PRIMARY CILIA INCIDENCE IN A MYOBLAST CELL LINE (C2C12)

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Introduction

The primary cilium (PC) is a sensory, solitary, non-motile microtubule-based structure that arises from the centrosome and projects from the surface of the majority of human cells. The presence of a primary cilium on the surface of a cell is transient, limited to the quiescent G1 (G0) phase and early S phase of the cell cycle. Primary cilia, typically 200-300 nm wide and 2-10 microns long are present on most vertebrae cell types [1]. A number of different pathologies are associated with compromised cell-to-cell contact and their microenvironment. Primary cilia are one of the organelles involved in this process [2].

Aims

In this study, we used an in vitro model to evaluate the effect of ionizing radiation on cilia incidence. Ionizing radiation promotes DNA double-strand breaks in the affected cells, how this affects cell interaction with their microenvironment, at primary cilia level, remains to be seen.

Methods

The myoblast cell line C2C12 was cultured in DMEM and incubated in a 5% CO_2 atmosphere at 37°C. Cells were irradiated 48 hours after plating using a ^{60}Co γ-ray source at a dose rate of 0,4 - 2 Gy/min. After irradiation, cells were incubated at 37°C/5% CO_2 according to the scheduled time (1, 3 and 6 days). Non-irradiated control cells were handled in parallel. C2C12 cells were starved under low serum conditions (0.5% FBS) during the predetermined time intervals (6, 12, 24, 48, 72 and 120 hours). Control cells (10% FBS) were analyzed at 72 hours after plating. WST-1 reagent was used to determine cells viability 72 hours after irradiation (2, 6, 10 and 20 Gy) and serum starvation (0.5% FBS). Cell cycle analysis was performed after treatment. Immunostaining was performed as follows: goat serum; anti-acetylated; anti-gamma tubulin; Cy3-conjugated donkey anti-mouse secondary; Alexa 488 conjugated donkey anti-rabbit secondary antibody. Transmission electron microscopy - Images were captured with Megaview G2 digital camera and iTEM software. The statistical analysis of differences between the groups was performed by the Student's t-test and P values of ≤ 0.05 were considered significant.

Results

In our experiments, the multiplication of primary cilia occurred after irradiation with a dose capable of inducing cell cycle arrest in G2 phase (20 Gy), while irradiation dose of 6 Gy, which leads to accumulation of the irradiated cells in G1 phase, only promotes formation of solitary cilia. This observation correlates well with previous findings that centrosome multiplication occurs during a prolonged G2 phase [3]. A significant number of ciliated cells was observed 1 day after the irradiation within the whole dose range (2-20 Gy), with the highest PC incidence three days after the treatment as compared to non-irradiated cells. However, PC incidence (%) observed in cells exposed to doses of 2, 6 and 10 Gy was significantly lower than that observed in cells irradiated by 20 Gy. Further dynamic of primary cilia formation was evaluated only after 20 Gy dose and it was found that PC incidence remained significantly higher than in non-irradiated cells even at 6 days post-irradiation. Unexpectedly, multi-ciliated cells were also detected in irradiated cells. In 2-4 % of irradiated cells two or more cilia were detected 1 day after the irradiation. A further increase in the number of cells with multiple cilia was observed 3 days after irradiation by the doses of 10 and 20 Gy (15% and 35% of cells, respectively). Cell proliferation was inhibited after serum starvation stress, decreasing the number of cells in S phase after 24 h of starvation. Cell viability was not affected and apoptotic cells were not observed. The percentage of ciliated myoblasts increased significantly as soon as after 6 hours of serum starvation as compared with the control. The PC incidence increases further during starvation, peaking at 24 hours and reaching nearly 80%. Thereafter, the percentage of ciliated cells was increased for the remaining duration of the experiment (120 hours of starvation).

Discussion

Assembly and disassembly of primary cilium is closely tied up with centrosome duplication cycle, as the basal body is formed from the mother centriole. Under normal conditions, centrosome duplication occurs precisely once per cell cycle. During S phase the mother and daughter centriole disengage and each of them nucleates a procentriole. These procentrioles elongate and mature during late S and G2 phase. Thus duplicated centrosomes are tethered together. This tether provides a loose connection between the proximal ends of the two parental centrioles and is removed during late G2 phase to allow mitotic spindle formation [4]. It seems that the formation of primary cilium requires functional appendages of mother centriole as well as a mature pericentriolar matrix [5, 6, and 7]. However, irradiated myoblasts were not only fully capable of ciliogenesis, but also formed multiple primary cilia. Our findings are in agreement with that of Conroy et al., [6], who observed occasional multiple cilia on irradiated hTERT-RPE1 retinal epithelial cell line. These cilia seem to originate from a single ciliary pocket. These observations suggest that centrosome multiplication after DNA damage could be followed by multiple primary cilia formation.

Conclusions

Taken together we suggest that centrosome multiplication and formation of multiple primary cilia is a regulatory step involved in regulation of G2/M cell cycle arrest after DNA damage induced by ionizing radiation. Although our results seem to indicate that such strong stress threatening cell survival promotes a high ciliation rate in the treated cells, further study on these results still needs to be approached as the reason behind the increased cells' multi ciliation rate under ionizing radiation is not yet completely clear, and although it coincides with a high caspase activity, care must be taken before trying to stablish any direct involvement between the two processes without further evidence indicating it. Though this work raises more questions than it answers, it hints at ciliation processes not yet studied and that further research will undoubtedly clarify.

