, USA) for 6 hours in both groups of cells. The resulting four groups of cells (with and without DMSO and STS) were used for immunocytochemistry and detection of caspase activities.

Liver was isolated and treated as described for hepatocyte isolation. Offcuts of ~1 cm^3 in ice cold William's E medium were sliced further using a Leica VT1200 S vibrating blade microtome (Leica Microsystems GmbH, Wetzlar, Germany) into 80 μm thick slices and were processed as is described for immunocytochemistry.

Immunocytochemistry

Immunocytochemical analyses were performed using the standard protocols as described by the suppliers. The following antibodies were used: anti-caspase-9 (Cell Signaling Technology, Inc.) together with the appropriate secondary antibody conjugated to the fluorescent dye Alexa Fluor 488 (Molecular Probes). Cover slips were mounted with Vectashield Hard Set mounting medium with DAPI (Vector Laboratories, Burlingame, USA). Non-specific labeling by antibodies was tested by staining the cells with fluorescent secondary antibodies only. The cells were visualized using a Leica SP5 confocal microscope (Leica Microsystems GmbH, Wetzlar, Germany) with an oil immersion objective (63x magnification and numerical aperture 1.25).

Cytotoxicity assessment by MTT

MTT test indicate metabolic activity of viable cells by following the reduction of tetrazolium salt 2-(4,5-dimethyl-2-thiazolyl)-3,5-diphenyl-2H-tetrazolium bromide (MTT) to formazan by cellular dehydrogenases, including mitochondrial dehydrogenases [28]. The experiment was performed in 96-well microtiter plates. Material from three independent experiments in quadruplicate was analyzed for each sample (blank, untreated, and treated cells) and time points of 4, 8, and 10 hours. Thiazolyl blue tetrazolium bromide (Sigma, Missouri, USA, 0.5 mg/ml) in the cell medium was added to hepatocytes for 2 hours and incubated in a humidified atmosphere at 37ºC with 95% air and 5% CO_2. The insoluble formazan salt produced was dissolved in DMSO. Absorbance was measured at 550 nm on Victor^3, Multilabel Counter 1420 (Perkin Elmer, Turku, Finland).

Detection of caspase-3 and caspase-9 activities

The cells were harvested by TrypLE Express (Gibco), counted, and solubilized in Cell culture lysis buffer (Promega). Protein concentrations were determined by BCA Protein Assay Kit (Pierce, Thermo Scientific, Rockford, USA). Each sample for detection of caspase activity contained 20 μg of protein. The

activities of caspase-3 and caspase-9 were deduced from formation of appropriate luminescent substrates by using Caspase-Glo 3/7 Assay and Caspase-Glo 9 Assay (Promega), respectively, as described by the supplier.

Statistical analyses

Numerical data for all samples were expressed as a percentage of the mean of appropriate untreated samples. Data from at least three independent experiments were plotted using Sigma Plot 11.0 (Systat Software, San Jose, CA, USA). Statistical analyses were performed using Statistical Package for the Social Sciences, version 15.0 (SPSS Inc., Chicago, IL, USA). Unpaired two-tailed Student t-test was used to compare two groups. To compare more than two groups we used: one or two way ANOVA for groups with equal variances and Kruskal-Wallis rank sum test for groups with unequal variances. When indicated, we used the Tukey *post hoc* test for groups with equal variances or the Dunnett T3 *post hoc* test for groups with unequal variances. We considered values of samples as statistically significant when $P < 0.05$.

RESULTS

To study the effect of DMSO on preapoptotic cell stress response, we followed the intracellular migration of caspase-9. Within the intact tissue, caspase-9 is only in the cytoplasm, however, upon culturing hepatocytes some of caspase-9 moves to the nuclei of isolated hepatocytes (Fig. 1A, the first two panels).

