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Original Article

The effects of adipose-derived mesenchymal stem cells combined with sodium selenite on Hashimoto's thyroiditis

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Abstract: Recent research found that sodium selenite (Na_2SeO_3) could ameliorate oxidative damage in patients with Hashimoto's thyroiditis (HT). Additionally, the effects of adipose-derived mesenchymal stem cells (AMSCs) in an animal model of HT were also reported. However, the effects of AMSCs combined with Na_2SeO_3 on HT are unknown. We investigated the combined effects of AMSCs and Na_2SeO_3 in a rat model of HT and the in vitro effect of Na_2SeO_3 on AMSCs using gene microarray analyses. In the HT rat model, the combination of AMSCs and Na_2SeO_3 restored thyroid tissue structure to that of normal controls and increased the levels of most antioxidant and inflammatory cytokines examined, but decreased the levels of interleukin 10 (IL-10) in HT thyroid tissues. At 0.5-20 μM, Na_2SeO_3 promoted AMSC growth and increased the levels of reduced glutathione and total antioxidant capacity in AMSCs ($P<0.05$). Na_2SeO_3 increased the levels of hepatocyte growth factor (HGF), transforming growth factor beta (TGF-β), and stem cell factor (SCF) in AMSC culture supernatants. The results of the gene microarray analyses showed that the expression levels of certain genes involved in mitosis, DNA replication and repair, ubiquitination, synthesis and metabolism, and mitochondrial transport changed in response to Na_2SeO_3 treatment. In conclusion, the combination of AMSCs and Na_2SeO_3 restored the function and structure of the thyroid in an HT model, and Na_2SeO_3 promoted the growth, improved the secretion, and the antioxidant capacity of AMSCs in vitro. This combination treatment may provide a new therapy for patients with HT.

Keywords: Hashimoto's thyroiditis, sodium selenite, adipose-derived MSCs, antioxidant, immunomodulation

Introduction

Hashimoto's thyroiditis (HT) is a common autoimmune thyroid disease and is especially prevalent in coastal areas [1]. The cause of HT involves many factors, including genetic susceptibility, inflammation, excess iodine intake, and infection [2]. Presently, treatment for HT is limited to antithyroid agents or hormone replacement therapy for hypothyroidism. As the pathogenesis of HT is unclear, its etiology needs to be further explored.

Consumption of excess iodine is a high-risk environmental factor in HT development, but the mechanism by which excess dietary iodine induces autoimmune thyroiditis is still unclear [3]. Previous reports indicated that excess iodine intake might lead to thyroid tissue injury by inducing immune cell infiltration into the thyroid [4], causing oxidative stress and eventually triggering apoptosis-signaling pathways [5]. Decreased autophagy and enhanced apoptosis were observed in the thyroid tissues of patients with HT [5].

Selenium is an essential nutrient for human health and plays an important role in human thyroid physiology [6]. Selenium-dependent enzymes, such as glutathione peroxidase, act

as antioxidants and modify redox status in the thyroid. The lack of glutathione peroxidase activity might contribute to oxidative damage in thyroid cells and the initiation of thyroid autoimmunity [7]. In rats, selenium supplementation was shown to prevent oxidative damage in the thyroid [8]. A recently published clinical trial reported that selenium compounds played significant roles in L-thyroxine therapy by serving as adjuvants and reducing the serum levels of antithyroid peroxidase antibodies in patients with autoimmune thyroiditis [9].

Adipose-derived mesenchymal stem cells (AMSCs) have low immunogenicity in vivo and a high capacity for rapid expansion in vitro. Previous research found that MSCs could migrate into areas of inflammation, release high levels of chemokines, and interact directly with immune cells to regulate abnormal immunity [10]. The administration of AMSCs (both syngeneic and allogeneic) to mice with autoimmune thyroiditis has been shown to suppress T-lymphocyte infiltration into the thyroid [11]. However, the therapeutic roles and specific mechanisms by which these cells function still need to be clarified.