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COGNITIVE IMPAIRMENT IN BIOPOLAR DISORDER IS CONNECTED WITH METABOLIC ABNORMALITIES AND A TYPE OF MEDICATION

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Introduction

Bipolar affective disorder (BD) is a chronic disease that has been thought to impair patients' social and working abilities together with cognitive functions only in acute phases. Patients were considered completely healthy when not currently suffering from a depressed or manic state. Such a conviction has been disproved by intensive research in the last decade scrutinizing above mentioned fields. We can now state that BD is associated with cognitive impairments representing a significant source of functional disability (i.e. ability to manage one's finances, to involve in interpersonal relationships and social activities, planning leisure time activities). Although the cognition in BD is impaired globally, majority of malfunction lies in domains such as attention, verbal memory, psychomotor speed and executive functions. [1] Incidence of metabolic syndrome and other cardiovascular risk factors is two to three times higher in bipolar population in comparison with healthy population and represent another debilitating condition increasing mortality. [2]

Aims

Aim of our study was to search for a linkage between cardiovascular risk factors and cognitive functions in order to suggest somatic interventions promising in improving patients' cognition and functional abilities.

Methods

We have enrolled 40 bipolar patients from outpatient clinic of our Dept. of Psychiatry that were in remission of BD. Remission was assessed by an absence of pathological mood symptoms and a score below 9 points in objective scales (HDRS, YMRS). Parameters of metabolic syndrome according to National cholesterol education program Adult Treatment Panel III were examined. [3] Neuropsychological examination consisting of a battery of tests was performed at the same time. Patients' neuropsychological performance was compared with normative values for healthy population. History of past and present psychofarmacotherapy was taken.

Results

Metabolic syndrome was found in 15 patients. Inferior performance in tests of cognition was significantly connected with presence of metabolic syndrome ($p=0.01$). Abdominal obesity ($p=0.04$) and pathological glycaemia ($p=0.02$) were another circumstances connected with impaired cognition. The most prominent finding linked to cognitive impairment was arterial hypertension ($p<0.01$). Patients treated by lithium performed significantly worse ($p=0.03$) in tests of cognition than patients treated by valproic acid or carbamazepine. We should state that lithium users were using it significantly longer ($p<0.01$) than carbamazepine or valproic users.

Discussion

We have found a significant connection between metabolic/cardiovascular risk parameters and cognitive functions and type of thymostabilizing medication. These results however do not implicate a simple solution to improving patients' functionality because it is not clear whether treating metabolic parameters would improve patients' cognition. This study was performed in a cross-sectional design and only longitudinal study could reveal if significant connections will remain. Also interventional study scrutinizing the effect of metabolic parameters treatment on cognition could clearly show if this would be the way to improve functionality in BD patients.

Conclusion/Summary

Bipolar patients with impaired metabolic/cardiovascular parameters are significantly more affected by cognitive impairment then patients with non pathological parameters. Longitudinal and also interventional studies are necessary to reveal possible ways of improving functionality in BD population.

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BISPHOSPHONATE-RELATED OSTEONECROSIS OF THE JAW: A REVIEW OF 20 CASES

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Introduction

Bisphosphonates (BP) are inhibitors of bone resorption used mainly in the treatment of metastatic bone disease and osteoporosis. They prevent complications as pathological fracture, pain, tumor-induced hypercalcemia. Even though patient‘s benefit of BP therapy is huge, various side effects may develop. Bisphosphonate-related osteonecrosis of the jaws (BRONJ) is among the most serious ones. Oncologic patients receiving high doses of BP intravenously are at high risk of BRONJ development. BP impair bone turnover leading to compromised bone healing which may result in the exposure of necrotic bone in the oral cavity frequently following tooth extraction or trauma of the oral mucosa. Frank bone exposure may be complicated by secondary infection leading to osteomyelitis development [1]. No ideal treatment strategy of BRONJ has been found so far. Combination of conservative therapy aiming to reduce the symptoms and resective surgery may lead to complete recovery, provided that the procedure is correctly indicated [2].

Aims

The aim of this study was to analyse the authors‘ experience with BRONJ in 20 individuals.

Methods

A retrospective study of patients diagnosed and treated for BRONJ at the Department of Dentistry, University Hospital Hradec Králové during the period 2006 to 2013 was performed. Data collected and analysed were: age, gender, indication of BP therapy, type of BP and route of administration, localisation of osteonecrosis, triggering factor, type of treatment and results. Diagnosis of BRONJ was based on the results of clinical examination and radiological examination (ortopantomogram or cone-beam computed tomography). Definition and staging system published by the American Association of Oral and Maxillofacial Surgeons in 2009 has been used to determine the stage of the disease [3]. The treatment protocol consisted of antimicrobial mouth rinses (chlorhexidine, hexetidine, benzydamin) and systemic antibiotic treatment (amoxicillin, clindamycin) according to the stage of the disease. Additional surgical intervention – resection of necrotic bone with soft tissue closure in combination with systemic antimicrobial therapy was performed in two cases.

Results

During the years 2006 to 2013, BRONJ was diagnosed in 20 patients. The patients‘ age ranged from 43 to 84 years with a mean age 65 years. The male-female ratio was 2 : 18. Postmenopausal osteoporosis was the indication of BP administration in 11 patients, while 9 patients received BP for one of the following oncologic diseases: renal cancer (1), breast cancer (6), multiple myeloma (2). All the oncologic patients were exposed to intravenous zolendronate as the most potent BP, whereas oral ibandronate has been most frequently used for the treament of osteoporosis. The putative cause of BRONJ was tooth extraction in 15 cases, while in 2 cases it was a spotaneous occurrence and chronic denture trauma was the presumable triggering factor in 3 cases. Necrotic bone was most frequently found in mandible (13 patients), in 3 patients maxilla was involved, 4 patients manifested with osteonecrosis in both jaws. Complete healing defined as the absence of any mucosal breaches and exposed necrotic bone, absence of any signs of inflammation and absence of subjective complaints was achieved in 11 patients. Satisfactory healing was achieved in 3 patients. In these patients a small area of asymptomatic exposed necrotic bone up to 5 mm in diameter persisted. In 1 oncologic patient, rapid progession with the development of necrotic bone in all quadrants with severe complaints and slow sequestration process was noted. In 4 cases the therapy led to stabilisation of the disease with stationary extent of necrotic bone. In 1 case, the patient discontinued the treatment, therefore treatment results were not evaluated.