The addition of 1% DMSO immediately after isolation of primary hepatocytes resulted in distribution of caspase-9 in the nuclei in addition to the cytosol after 24 hours (Fig. 1A). No difference in distribution of caspase-9 was observed in the presence and absence of DMSO under these conditions; however considerably fewer cells remained after immunocytochemistry in DMSO-treated samples. The addition of DMSO did not result in reduced cell numbers when the cells were left to attach in the medium without DMSO for the first 24 hours after isolation. Then, caspase-9 was present in the nuclei of all hepatocytes. When these hepatocytes were subsequently treated with 1% DMSO for 4, 8, and 10 hours, caspase-9 was in the nuclei of the cells after 4 and 8 hours exposure to DMSO. However, at 10 hours, it was solely cytosolic in about 50% of the cells (48 ± 14%, Fig. 1B). Caspase-9 is cytosolic in intact tissue (Fig. 1A, liver slice) and in untreated hepatocytes of about 4–6 days after isolation (Fig. 1B, 144 h, middle panel). Treatment with 1% DMSO for longer than 48 hours resulted in redistribution of caspase-9 into the nuclei. The distribution of caspase-9 could not be evaluated after 168 hours (7 days), due to the very small numbers of DMSO-treated cells that remained after immunocytochemistry. The most probable reason was that DMSO reduced the adhesion of hepatocytes to the collagen

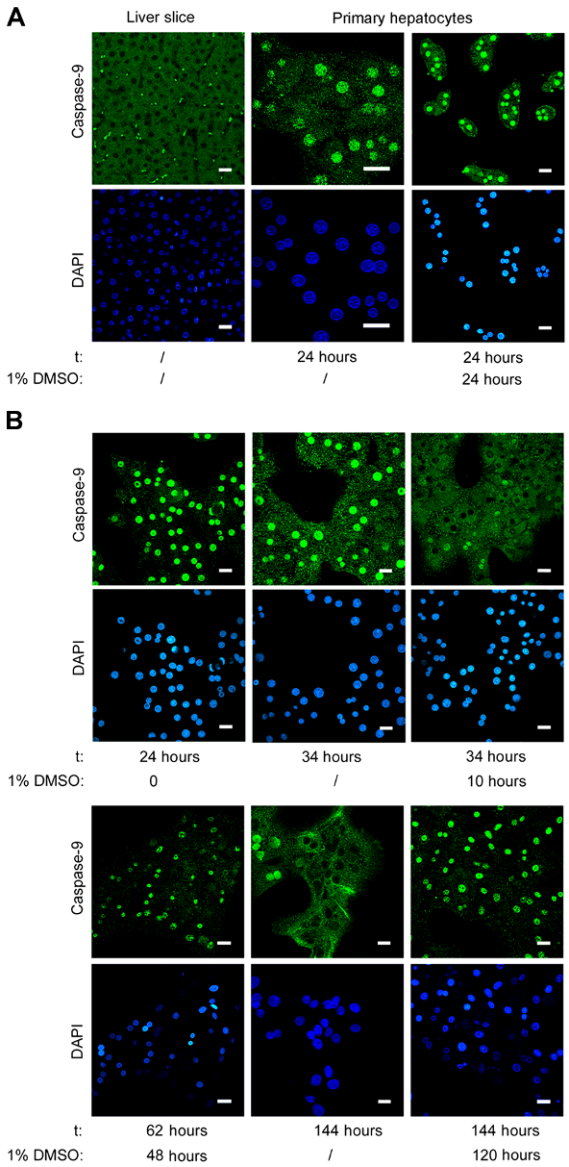

Fig 1. Effect of DMSO on the location of caspase-9. Confocal microscopy images of primary hepatocytes. Caspase-9 (green) was detected by specific polyclonal antibodies to full-length protein, followed by the secondary antibodies conjugated to Alexa Fluor 488. Chromatin was stained by DAPI (blue). t: time after isolation of primary hepatocytes; 1% DMSO: time after the treatment with 1% DMSO (hours after DMSO treatment are shown), 0: the point of addition of DMSO; /: untreated). Bars: 20 μm. A – Images of liver slice (left panels) and hepatocytes treated with 1% DMSO immediately after isolation for 24 hours (right panels) and their respective untreated control (middle panels). B – Images of hepatocytes treated with 1% DMSO starting 24 hours after isolation. The times of treatment are indicated in the row labeled 1% DMSO.

