Biochemical Effects of Retinoid Derivatives on Mesenchymal Stem Cells *In Vitro*

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The studies we performed targeted the effects of all-trans retinol (vitamin A) and some retinoid derivatives (including tretinoin or all-trans retinoic acid, retinyl propionate, 9-cis retinoic acid, 13-cis retinoic acid), as well as of tazarotenic acid on apoptosis of rat mesenchymal stem cells, cultured after isolation. Tazarotenic acid is considered to be relatively selective and a potent agonist for RARβ and RARγ and less for RARα. The same time, tazarotenic acid is not binding to RXRs (retinoid X receptors). The relevant analysis of our experimental results demonstrated that 13-cis retinoic acid was the most potent inducer of apoptosis of cultured mesenchymal stem cells of rat origin when compared to other retinoid derivatives, as follows: 13-cis retinoic acid > 9-cis retinoic acid > tazarotenic acid > all-trans retinoic acid > retinyl propionate > retinol (or vitamin A). Very interesting and unexpected were the apoptotic effects of 1 μM tazarotenic acid for 24 hours in our experiments, very close to those induced by all-trans retinoic acid (tretinoin). The apoptosis induced by 13-cis retinoic acid, a principal activator of RARβ and RARγ, and that induced by 9-cis retinoic acid, a major activator of RXRs, suggests different pathways activated by these retinoid derivatives.

Keywords: mesenchymal stem cell, rat, apoptosis, 13-cis retinoic acid, 9-cis retinoic acid

The sacro-caudal region is born as a result of the development and differentiation of mesenchymal stem cells with high pluripotent capacities, located in the tail bud, which represents the caudal part of the vertebrate embryo. The agenesis of this caudal part could be studied using a mice model, obtained after the treatment with retinoic acid at 9.5 days after the pregnancy settlement. Retinoic acid treatment is inducing a reliable and harsh truncation of the body axis. The very first observation was that retinoic acid induced broad apoptosis of cells located in tail bud in the initial 24 hours following the administered treatment. After the removal of resulted apoptotic cells, the remnant mesenchymal cells were able to differentiate and adopted an obvious neural fate. The developed neurons were positive for neurofilaments and expressed Pax-3 and Pax-6 as a characteristic regional paradigm. Furthermore, it was demonstrated that the treatment with retinoic acid was able to down-regulate the Wnt-3a gene expression, extremely important for the development of tail bud. On the other hand, Wnt-5a and RAR-γreceptors were not highly affected by the retinoic acid treatment. The above obtained results demonstrated that retinoic acid treatment induced a severe down-regulation of Wnt-3a gene, associating a central role in axial truncation pathologic mechanisms, resulting in an enhanced apoptosis and tail bud mesenchymal cells fate alteration, followed by the forming of multiple ectopic neural tube [1].

One of the important cell populations with heterogeneous characteristics is represented by the so-called mesenchymal stromal cells variant. This population is including various cell types as mesenchymal stem cells, fibroblasts and fibroblast cell-types, many progenitor cells, as well as other types of cells. When taking into account the tissues as bone marrow, adipose and dermal ones, we must keep in mind that these types are including a specific population known as multilineage-differentiating stress enduring cells or Muse. These multilineage-differentiating stress enduring cells could represent the population of mesenchymal stem cells responsible for their associated pluripotent-related properties. These multilineage-differentiating stress enduring cells also expressed genes bearing features of pluripotency and were able to differentiate in triploblastic cells starting from single cell and to be self-renewable. Mesenchymal stem cells are well-known types of cells being able to liberate huge amounts of active factors in local medium, altering the development dynamics of surrounding fate of cells. Revealing the secretomes of multilineage-differentiating stress enduring cells and mesenchymal stem cells will allow us to shed light on the internal biological pathways phenotypically responsible for their development and functional characteristics. The actual studies established that the secretomes of multilineage-differentiating stress enduring cells include biologically active factors effective in remodeling of extracellular milieu, regulation of ox-redox processes and immune system modulation. Moreover, the multilineage-differentiating stress enduring cells seem to release biologically active factors able to maintain their broad stem features, to empower the survival when facing stress conditions and to sustain their immunomodulatory capacities. The described features of multilineage-differentiating stress enduring cells are basically involving pathways as PKA signaling, as well as FXR/RXR and LXR/RXR activations [2].