Discussion

BRONJ is a relatively rare complication which may negatively influence patients‘ oral health. Especially oncologic patients receiving high doses of intravenous BPs are at high risk of developing osteonecrosis. Management of the disease is a complicated task since no ideal treatment strategy has been suggested so far. Conservative treatment strategy leads to symptom reduction and decrease in the frequency of infectious complications. Surgical treatment approach with wide bone resection followed by primary soft tissue closure with pre- and postoperatively administered antiobiotics may lead to complete healing. However BPs affect the whole jaw and surgical trauma to the bone could trigger progression of the osteonecrosis. Therefore, careful indication of resective surgery as well as accurate diagnotic procedure and preoperative assessment are necessary [4]. The patient’s morbidity and life expectancy should be taken into account when planning surgical intervention. BRONJ seems to be a preventable complication to a certain degree since osteonecrosis is frequently a result of tooth

extraction. Less often it may develop spontaneusly or as a result of chronic trauma to the oral mucosa, often caused by ill-fitting dentures. Adverse effects and possible risks should be explained to the patient by the prescribing physician before the therapy onset and dental preventive measures should be taken. This requires adequate communication between the prescribing physician, the patient and the dentist [5].

Conclusion

From these data it was concluded that conservative approach in the treatment of BRONJ led to symptom regression but was not always curative. Surgical intervention, however, bears the risk of further progression of the osteonecrosis and must be carefully planned with respect to the patient‘s general health status and life expectancy. The treatment of BRONJ is generally difficult. Prevention plays a predominant role in the management of the this condition.

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TRANSDERMAL ABSORPTION OF POLYCYCLIC AROMATIC HYDROCARBONS

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Introduction

Coal tar (CT) is frequently used in various fields of human activities, including medical therapies. CT contains mixture of mutagenic and carcinogenic polycyclic aromatic hydrocarbons (PAHs) which can be absorbed in the skin during dermal exposure. The values of basic characteristics of dermal absorption (flux, lag time) of individual PAH may be significantly changed during mixed PAHs exposure[1,2]. So far, the issues of differences between dermal absorption of individual PAH and mixtures of PAHs were studied very little.

Methods

The basic characteristics of dermal absorption of individual PAH (anthracene, benz[a]anthracene, benzo[a]pyrene, fluoranthene, fluorene, naphtalene, phenanthrene and pyrene) and basic characteristics of dermal absorption of PAH mixture were determined. The determination was performed using static vertical Franz diffusion cells and full pig ear skin. The samples of receptor fluid were collected during 76 hours and the concentrations of PAHs were determined by using of HPLC.

Results

Table 1 summarizes basic characteristics (flux and lag time) of individual and mixed application of PAHs. The presented values are expressed as arithmetic means and standard deviations.

Table 1. Basic characteristics of dermal absorption of PAHs

PAH	Molecular weight	Flux (nmol/cm^2/h)			Significance (p)	Lag time (h)			Significance (p)
		Individual application (IA)	Mixture (M)	IA/M		Individual application (IA)	Mixture (M)	M/IA	
Naphthalene	128,17	0,6393±0,5833 (n=6)	0,5296±0,3149 (n=6)	1,21	0,6937	0,86±0,65 (n=6)	0,98±1,06 (n=6)	1,14	0,8241
Fluorene	166,20	0,0420±0,0315 (n=6)	0,0405±0,0256 (n=5)	1,04	0,9353	8,14±8,10 (n=6)	7,00±1,77 (n=5)	0,86	0,7680
Phenanthrene	178,23	0,2981±0,2085 (n=6)	0,1486±0,1040 (n=6)	2,01	0,1471	14,87±5,49 (n=6)	16,88±6,06 (n=6)	1,14	0,5596
Anthracene	178,24	0,0348±0,0168 (n=6)	0,0141±0,0069 (n=5)	2,47	0,0305*	16,97±2,55 (n=6)	34,12±6,59 (n=5)	2,01	0,0002**
Fluorantene	202,26	0,1170±0,1229 (n=6)	0,0540±0,0314 (n=5)	2,17	0,2743	28,77±9,97 (n=6)	37,93±4,22 (n=5)	1,32	0,0894
Pyrene	202,26	0,1372±0,0905 (n=6)	0,0342±0,0268 (n=6)	4,01	0,0377*	30,78±7,15 (n=6)	33,11±4,05 (n=6)	1,08	0,5064
Benz[a]anthracene	228,29	0,0058±0,0082 (n=6)	0,0005±0,0003 (n=5)	11,6	0,1320	37,95±25,02 (n=6)	38,73±4,42 (n=5)	1,02	0,9474
Benzo[a]pyrene	252,32	0,0017±0,0012 (n=6)	-	-	-	50,98±13,28 (n=6)	-	-	-

Legend:
statistical significance at $\alpha = 0.05$ * and $\alpha = 0.001$ **
individual PAHs are sorted with increasing molecular weight
absorption of benzo[a]pyrene in mixture was below detection limit (flux and lag time could not be determined)

When compared the data, the smallest differences in flux-values (individual vs. mixture) were seen in the cases of fluorene and naphthalene (absorption during individual exposure was only

1.04, resp. 1.21 times higher). The time course of absorption of naphthalene is shown in Figure 1. The greatest differences in flux-values (individual vs. mixture) were seen in the cases of pyrene and benz[a]anthracene (absorption during individual exposure was 4.01, resp. 11.6 times higher). The time course of absorption of pyrene is shown in Figure 2. Statistically significant differences in flux level were found in pyrene and anthracene ($p<0.05$).