surface; this effect has been previously reported [12]. Also, longer incubation of hepatocytes with DMSO resulted in multilayer aggregates or spheroids [12]. These were also observed in our study and can be detached easily during immunocytochemistry. In conclusion, there was a shift of caspase-9 out of the nuclei (resembling the conditions *in vivo*), when hepatocytes were treated with DMSO 24 hours after isolation; however, this shift was transient. DMSO added immediately after isolation of primary hepatocytes did not prevent the migration of caspase-9 into the nuclei. This treatment resulted in a consistently low number of cells after immunocytochemistry.

To estimate, whether the observed loss of hepatocytes was also due to increased cell death in the DMSO-treated samples, the supernatants were screened for released cells. These were stained with Trypan blue, while the cytotoxicity of the attached hepatocytes was assessed by the MTT test. The rate of apoptosis was estimated from measuring activities of caspase-3 and caspase-9.

Overall, similar proportions of cells in the supernatants were dead (always under 1% of the cells seeded), as detected by Trypan blue staining, regardless of whether the cells were untreated (85 ± 10%) or treated with 1% DMSO (86 ± 11%). Specifically, no statistical significance was observed between the numbers of dead cells in supernatants of DMSO-treated and untreated samples of any time point (of 4, 8, and 10 hours). This was true when the cells were treated immediately after isolation (Fig. 2A, $P = 0.057$, one way ANOVA) or 24 hours after isolation (Fig. 2B, $P = 0.171$, one way ANOVA).

The possible cytotoxicity of the attached hepatocytes was measured by MTT test at 4, 8, and 10 hours after the addition of DMSO (Fig. 2B). In addition, the supernatants of these cells were screened for dead cells (above). A statistically significant difference was observed between the time points when DMSO was added immediately after isolation ($P = 0.002$, Kruskal-Wallis rank sum test). *Post hoc* analysis revealed that the DMSO-treated sample of 8 hours was the only sample that statistically significantly differed to the other samples ($P = 0.041$, Dunnett T3 *post hoc* test). It turned out that the treatment with DMSO for 8 hours was statistically significantly more toxic than if the cells were left untreated; however, this difference was small (Fig. 2B, left panel). We concluded that there was no meaningful difference in cytotoxicity between the samples treated with and without DMSO immediately after isolation. Likewise, the statistical significance was observed by the first analysis between the DMSO-treated and untreated samples of hepatocytes 1-day post isolation ($P = 0.00057$, Kruskal-Wallis rank sum test, Fig. 2B, right panel). Again, the DMSO-treated sample of 8 hours was somewhat different to all untreated samples, including to its control of 8 hours ($P = 0.012$, Dunnett T3 *post hoc* test). Since this difference was small, we concluded that there were no meaningful differences in cytotoxicity when cells were treated with DMSO.