One of the most common type of primary malignant tumours is represented by the osteosarcoma, affecting the bones. The survival of the patients with osteosarcoma is entirely related to our capabilities and abilities to treat the recurrent lesions and metastasis. Overexpression or applied ligands of some types of receptors as PPARγ or retinoid receptors are both able to induce important apoptosis of osteosarcoma cells. The vast majority of studied osteosarcoma cells were capable to express endogenous PPARγ and several isotypes of retinoic acid receptors and retinoid X receptors. Apoptosis and inhibition

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of the proliferation of osteosarcoma cells were extensively induced by 9-cis retinoic acid, all-trans retinoic acid, troglitazone, as well as by the overexpressed PPARγ, RARα and RXRα. Cumulative inhibitory effects on proliferation were observed for troglitazone and retinoid derivatives. Similar effects were obtained when pairs as PPARγ/RARα and PPARγ/RXRα were overexpressed in osteosarcoma cells. It is very clear that overexpressed PPARγ, RARα, RXRα or their combinations were able to promote osteosarcoma cells apoptosis, reducing thus their proliferation. In addition, retinoid derivatives were efficient to induce the differentiation of osteosarcoma and mesenchymal stem cells. Such a positive effect was less important for troglitazone [3].

The studies we performed targeted the effects of *all-trans* retinol (vitamin A) and some retinoid derivatives (including tretinoin or *all-trans* retinoic acid, retinyl propionate, 9-*cis* retinoic acid, 13-*cis* retinoic acid), as well as of tazarotenic acid on apoptosis of rat mesenchymal stem cells, cultured after isolation. Tazarotenic acid is considered to be relatively selective and a potent agonist for RARβ and RARγ and less for RARα. The same time, tazarotenic acid is not binding to RXRs (retinoid X receptors).

Experimental part

Our thorough experiments were accomplished using isolated mesenchymal stem cells obtained from rats and cultured in well-established and tested conditions as we previously reported [4]. The 24 h effects of 1 μM *all-trans* retinol and some retinoid derivatives (tretinoin or *all-trans* retinoic acid, retinyl propionate, 9-*cis* retinoic acid, 13-*cis* retinoic acid) and tazarotenic acid on chemically-induced apoptosis of rat isolated mesenchymal stem cells were highlighted using flow cytometry techniques [5-7]. Retinol or vitamin A is synonym with all-*trans*-3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraen-1-ol. Retinol is the alcoholic circulating form of vitamin A and is not biologically active. It is the precursor for active retinoid derivatives. Tretinoin or *all-trans* retinoic acid activates both types of receptors, RARs and RXRs. The activated RARs and RXRs work as transcription factors in normal as well as malignant cells. Retinyl propionate is an ester form of vitamin A. Beside the other esters, acetate and palmitate, it represents an important pool for vitamin A to be transformed in retinoid derivatives as needed. 9-*cis* Retinoic acid, synonym with 9-*cis* tretinoin or alitretinoin is able to alike activate RARS and RXRs. 13-*cis* Retinoic acid, synonym with isotretinoin, extensively activates RARβ and RARγ. Tazarotenic acid is synonym with 6-(2-(4,4-Dimethylthiochroman-6-yl)ethynyl)nicotinic acid or 6-[2-(3,4-Dihydro-4,4-dimethyl-2H-1-benzothiopyran-6-yl)ethynyl]-3-pyridinecarboxylic acid.

The Ethics Committee of the Grigore T. Popa University of Medicine and Pharmacy from Iasi approved the described experimental rat cells protocols.

Results and discussions

The relevant analysis of our experimental results demonstrated that 13-*cis* retinoic acid was the most potent inducer of apoptosis of cultured mesenchymal stem cells of rat origin when compared to other retinoid derivatives, as follows: 13-*cis* retinoic acid (fig. 1) > 9-*cis* retinoic acid (fig. 2) > tazarotenic acid (fig. 3) > *all-trans* retinoic acid (fig. 4) > retinyl propionate (fig. 5) > retinol (or vitamin A) (fig. 6).

All the above results are to be compared to control group (data not showm), in which the natural and culture conditions-induced apoptotic proportion is 9.2±1.51% for 24 h.