In all cases (except fluorene) we found longer lag time during mixed exposures. When compared the data, the smallest difference in lag time values (individual vs. mixture) was found in the case of benz[a]anthracene (lag time for mixed exposure was only 1.02 times longer). The greatest difference in lag time values (individual vs. mixture) was seen in the case of anthracene (lag time for mixed exposure was 2.01times longer). Statistically significant difference in lag time was found in anthracene ($p<0.001$).

Figure 1. Absorption of naphthalene

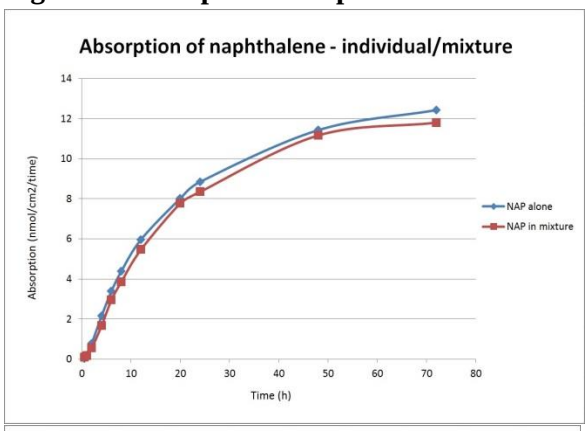
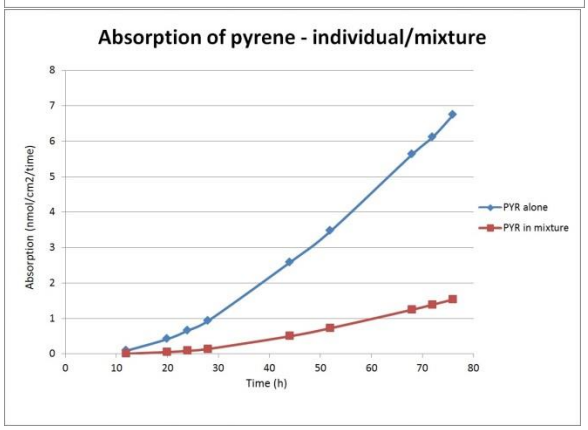


Figure 2. Absorption of pyrene



Conclusion

Passive penetration of PAHs through the intercellular spaces is considered to be the major pathway for dermal absorption. Mixed exposures (PAHs) probably reduce the individual rate of penetration through this pathway. PAHs with higher molecular weight showed greater differences in fluxes (individual vs. mixture) however this tendency was not observed in lag time. The variations in the intensity and speed of absorption, related to the individual and mixture exposure scenarios, naturally increase the uncertainty of the risk assessment of dermal exposure to PAHs.

Acknowledgements

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COMPARISON OF ACETAMINOPHEN TOXICITY IN PRIMARY HEPATOCYTES ISOLATED FROM TRANSGENIC MICE WITH DIFFERENT APOLIPOPROTEIN E ALLELES

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Introduction & Aims

Nrf2 is a transcription factor important for combating electrophilic and oxidative stress in liver and other organs[1]. This encompasses detoxification of hepatotoxic drugs including paracetamol, also known as acetaminophen (APAP)[2]. Recently, an association between apolipoprotein E (ApoE) genotype and Nrf2 expression was described[3].

Methods

Primary hepatocytes were isolated by two-step collagenase perfusion from female transgenic mice carrying human ApoE3 or ApoE4 allele, and from C57Bl/6 wild-type controls. The cells were exposed to APAP in concentrations from 0.5 to 4 mmol/l for up to 24 hours. Morphological criteria, markers of cellular damage, oxidative stress, APAP metabolism and apoptosis were evaluated.

Results

APAP led to a dose-dependent hepatotoxicity from 1 mmol/l after 16 h exposure in all mice tested. The toxicity was higher in hepatocytes isolated from both transgenic strains than in wild-type controls and most pronounced in ApoE3 mice. Concurrently, there was a decline in mitochondrial membrane potential, especially in ApoE3 hepatocytes. Activity of caspases 3 and 7 did not differ among groups and was minimal after 24 hour incubation with 4 mmol/l APAP.

Formation of reactive oxygen species was increased after 24 hours with 2.5 mmol/l APAP in hepatocytes of all strains tested; the steepest increase was observed in the ApoE3 genotype. Furthermore, we observed higher lipid accumulation in hepatocytes isolated from both transgenic strains than in wild-type controls. The expression of Nrf2-dependent genes was higher in ApoE3 than in ApoE4 hepatocytes.

Discussion & Conclusions

Hepatocytes isolated from transgenic mice with ApoE4 and ApoE3 alleles displayed higher susceptibility to acute APAP toxicity *in vitro* than hepatocytes from wild-type mice. Of the two transgenic genotypes tested, ApoE3 allele carriers were more prone to the injury. Apoptosis was not the main mechanism of cell death in the present study. *In vivo* studies would be advisable to compare the toxicity in whole animals.