There are no reports on the effects of AMSCs combined with sodium selenite (Na_2SeO_3) on autoimmune thyroiditis. In the present study, we investigated the effects of AMSCs combined with Na_2SeO_3 in a rat model of HT and further explored the effects and mechanisms of Na_2SeO_3 on AMSCs in vitro.

Materials and methods

Preparation of AMSCs

This study was approved by the Institutional Animal Ethical Committee, Qingdao, and the Ethics Committee of the Affiliated Hospital of Qingdao University. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Inguinal fat pads were excised from 8-week-old female Wistar rats for the preparation of AMSCs. Briefly, the fat tissue was cut into pieces and digested with 0.05% collagenase type I (Sigma-Aldrich, St. Louis, MO, USA) in 5% bovine serum albumin (Sigma-Aldrich), and subsequently filtered through a 250-μm nylon mesh and centrifuged. The cell pellet was resuspended in AMSC medium containing DMEM (Gibco, Waltham, MA, USA) and 10% fetal bovine serum (Gibco) and cultured at 37°C in 5% CO_2 and 95% O_2.

AMSCs were passaged 3 times prior to harvest. Cell surface marker analysis was performed using flow cytometry for CD31, CD45, CD73, CD44, CD105, and CD90 (Chemicon, Temecula, CA, USA). The specific fluorescence of 10,000 cells was analyzed with a FACScalibur instrument (Becton Dickinson, Franklin Lakes, NJ, USA) using Cell Quest Pro software. Adipogenic and osteogenic differentiation markers were analyzed, as previously described [12, 13]. Calcium deposition was determined using von Kossa staining (Gibco), and intracellular droplets of adipocytes were visualized using oil red O staining (Sigma).

Experimental rat model of autoimmune thyroiditis

A total of 70 female Wistar rats (6 weeks of age), weighing 80-100 g, were purchased from the Nanjing Laboratory Animal Center (Nanjing, China). Rats were fed specific pathogen-free mouse chow and water *ad libitum* and housed at a temperature of 22-23°C and a humidity of 60% with a 12-h light/dark cycle.

Experimental autoimmune thyroiditis (EAT) was induced by the injection of porcine thyroglobulin (Tg, Sigma-Aldrich) and maintained with high iodine water (500 mg/L) feeding. For induction, 50 mg of porcine Tg in deionized water (0.5 mL) was emulsified in Freund's complete adjuvant (1:1, v/v, Sigma-Aldrich), and 1 mL was administered subcutaneously (s.c.) to each rat. The booster injections on days 7, 21, and 28 were given s.c. using 50 mg porcine Tg in incomplete Freund's adjuvant (Sigma-Aldrich), using the same dosage and injection method. The success of the EAT model was confirmed by examining the serum levels of FT3, FT4, TSH, Thyroid peroxidase antibody (TPO Ab), and Thyroglobulin antibody (Tg Ab), and images of the thyroid gland.

Fifty rats were injected with porcine Tg, which successfully induced EAT in 90% of the animals. Forty-four EAT rats were randomly divided into 4 groups: HT control group (n=10), AMSC group (n=12), Na_2SeO_3 group (n=10), and AMSC + Na_2SeO_3 group (n=12). Rats fed normal water

and chow throughout the experiments were considered normal controls (n=10). Rats in the HT control group were fed high iodine water (500 mg/L sodium iodide). Rats in the AMSC group were treated with 4 AMSC infusions via the vena caudalis from the third day after diagnosis of EAT (1×10^6 AMSCs per rat, one injection each week) for 4 weeks. Rats in the Na_2SeO_3 group were treated with 0.2 mg Na_2SeO_3/kg/day (S5261, Sigma-Aldrich) by intragastric administration from the third day after diagnosis of EAT for 35 days. Rats in the AMSC + Na_2SeO_3 group were treated with the AMSC infusions as described above and 0.2 mg Na_2SeO_3/kg/day for 35 days. All rats were sacrificed on day 63 for analysis.