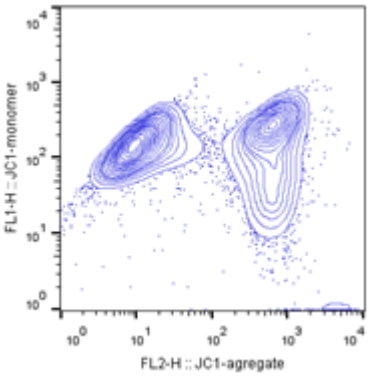

Fig. 1. The proportion of apoptotic mesenchymal stem cells was 72.12±4.34% when 1μM 13-*cis* retinoic acid was administered in the culture medium for 24 h (7 experiments, representative experiment showed)

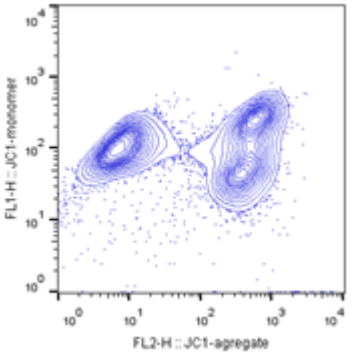

Fig. 2. The administration of 1μM 9-*cis* retinoic acid for 24 h triggered mesenchymal stem cells apoptosis in a proportion of 54.78±2.97% (7 experiments, representative flow cytometry)

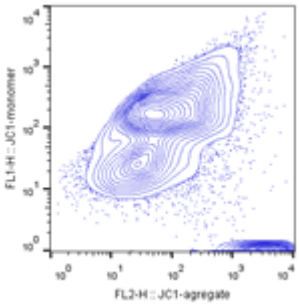

Fig. 3. Apoptotic rat mesenchymal stem cells were found in a proportion of 38.17±2.71% when were cultured in the presence of 1μM tazarotenic acid for 24 h (7 experiments, representative experiment showed).

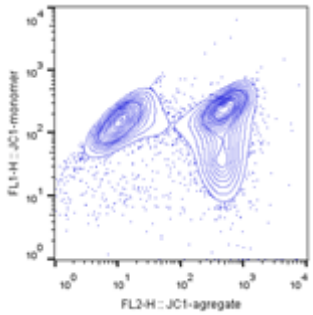

Fig. 4. *all-trans* Retinoic acid, 1μM, induced an apoptotic proportion of 31.12±2.23% of rat isolated and cultured mesenchymal stem cells (7 experiments, showing representative flow cytometry)

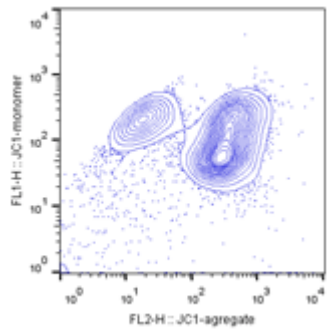

Fig. 5. The treatment for 24 h with 1μM retinyl propionate, an ester form of vitamin A, generated a reduced rate of apoptotic mesenchymal cells of rat origin in culture, of only 15.63±1.98% (7 experiments, showing representative experiment)

The apoptosis induced by 13-*cis* retinoic acid, a principal activator of RARβ and RARγ, and that induced by 9-*cis* retinoic acid, a major activator of RXRs, suggests different pathways activated by these retinoid derivatives. As

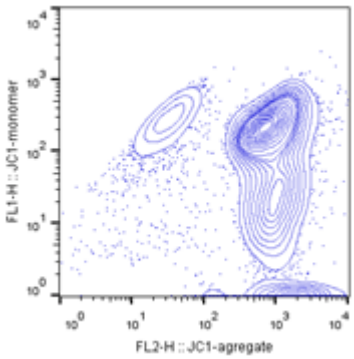

Fig. 6. Retinol, the alcoholic form of vitamin A, 1μM for 24 h in culture medium, induced apoptosis of 13.91±1.37% of isolated rat mesenchymal stem cells (representative flow cytometry showed)

average, 9-*cis* retinoic acid is less effective with 24.04% than 13-*cis* retinoic acid.

Tretinoin or *all-trans* retinoic acid, which activates both types of receptors, RARs and RXRs, is less effective to induce apoptosis of isolated rat mesenchymal stem cells in culture. To be mentioned that, as average, *all-trans* retinoic acid is less effective with 56.84% than 13-*cis* retinoic acid and with 43.19% than 9-*cis* retinoic acid.

Very interesting and unexpected were the apoptotic effects of 1 μM tazarotenic acid for 24 h in our experiments, very close to those induced by *all-trans* retinoic acid (tretinoin). Although, as average, tazarotenic acid is less effective with 47.07% than 13-*cis* retinoic acid and with 30.32% than 9-*cis* retinoic acid.