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THE USE OF FIDUCIAL MARKERS FOR PATIENT SETUP ALLOWS SAFETY MARGIN REDUCTION AND BETTER SPARING OF ORGANS AT RISK DURING PROSTATE CARCINOMA EXTERNAL BEAM RADIOTHERAPY

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Introduction

To account for geometric uncertainties during external beam radiotherapy, safety margins are applied. Image-guided radiotherapy (IGRT) gives the possibility to apply tighter margins as with conventional RT. This can be beneficial especially in prostate cancer, where the dose to the rectum limits dose escalation. Nonpredictable prostate position variation is the challenge for comparison of different IGRT strategies. Techniques of patient setup relative to external beam's isocenter have developed during the last decade. Historically, skin marks and setup lasers have been used. These are not adequate surrogates for prostate position and require extensive safety margins, which are incompatible with the delivery of the high radiation doses above 70 Gy that are currently used in routine practice. [1] Planar X-ray imaging techniques have enabled registration with skeletal anatomy, but recent studies showed a poor correlation of prostate position and bony anatomy. [2] Prostate location variations were studied relative to the adjacent bony anatomy by Schallenkamp et al. [3] with the conclusion that significant interfractional motion exists between the prostate and the pelvic bony anatomy. These move independently, therefore the pelvic bony anatomy should not be used as a surrogate for prostate motion. The authors also suggest that fiducial markers are stable within the prostate and allow significant margin reduction when used for online localization of the prostate. "The limited interuser variability and the marker stability make markers an ideal surrogate for the prostate position". [1] While the planning target volume (PTV) margin required for bony anatomy alignment should be within the range of 10 mm, the PTV margin required for marker alignment can be lowered. [4] IGRT systems provide more information than that which is required for simple patient positioning. Utilization of cone-beam CT (CBCT) can provide 3D anatomic information directly in irradiation position. Such information enables reconstruction of a current dose distribution. CBCT was evaluated for treatment planning by Yoo and Yin [5] and Yang et al. [6] with the conclusion that CBCT could be used for verification planning to verify treatment delivery retrospectively.

Aims

The aim of this study was to utilize the CBCT scans acquired before treatment for dose reconstruction purposes and hereby to compare two different styles of IGRT: bony landmark setup vs. fiducial markers setup. Comparison of isotropic 10 mm and 7 mm margin for both types of image guidance was made.

Methods
We used CBCT to compare two different styles of IGRT: 29 patients (134 CBCTs) with bony landmark (BL) setup vs. 30 patients (177 CBCTs) with fiducial markers (FM) setup were assessed. We delineated clinical target volumes (prostate - CTV2, seminal vesicles - CTV1-2) and organs at risk (rectum, bladder) on CBCTs acquired directly before the patient's treatment. Then, the dose distribution was reconstructed using the fluence maps from the original treatment plan assuming 10mm margin. Fluences from hypothetical alternative plans considered tighter 7mm margins were also used for the dose reconstruction. Possibility of margin reduction was evaluated by means of calculated target coverage by the 95% isodose.

Results
CTV2 was underdosed in 9 % of reconstructed plans in case of BL and 10mm margin, 20 % in BL-7mm and 3 % in FM-7mm. CTV1-2 was underdosed in 2 % in BL-10mm, 10 % in BL-7mm and 7 % in FM-7mm. While the margin reduction in case of BL setup makes both CTV2 and CTV1-2 coverage significantly worse ($p = 0.0003$ and $p = 0.0044$, respectively, McNemar's test), in case of FM setup with the reduced 7 mm margin, the CTV2 coverage is even better compared to BL setup with 10 mm margin ($p = 0.049$, Fisher's exact test). There was no significant difference in CTV1-2 coverage between BL-10 mm and FM-7 mm group.

Cunclusions/Summary
Reducing of safety margin is not acceptable in case of bony landmark setup, because of the interfractional motion between the prostate and the pelvic bony anatomy. The margin can be lowered from 10 mm to 7 mm in case of fiducial markers setup, which should enable better rectum and bladder sparing. This creates a potential for dose escalation in the future treatments. Another promising way is the use of CBCT for daily setup, but fiducial imaging requires less daily physician input, is less time-consuming, and patient exposure to additional ionizing radiation is much lower.

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EFFECT OF FGF-1 ON LIVER MYOFIBROBLASTS CULTURED IN VITRO

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Introduction

A precise equilibrium between cellular differentiation and proliferation or extracellular matrix synthesis and degradation is essential for liver tissue homeostasis. Liver MFBs are differentiated fibroblasts, the primary source are hepatic stellate cells (HSC). MFB are phenotypically characterized by the presence of alpha smooth muscle actin (α-SMA). They are known to synthesize fibrogenic cytokines (Novo et al. 2009, Knittel et al. 1999) and extracellular matrix (ECM) components, including COL1A1 (collagen) and FN1 (fibronectin). When stimulated, fibroblasts undergo phenotypical change to myofibroblasts. The change could be induced by transforming growth factor β1 (TGF-β1) (Ramos et al. 2010) and other cytokines and develop the autocrine activation loop in liver myofibroblasts.

We explored suggested reversal effect of fibroblast growth factor-1 (FGF-1) on rat liver myofibroblasts, both in standard cell culture and in three-dimensional collagen matrices. FGF-1 is known as a member of the fibroblast growth factor family. FGFs control cell proliferation, migration and differentiation in various organs but the effect on myofibroblastic conversion has not been elucidated.

Aims

The aim of this study was to determine the effect of heparin stabilized FGF-1 on liver myofibroblasts.

Methods

Liver myofibroblasts were isolated from nonparenchymal liver rat cells by repeated passaging. Cells were cultivated in the collagen gel and on the plastic Petri dishes. The concentration of FGF-1 was 4 ng/ml and concentration of heparin was 10 µg/ml. Expression of proteins was determined by Western blot analyses and by ELISA. Statistical analysis was performed using student T-test.