Determination of serum selenium levels and thyroid function

Serum selenium concentrations were measured in duplicate using inductively coupled plasma mass spectrometry (ICP-MS, Perkin-Elmer SCIEX ELAN 6000, Waltham, MA, USA). A calibration curve was established for 1-400 μg selenium/L (r^2>0.99). The limit of detection was 0.5 μg/L. Serum levels of FT3, FT4, TSH, TPO Ab, and Tg Ab were examined, according to the manufacturer's protocol (Roche Diagnostics GmbH, Mannheim, Germany). All tests were repeated 3 times.

Pathological examination and AMSC homing assay

Thyroid samples were fixed in 10% neutralized formalin, embedded in paraffin, and cut into 4-μm sections. Tissue sections were counterstained with hematoxylin and observed using optical microscopy. The homing efficiency of injected AMSCs was assayed by labeling AMSCs with the red fluorescent dye CM-DiI (Life Technologies, Carlsbad, CA, USA), according to the manufacturer's instructions. Animals were sacrificed 2 days after injection, and the presence of CM-DiI-labeled cells was examined in thyroid tissue using fluorescence microscopy.

Cytokine analysis

Supernatant samples of the thyroid tissue extract were assayed to determine levels of cytokines in T helper type 1 (Th1) and Th2 cells using a multiplex cytokine/chemokine kit for tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), hepatocyte growth factor (HGF), stem cell factor (SCF), transforming growth factor beta (TGF-β), and interleukins (IL-2, IL-4, IL-6, and IL-10), according to the manufacturer's instructions (Millipore, Bedford, MA, USA). To determine cytokines released by AMSCs, cell culture supernatants were used. Levels of vascular endothelial growth factor (VEGF), TGF-β, Indoleamine 2,3-dioxygenase (IDO), SCF, and HGF were measured using enzyme-linked immunosorbent assays, according to the manufacturer's instructions.

AMSCs cultured with sodium selenite

AMSCs (at passage 4) at a density of 10,000 cells/well were seeded onto 6-well cell culture plates and treated with different concentrations (0.1-50 μM) of Na_2SeO_3 (Sigma-Aldrich), as previously described [14]. Untreated cells were considered the control group. AMSCs (2×10^5) were plated into 60-mm dishes one day prior to exposure to Na_2SeO_3. The number of AMSCs was counted each day using a Coulter counter (Beckman Coulter, Brea, CA, USA) and photographed under microscopy after 4 days (Nikon, Tokyo, Japan).

Gene microarray analysis

Microarray analysis was carried out, as previously described [15]. Briefly, RNA was extracted using an RNeasy Mini kit (Qiagen, Venlo, Netherlands), according to the manufacturer's protocol. Array analysis was carried out using cRNA prepared from equal amounts of RNA (1 μg) pooled from 3 replicates per group. Biotin-labeled cRNA was fragmented at 94°C for 35 min, hybridized overnight onto an Affymetrix mouse 430A 2.0 GeneChip® (Affymetrix, Santa Clara, CA, USA), and scanned using an Agilent Gene Array scanner (Agilent Technologies, Santa Clara, CA, USA). Grid alignment and raw data generation were performed using Affymetrix GeneChip Operating software, version 1.1. A noise value, based on the variance of low-intensity probe cells, was used to calculate the minimum threshold for each GeneChip. Samples were averaged, and the data refined by eliminating genes with signal intensities less than 300 in both groups. Heat maps were generated from greater than or equal to 3-fold changes in gene expression normalized to control tissue using an unsupervised hierarchical cluster analysis.