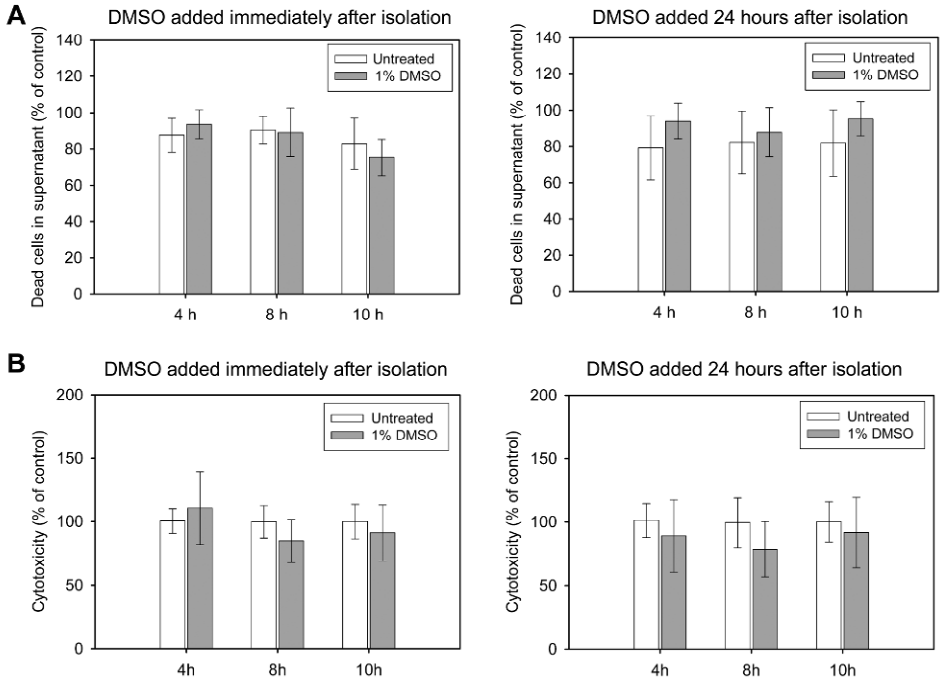

Fig. 2. Effect of DMSO on cell death. Hepatocyte cultures were treated with 1% DMSO for 4, 8, and 10 hours. A – The supernatants were screened for dead cells by Trypan blue staining. There is no statistically significant difference among the samples of left panel ($P = 0.057$, one way ANOVA) and of right panel ($P = 0.171$, one way ANOVA). B – The cytotoxicity of hepatocytes attached to the collagen surface was assayed by MTT test. Left panel: there is a statistically significant difference between all samples ($P = 0.002$, Kruskal-Wallis rank sum test). Specifically, a statistically significant difference is found between the untreated and DMSO-treated samples 8 hours after isolation ($P = 0.041$, Dunnett T3 *post hoc* test). Right panel: there is a statistically significant difference in cytotoxic effect between all samples ($P = 0.00057$, Kruskal-Wallis rank sum test); specifically, statistically significant difference is only between the samples of 8 hours ($P = 0.012$, Dunnett T3 *post hoc* test).

The activities of caspase-3 and caspase-9 were measured from combined pools of attached cells and of those from supernatants 10 hours after the addition of DMSO (Fig. 3A). The changes in activity of caspase-3 appeared to differ statistically significantly, between when DMSO was added to hepatocytes immediately after isolation and when it was added 24 hours later ($P = 0.035$; two way ANOVA). However, the following analysis revealed that there was no statistically significant difference between any of the groups (Tukey *post hoc* test, see legend to Fig. 3). The activities of caspase-9 from the same samples were calculated also as statistically significant ($P = 0.019$; two way ANOVA). Further analysis revealed no significance between the relevant untreated and treated groups, neither when DMSO was added to hepatocytes immediately after isolation nor when it was added 24 hours later (Tukey *post hoc* test, see legend

to Fig. 3). The mean values of activities of caspases -3 and -9 were even slightly lower in DMSO-treated samples, when DMSO was added 24 hours after isolation of hepatocytes (Fig. 3A). This may indicate a trend; however, the differences are small and are not statistically significant. We concluded that DMSO had no influence on caspase activation. No effect of DMSO was observed on the frequency of cell death. The consistently low number of cells after immunocytochemistry, when DMSO was added immediately after isolation, is most probably due to reduced adhesion of hepatocytes to collagen surface in the presence of DMSO. This is in agreement with reports by other authors [12].