Results

We found that expression of proteins which are typical for myofibroblastic conversion varies according to the type of culture environment. Protein expression of TGF-β1, α-SMA and FN-EDA was significantly lower in the cells cultured in collagen gel than in the cells cultured on plastic. The influence of FGF-1 on MFBs was tested in two different environments: on plastic Petri dishes and in the collagen gel in four different intervals. The expression of α-SMA in MFB on plastic was downregulated 24h after FGF-1 treatment but longer incubation had no effect. FN-EDA increases up to 72h and then drops. In contrast FGF-1 in MFB in collagen gel induced expression of both α-SMA and FN-EDA after 48h treatment. Addition of FGF-1 and heparin had no effect on the expression of extracellular TGF-β1 and MMP-2.

Discussion

We found that collagen gel cultivation has calming effect on MFB accompanied with downregulation of α-SMA and FN-EDA. We hypothesize that it is due to lower intracellular and extracellular levels of TGF-β1 in the cells cultured in collagen gel. TGF- β1 is known to stimulate the expressions of EDA-FN and α-SMA in human fibroblasts (Serini et al. 1998). TGF-β1 synthesis depends on the rigidity of environment. Collagen gel has low rigidity than plastic, therefore expression of TGF-β1 is lower (Vaughan et. al 2000).

FGF-1 had no effect on TGF-β1 expression in MFB. FGF-1 and heparin induces antifibrotic phenotype in human lung fibroblasts (Becerril et al. 1999). FGF-1 antagonizes TGF-β via inhibition of TGFBR dependent Smad phosphorylation (Ramos et al. 2010). Combination of FGF-1 and heparin inhibits α-SMA expression in rabbit corneal myofibroblasts (Maltseva et al. 2001). Liver MFB in our experiments responded to FGF-1 in combination with heparin by the decrease of α-SMA protein in cells both on plastic and in gel with exception of 48h interval. The correlation of EDA-FN and α-SMA expression was better in the cells in collagen gel than in the cells on plastic.

Conclusion

Our results demonstrate that collagen gel cultivation can reduce myofibroblastic phenotype in liver MFB. Although FGF-1 was suggested to have similar effect on myofibroblastic cell of different origin liver MFB respond with transient decrease of α-SMA levels. The expression of α-SMA in MFB in collagen gel is lower than in cells on plastic and FGF-1 is able to enhance and then diminish. We conclude that three-dimensional collagen cultivation affects the phenotype and behavior of liver MFB.

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ADMINISTRATION OF HIGH-DOSE INTERPHERON ALPHA 2B IN ADJUVANT TREATMENT OF MALIGNANT MELANOMA AND MONITORING OF ITS ANTIANGIOGENIC POTENTIAL

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Introduction

Malignant melanoma is thought to be one of the most fatal malignant diseases affecting even young adults. Radical surgical procedure is considered to be curative; however only in case that the tumour hasn´t reached angiogenic switch. Otherwise, it must be considered as a systemic disease. Adjuvant treatment is indicated in patients stage IIb, IIIa and IIIb. The aim of systemic adjuvant therapy is to reduce the risk of disease recurrence and metastasis, thereby improving survival [1]. Interpheron alpha2b was approved by FDA for adjuvant treatment of malignant melanoma in 1995. Its activity is multifunctional – antiviral, antiproliferative, immunomodulative and antiangiogenic. Vascular endothelial growth factor is thought to have a key role in angiogenesis. Soluble VEGF molecule binds to its receptor which activates cascades of signalling ways included in cell migration, proliferation, survival, permeability and angiogenesis. VEGF receptor is expressed on endothelial cells, but also on tumour cells; not on healthy melanocytes [2].

Aims

The aim of this study was to confirm antiangiogenic function of interpheron alpha2b administrated in high dose intravenous scheme by monitoring serum levels of VEGF A.

Methods

High dose interpheron alpha2b intravenous in dose 20 MIU/m^2, 5 days in week for 4 weeks („Kirkwood´s scheme“) was administered to 29 patients in Department of Clinical Oncology, University Hospital Hradec Kralove in 2010 – 2014. In these patients we monitored serum levels of VEGF A and MMP-8 before and after intravenous high-dose treatment. Serum levels of VEGF A and MMP-8 were measured by ELISA method in Department of Clinical Immunology, University Hospital Hradec Kralove.

Results

After high dose intravenous treatment with interpheron alpha2b levels of VEGF A decreased in more than half patients (66 %, 19/29 patients), no matter the disease stage. 17 of them (90 %) has long-term therapeutic response – complete remission. 2 of them died. Initiation mean level of VEGF was 779.4 pg/ml, mean level after treatment was 446.2 pg/ml. Decrease of this level goes hand in hand with decrease of MMP8 (80 %). In 10 patients, whose levels of VEGF A didn´t decrease or even increased, 8 died, 2 are in CR. Initiation mean level of VEGF A in this group

was 408 pg/ml. Mean level after treatment was 500 pg/ml. No certain correlation between VEGF and MMP-8 levels was observed in this group.

Discussion

Angiogenesis is an important step in progression of cancer. Moreover, pharmacological targeting of angiogenesis is an effective approach in treatment of many types of solid tumours (bevacizumab, sunitinib, sorafenib, pazopanib, axitinib, aflibercept etc.). VEGF is involved in several steps of angiogenesis (main pro-angiogenic factor released from tumour cells, liberation of proteolytic enzymes to degrade the basal lamina, proliferation of endothelial cells and their differentiation into capillaries [3]. Therefore VEGF is under investigation as a possible predictive biomarker for antiangiogenic treatment and prognostic marker for patient´s survival. There were some studies having tried to monitor VEGF A levels during treatment or during disease progression. Higher levels of VEGF are usually associated with worse prognosis [4, 5]. We observed antiangiogenic potential of high dose interpheron alpha. Patients whose levels of VEGF A didn´t decrease or even increased during treatment did worse in general after-treatment outcome. This therapeutic outcome could correspond with Kirkwood´s last extended analysis of E1694 and improved survival of certain subgroup of patients after high-dose interpheron alpha treatment [6].