Retinyl propionate and retinol, the alcoholic form of vitamin A, both 1μM for 24 h, did not induce statistically significant apoptotic effects, when administered as treatment of isolated cultured rat mesenchymal stem cells.

These are precursory studies and we did not extend the experiments toward the molecular pathways underlying the above observed effects.

The best known mesenchymal stem cells, presenting features of fetal and pluripotent stem cells, are those of human origin and derived from umbilical cord. Also, they are associating the capabilities to differentiate into various other cell lineages. During the last decade they became very important from the point of view of possible therapeutic applications. Therefore, there are few data on their sensitivity to retinoic acid. Retinoic acid was clearly known as a powerful teratogen, inducing craniofacial and limb abnormalities in embryos of vertebrates. The studies on mesenchymal stem cells from umbilical cord demonstrated that retinoic acid was able to upregulate the enzyme caspase expression and also to increase Bax/BCl2 ratio. The cytotoxicity of retinoic acid on mesenchymal stem cells derived from umbilical cord is highly dependent on the duration of treatment, status of cells, passages, time of culture, etc. [8, 9].

Some recent studies suggested that tazarotene, a new component of retinoids with selective activity at RAR γ/β presents an inhibitory effect against the proliferation of human basal carcinoma cell through the induction of apoptosis involving caspase pathways. Anyway, the deep molecular pathways involved in the anti-proliferative effects of tazarotene are not clear understood. The present studies showed that tazarotene was able to induce cleavage of caspases 9 and 3 as well as of PARP in human basal carcinoma cells, in a mitochondrial dependent manner, these processes being activated by enhancing reactive oxygen species quantitatively and caspase 8 activation. Caspase 8 activation was induced through reactive oxygen species as well as through death receptor pathways triggering. Meanwhile, the BCl-2 and BCl-XL, anti-apoptotic proteins, are decreasing. Interestingly, tazarotene is inducing a convergency of extrinsic and intrinsic pathways of apoptosis, involving caspase 8 and Bid signaling [10].

The derivatives of vitamin A, named retinoids, are biologically active molecules, natural or synthetic, with a variety of induced effects, regulating the differentiation of cells, cellular proliferation and cellular death through apoptosis. Retinoic acid and 9-*cis* retinoic acid, natural components of retinoids system, are synthetized from retinal, retinaldehyde being the intermediary reaction product. Retinoic acid is the natural ligand for RARs and 9-cis retinoic acid is the natural ligand for the RXRs. Concerning the synthetic derivatives, RAR ligands include isotretinoin, tazarotene and adapalene. The first ligand of RXRs is bexarotene. Each family of receptors is including three isotypes, namely α, β and γ, and several isoforms exist for each isotype. Nuclear retinoid receptors of type RXRs require for activation the dimerization process, as hetero- or homodimers. Resulting dimers bind specifically to DNA sequences and recruit coactivators, the activation of transcription factors being induced [11].

Taking into account our results we should emphasize the involvement of retinoid derivatives functioning into all the biologic systems, knowing that they are involved in cellular differentiation, cellular proliferation, apoptosis and immunomodulation. All the tissues and organs might be influenced by the alterations of the retinoid derivatives systems [12-22].

The most important modern threatening of our life is the interaction of released components of plastics, spread all over the environmental milieu, with the molecular systems of the human body [23,24]. Through the reduction of aggressiveness from the environment we will be able to empower the quality of our life [25-30].

Conclusions

The studies we performed targeted the effects of *all-trans* retinol (vitamin A) and some retinoid derivatives (including tretinoin or *all-trans* retinoic acid, retinyl propionate, 9-*cis* retinoic acid, 13-*cis* retinoic acid), as well as of tazarotenic acid on apoptosis of rat mesenchymal stem cells, cultured after isolation. Tazarotenic acid is considered to be relatively selective and a potent agonist for RARβ and RARγ and less for RARα. The same time, tazarotenic acid is not binding to RXRs (retinoid X receptors).

The apoptosis induced by 13-*cis* retinoic acid, a principal activator of RARβ and RARγ, and that induced by 9-*cis* retinoic acid, a major activator of RXRs, suggests different pathways activated by these retinoid derivatives.

These are precursory studies and we did not extend the experiments toward the molecular pathways underlying the above observed effects.

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