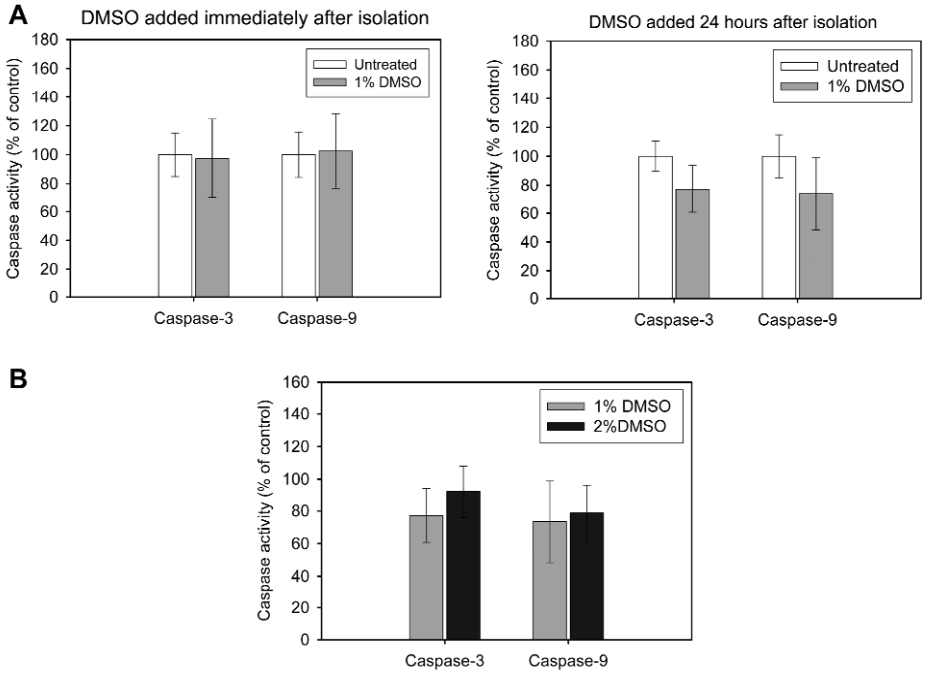

Fig. 3. Effect of DMSO on caspase activity. A – The effects of DMSO on activities of caspase-3 and caspase-9 measured 10 hours after its addition to cell medium. The activities of caspase-3: $P = 0.035$, two way ANOVA; *post hoc* analysis detected no statistically significant differences neither between the DMSO-treated and untreated samples of the cells immediately after isolation ($P = 0.989$, Tukey *post hoc* test) nor between the equally treated groups of cells treated 1 day after isolation ($P = 0.059$, Tukey *post hoc* test). The activities of caspase-9 in the presence and absence of 1% DMSO are statistically significant ($P = 0.019$, two way ANOVA). *Post hoc* analysis detected no statistically significant differences in caspase-9 activities neither between the DMSO-treated and untreated samples of the cells treated immediately after isolation ($P = 0.994$, Tukey *post hoc* test) nor between the equally treated groups of cells treated one day after isolation ($P = 0.055$, Tukey *post hoc* test). B – Hepatocytes in culture for 24 hours post isolation treated with 1% or 2% DMSO for 24 hours. There are no statistically significant differences neither in the activity of caspase-3 ($P = 0.073$, unpaired two-tailed Student t-test), nor in the activity of caspase-9 ($P = 0.618$, unpaired two-tailed Student t-test).

The concentration of DMSO added to primary cultures was between 1% and 2% in the studies that reported beneficial effect of DMSO on the longevity of hepatocytes. Effect of 2% DMSO on caspase activity was compared with that of 1% (Fig. 3B). Hepatocytes were treated one day after isolation. There were no statistically significant differences neither in the activity of caspase-3 (P = 0.073; unpaired two-tailed Student t-test) nor in the activity of caspase-9 (P = 0.618; unpaired two-tailed Student t-test) after 10 hours. Therefore, 1% DMSO was used in all subsequent studies.

The effect of DMSO on preapoptotic cell stress response

The changes in distribution of caspase-9 from cytosolic to nuclear result in different ways of triggering apoptosis [17]. We examined the influence of DMSO treatment on the pathway of apoptosis triggering, when 1% DMSO was added to hepatocytes 24 hours post isolation. Then, the cells were divided into four groups, of which two were left untreated and two treated with DMSO (Fig. 4).