Conclusion

In our study, we describe decrease of VEGF A levels in 66 % of patients undergoing treatment with high-dose interpheron alpha. This trend suggests antiangiogenic potential of high-dose interpheron alpha. Decrease of VEGF A levels seems to be prognostic positive (90 % of patients, whose levels of VEGF A decreased, is in long-term complete remission). We are fighting small numbers; therefore another investigation is necessary to fully understand mechanisms of angiogenesis and metastasis, especially to define the correlation between VEGF and MMP and their particular roles in these mechanisms. Even the very incipient stage of malignant melanoma can be fatal. The key is in its angiogenic potential to rule the patient´s vasculature system, not in the size of melanoma.

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CHRONIC INFLAMMATION IN PSORIASIS: COMPARISON OF INFLAMMATORY PARAMETERS IN PATIENTS WITH PSORIASIS WITH THE CONTROL GROUP

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Introduction

Psoriasis is a chronic multisystemic inflammatory disease with manifestation primary on the skin and / or joints. Affects 2-3% of the population (1) and it is characterized by hyperplasia of keratinocytes resulting in erythematous oval maculopapules with silver scales on the surface. A standarized clinical indicator of severity of psoriasis is measurement of PASI (Psoriasis Area and Severity Index). PASI score ranges from 0 to 72, higher scores indicate higher severity of disease. PASI index is important in assessing of involvement of the skin surface, but there are also known complications of psoriasis which are localized not only on the skin- comorbidities including Crohn's disease, ulcerative colitis, diabetes (2), chronic neuropathy (3), prone to depression, metabolic syndrome. (4) Patients with psoriasis also have an increased risk of cardiovascular diseases- myocardial infarction, stroke and arterial hypertension (5), malignancy, particularly lymphoproliferative non- melanoma skin cancers (6). Due to comorbidities and mainly because of the increased risk of cardiovascular disease is in psoriasis seen shortening of life in about 5 to 10 years. (7)

Aims

It would be advisable to find a common cascade of inflammatory parameters in psoriasis and its comorbidities, and standardised measurement of another parameters, except PASI, which would indicate the severity of systemic involvement in psoriasis. We decided to monitor the pro- inflammatory markers (C-reactive protein- CRP, lipoprotein- associated phospholipase A2- Lp-PLA2, adiponectin, leptin, lipocalin, resistin and retinol binding protein 4- RBP 4). This parameters seem to be critical in psoriasis.

Methods

Measurement of pro- inflammatory markers in patients with psoriasis and in healthy patients. Venosus blood was taken from 32 patients with psoriasis who were hospitalized at the Department of Dermatology and Venereology University Hospital in Hradec Králové, then the serum was stored at - 70 ° C, until the ELISA tests (Enzyme-Linked Immunosorbent Assay) were performed. Then the inflammatory markers were compared with the markers of the control group. The control group was made up of 24 voluntary blood donors of Hematology Clinic of the University Hospital in Hradec Králové, who are not suffering from psoriasis. The groups were also divided into smokers and non-smokers and by gender. BMI, which is calculated as a proportion of body weight and height squared was also measured. The study was approved by the Ethical Committee of the University Hospital Hradec Králové, all patients were informed about the study and signed an informed consent.

Results

CRP, Lp-PLA2, lipocalin (level of significance 0,001), leptin (level of significance 0,05) were statisticaly significant higher in psoriasis vulgaris patients than those in healthy controls. In the groups of non-smoker we did not see the differences in the results we had already measured in the basic groups. Statisticaly significant positive were CRP, Lp-PLA2, lipocalin (level of significance 0,001), leptin (level of significance 0,05). We found differences in the values of inflammatory markers in the groups of smokers. Significant higher were Lp-PLA2 (level of significance 0,001), lipocalin (level of significance 0,01). The levels of other proinflammatory mediators were not significant higher. Regarding gender, in males with psoriasis were significant higher CRP, Lp-PLA2, lipocalin (level of significance 0,001), in

females were significant positive Lp- PLA2 (level of significance 0,001), lipocalin (level of significance 0,01), CRP, leptin (level of significance 0,05). In the patients with psoriasis was found negative correlation between BMI and PASI, adiponectin. Positive corellation between BMI and leptin.

Discussion

In patients with psoriasis we had measured significant higher levels of CRP, Lp-PLA2, lipocalin and leptin than the control group had. Between gender we had measured different values. In woman with psoriasis was also leptin higher (exept of CRP, Lp-PLA2, lipocalin) than in males with psoriasis. It is known that levels of leptin increase with the amount of adipose tissue, our results confirm this fact with significant positive correlation. Therefore every study should measure the levels of leptin always together with BMI and with the waist circumference. Also estrogen regulates the efficacy of leptin on endothelial cells, therefore it is suggested that hyperleptinemia is the problem mostly of female with psoriasis. In our study woman with psoriasis have significant higher levels of leptin, but in males with psoriasis was found significant positive correlation between BMI and leptin.

Conclusions/summary

CRP, Lp-PLA2, lipocalin and leptin are pro-inflammatory biomarkers, which are increased in psoriasis. They seem to have got predictable value of severity in psoriasis. Measured results are necessary to verify by another study following bigger group of patients and it is necessary to focus also on measurment of parameters of metabolic syndrome and BMI, because psoriasis and metabolic syndrome are related.

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HUMAN BLOOD PLASMA AND HUMAN BLOOD PLATELET-RICH PLASMA AS A SUBSTITUTE FOR FETAL CALF SERUM DURING LONG-TERM CULTIVATION OF MESENCHYMAL DENTAL PULP STEM CELLS

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Introduction

Fetal calf serum (FCS, FBS) became, due to its high concentration of growth factors and conversely a low content of inhibitory factors, the most widely used growth supplement in cultivation media. Particularly bovine serum proteins can be internalized by stem cells and stimulate immunogenicity (1, 2) in this context may also arise many problems due to viral and prion transmission (3), or immunological reactions as response to antigenic substrate (4). Therefore, the interest of the scientific community turns to derivatives of human blood or serum-free media containing artificial supplements. Although, it isn´t possible to standardize the human blood derivatives, their use avoids internalization of the animal substances, thus excluding the risk of zoonosis and immunogenicity (1, 2). The effect of platelet rich plasma (PRP) and (human plasma) HP serum derived from human blood is based on degranulation α granules from activated platelets, which contains growth factors. These growth factors are platelet derived growth factor (PDGF), transforming growth factors beta 1 and beta 2 (TGF β1, TGF β2), vascular endothelial growth factor (VEGF), platelet derived endothelial cell growth factor, interleukin -1 (IL-1), basic fibroblast growth factor (bFGF), and platelet activating factor-4 (PAF -4) (5).

Aims

The aim of this study was to confirm the possibility to cultivate human dental pulp stem cells in a cultivation media where the FCS was replaced by the HP or PRP.

Methods

DPSC were cultured under standard conditions in an incubator at 37 °C, in an atmosphere with 5% CO_2, in a culture medium consisting of α - MEM, 2% FCS, 10 ng/ml EGF, 10 ng/ml PDGF, L-ascorbic acid, 2% glutamine, penicillin/streptomycin, gentamycin, dexamethasone and supplemented with insulin- transferrin-sodium-selenite supplement (ITS) at a concentration of 10 µl//ml. After the DPSC bypassed the Hayflick limit; the DPSC ability to differentiate in osteoblast-like cells was verified. The cells obtained from the third passage were thawed and seeded into 5 different media. Medium 1 (standard medium): α - MEM, 2% FCS, 10 ng/ml EGF, 10 ng/ml PDGF, L-ascorbic acid, 2% glutamine, penicillin/streptomycin, gentamycin, dexamethasone and 10 µl of ITS/ml. Medium 2: α - MEM, 2% HP, 10 ng/ml EGF, 10 ng/ml PDGF, L-ascorbic acid, 2% glutamine, penicillin/streptomycin, gentamycin, dexamethasone and 10 µl of ITS/ml. Medium 3: α - MEM, 10% HP, L-ascorbic acid, 2% glutamine, penicillin/streptomycin, gentamycin, dexamethasone. Medium 4: α - MEM, 2% PRP, 10 ng/ml EGF, 10 ng/ml PDGF, L-ascorbic acid, 2% glutamine, penicillin/streptomycin, gentamycin, dexamethasone and 10 µl of ITS/ml. Medium 5: α - MEM, 10% PRP, L-ascorbic acid, 2% glutamine, penicillin/streptomycin, gentamycin, dexamethasone.
The proliferation activity was measured each passage and at the end of cultivation the phenotype profile was measured.

Results:

We cultivated DPSC in the various cultivation media over 15 population doublings except for the medium supplemented with 10% HP. Our results showed that DPSC cultivated in medium supplemented with 10% PRP had the shortest average population doubling time (DT) (28.6 +/- 4.6 hours), in contrast to DPSC cultivated in 10% HP which had the longest DT (156.2 +/- 17.8 hours); hence this part of the experiment had been cancelled in the 6th passage. DPSC cultivated in media with 2% FCS+ITS (DT 47.3 +/- 10.4 hours), 2% PRP (DT 40.1 +/- 5.7 hours) and 2% HP (DT 49.0 +/- 15.2 hours) showed almost the same proliferative activity.

DPSC's viability in the 9th passage was over 90% except for the DPSC cultivated in the 10% HP media.

The positivity for CD in media order 1,2,4,5 were for CD 29 - 86.1 % / 81.09 % / 89.49 % / 98.56 %, for CD 44 - 41.7 % / 64.78 % / 33.26 % / 10.66 %, for CD 73 – 67.81 % / 73.62 % / 34.94 % / 12.77 %, for CD 90 – 82.15 % / 85.49 % / 49.24 % / 12.05 %, for CD 105 – 26.92 % / 38.28 % / 10.2 % / 5.12 %, for CD 146 – 63.94 % / 68.66 % / 40.45 % / 41.72 %.

Discusion:

When the scientist firstly described the stem cells and presented their properties it was believed, that most of the diseases will be eradicated soon. Now, about 50 years ago, when the understanding of the stem cells is much deeper, still the clinicians cannot use the stem cell based therapy as standard and common way of treatment. Why did this happen? The answer is thanks to better understanding we haven´t just solved first questions, but we have moreover found new ones. One of the newly detected threats is the usage of xenogeneic supplements during cultivation of stem cells. In our study we successfully replaced the most widely used xenogeneic supplement which is FCS by HP and PRP. We have observed that medium with 10 % of HP leads to low proliferation activity which resulted in loosing of the lineage. Medium with 2% HP or PRP substitute by growth factors leads to proliferation activity similar to our reference media containing 2% FCS with growth factors, but the phenotype of DPSC cultivated in 2% PRP started to lose positivity for typical mesenchymal stem cells markers. PRP in the 10% concentration leads to increased proliferation capability, but it has side effect on the DPSC phenotype, where the positivity of typical stem cells markers was almost lost. The possible explanation of the phenotypical changes are:1) the stem cell differentiate into mature cell line, 2) the stem cells changed their phenotype closer to the ectomesenchymal phenotype under the influence of PRP or 3) the cell dedifferentiate to less mature line.

Conclusions:

We proved that human blood derivates are viable substitutes for FCS in cultivation media for long-term DPSC cultivation.

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