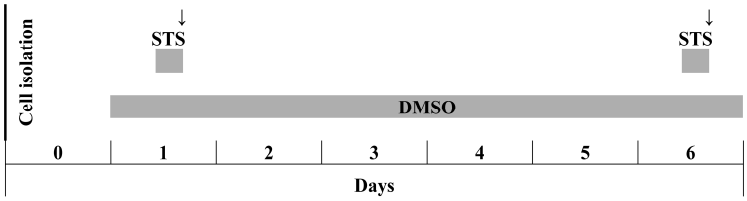

Fig. 4. Overview of the treatment regime. Abbreviations: Cell isolation – the isolation of primary hepatocytes; DMSO – treatment with 1% DMSO; STS – treatment with 1 μM staurosporine; ↓ – measurement of caspase activities.

Tab. 1. Statistical analyses of apoptosis triggering by STS.

Groups	Untreated:DMSO:STS:(STS+DMSO)			STS:(STS+DMSO)	
Day	1	6	1+ 6	1	6
Caspase-9	3.7×10^{-8}	1.2×10^{-5}	3.4×10^{-5}	0.005	9.2×10^{-5}
	A	K-W	K-W	T	D
Caspase-3	1.4×10^{-8}	1.6×10^{-7}	1.7×10^{-13}	0.023	1.6×10^{-7}
	K-W	K-W	K-W	D	D

Abbreviations: A – one way ANOVA; D – *post hoc* test Dunnett T3; K-W – Kruskal-Wallis rank sum test; T – *post hoc* test Tukey. The numbers represent *P*-values.

One of the two treated or untreated groups was subsequently treated with STS, to ascertain the role of DMSO-treatment on apoptosis triggering. These effects were observed on the cells incubated with DMSO for 10 hours (day 1) and for 5 days (day 6). See Tab. 1 for the results of statistical analyses. A statistically significant difference in activities of either caspase-3 or caspase-9 was observed among all samples treated 24 hours post-isolation. When apoptosis was triggered by STS after 10 hours of DMSO treatment, both caspase-9 and caspase-3 were

triggered more efficiently than in the cells, which were treated with STS only (Fig. 5, day 1). At that time, caspase-9 was solely cytosolic in about 50% of the DMSO-treated cells (Fig. 1B). Prolonged treatment of these cells with DMSO (total 48 hours) reversed the distribution of caspase-9 so that some shifted to the nuclei. Thereafter, caspase-9 was retained in the nuclei of DMSO-treated cells even after day 6, while at the same time, caspase-9 spontaneously relocated into the cytosol of the cells that were not treated with DMSO (Fig. 1B, 144 hours). The localization of caspase-9 effected the triggering of apoptosis; apoptosis was efficiently triggered through caspase-9 only in the cells with its cytosolic distribution. In hepatocytes of 6 days culture, both caspase-3 and caspase-9 activities were triggered more efficiently in the cells that were not treated with DMSO (Fig. 5, day 6).

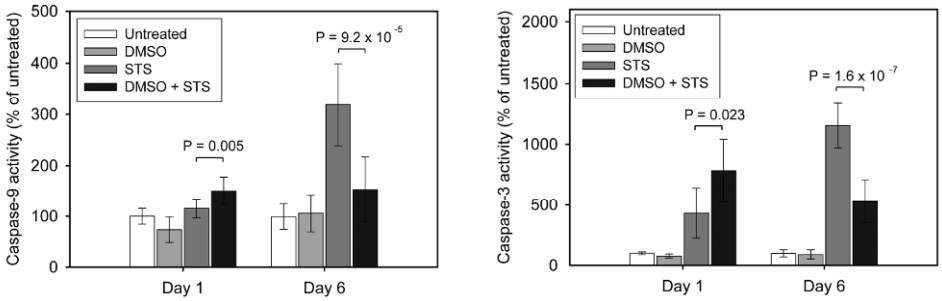

Fig. 5 Effect of DMSO on apoptosis induction. Cells were treated with 1% DMSO 24 hours after isolation. Apoptosis was induced by exposing the cells to 1 μM STS for 6 hours at 10 hours (day 1) and 120 hours (day 6) after the onset of DMSO treatment. The *P* values of calculated differences between the activities of caspase-9 and caspase-3 are shown in Tab. 1.

DISCUSSION

DMSO treatment has an effect on preapoptotic cell stress response. It cannot prevent the migration of caspase-9 into the nuclei when it is added to primary hepatocytes immediately after isolation; however, it can trigger the redistribution of caspase-9 from the nuclei into the cytosol when added 1 day after isolation. This redistribution is only temporary, since the caspase-9 returns to the nuclei within 48 hours of DMSO treatment. Thereafter, caspase-9 is found in the nuclei and in the cytosol in DMSO-treated cells. Surprisingly, caspase-9 is retained in the nuclei of these cells for longer than in the nuclei of untreated hepatocytes. We confirmed in this study that distribution of caspase-9 influenced the pathway of apoptosis triggering [17]. DMSO treatment did not trigger apoptosis *per se*, as it was reported for pre-T cells RPMI-8402 [22]; this effect may be specific to these cells, since the authors reported there were no effects on pre-B cell line, KM-3 cells, that were treated in parallel and reported in the same study. Moreover, our results agree with those in CHO cells, where DMSO treatment reduced apoptosis [21].

The addition of DMSO to primary hepatocytes modulated the pathway of apoptosis triggering through caspase-9. Upon the apoptotic stimulus, caspase-9 was efficiently activated only when it was in the cytosol (within 24 hours of DMSO-treated hepatocytes and 6 days after isolation in untreated hepatocytes). The DMSO treatment, therefore, had an effect on the shift of caspase-9 from the nuclei into the cytoplasm; interestingly caspase-9 was again found in the nuclei after the prolonged treatment with DMSO. We propose that DMSO is a stressor for the cell. Whilst a short treatment with DMSO appears beneficial, the prolonged treatment extends preapoptotic cell stress response. Preapoptotic cell stress response protects the cells from triggering apoptosis upon encountering a mild stressor. This may be another reason for improved long-term survival of DMSO-treated hepatocytes in primary cultures. It is of great importance to study the quality of primary hepatocytes and to compare their characteristics with that of hepatocytes *in vivo*, since the primary hepatocytes are an important model for studying metabolism. Moreover, there are already over 80 patients worldwide, who have received hepatocyte transplantation for the treatment of metabolic diseases and of acute liver failure [8]. Knowledge about the quality of hepatocytes is of utmost importance to obtain results that mimic the conditions *in vivo*, and also to improve assessment of the quality of cells for transplantation. It may be useful to investigate whether DMSO can extend the survival of cells other than hepatocytes in primary cultures. Knowledge about the effects of DMSO is crucial for the proper interpretation of experimental results when DMSO is used as a solvent. Understanding the effects of DMSO may accelerate the rate of searching for analogs with more specific functions to suit a particular need.

CONCLUSIONS

The distribution of caspase-9 influences the pathway of apoptosis triggering, since caspase-9 is activated efficiently only when it is distributed solely in the cytosol. The addition of 1% DMSO to a culture of primary hepatocytes 24 hours post-isolation temporarily enables the triggering of apoptosis through the intrinsic pathway, i.e. abolishes preapoptotic cell stress response. The prolonged preapoptotic cell stress response in DMSO-treated hepatocytes may contribute to improved long-term survival of DMSO-treated hepatocytes in primary cultures. Assessment of the quality of hepatocytes is of utmost importance because of the clinical use of hepatocytes in transplantations and for modeling human metabolism. Knowledge about the effects of DMSO on all types of cells is important due to its common use as a solvent and cryoprotectant.

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