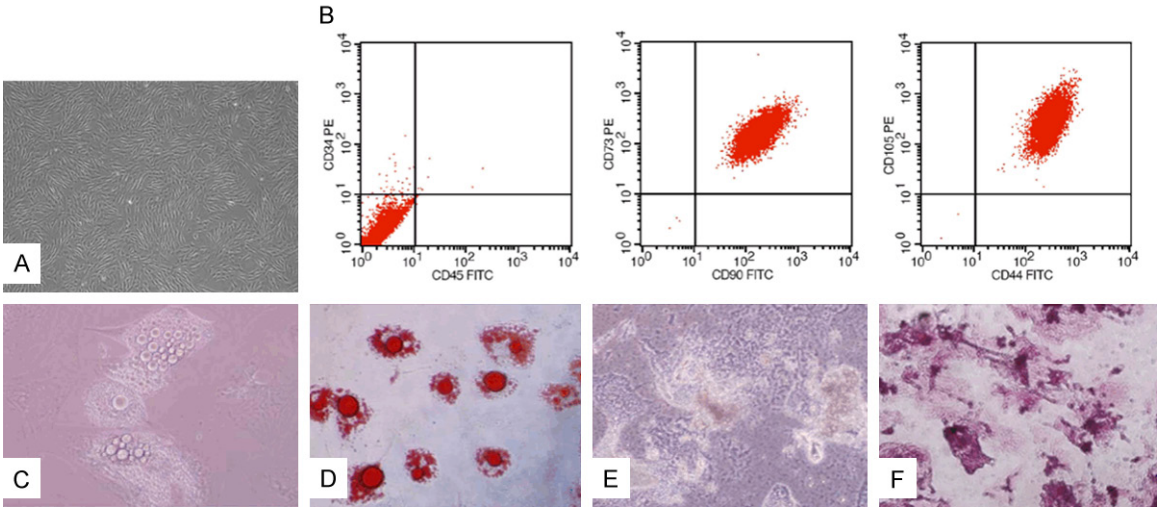

Figure 1. AMSCs and multilineage differentiations. A: AMSCs at passage 4; B: Flow cytometry analysis of AMSCs; C: AMSCs with adipogenic differen-tiation; D: Result of oil red O staining; E: AMSCs with osteogenic differentiation; F: Result of von Kossa staining.

Quantitative RT-PCR

Total RNA was extracted using the Trizol reagent (Life Technologies), and 1 μg of total RNA was reverse transcribed to generate cDNA using a cDNA synthesis kit (TransGene, Beijing, China). Real-time qPCR analysis was performed in triplicate, with GAPDH as the internal control, using the SYBR Green qPCR SuperMix and StepOnePlus Real-Time Detection System (Applied Biosystems, Waltham, MA, USA), following the manufacturer's instructions. PCR primers were designed using Primer 3, and their specificity was verified using BLAST (NCBI, Maryland, USA).

Determination of cellular antioxidant status and oxidative damage

Cellular antioxidant status was evaluated using the levels of total antioxidant capacity (T-AOC) and reduced glutathione (GSH). Levels of T-AOC were measured using a previously described colorimetric method [16]. Intracellular GSH concentrations were measured using a commercial assay kit (GSH assay kit, Beyotime). Absorbance was measured at 412 nm, and the data were normalized to cellular protein levels.

Statistical analysis

All statistical analyses were conducted using SPSS, version 17.0 (SPSS Inc., Chicago, IL, USA). All results were expressed as the mean and standard error of the mean. Group data were compared using the Student's *t* test or one-way analysis of variance, followed by Tukey's post hoc test. Differences with a confidence level of 95% or higher were considered statistically significant ($P<0.05$).

Results

Characterization of AMSCs and animals

AMSCs isolated from rat inguinal adipose tissue were adhered to the plastic surfaces of culture flasks and had a spindle-shaped morphology (**Figure 1**). After 3 passages, the cells were highly pure and expressed CD_{44}^+ (75.34%), CD_{105}^+ (79.26%), CD_{90}^+ (85.77%), CD_{73}^+ (89.63%), CD_{34}^- (0.34%), and CD_{45}^- (0.14%). The adipogenic and osteogenic differentiations are shown in **Figure 1**.

AMSCs combined with Na_2SeO_3 restore thyroid function and structure in HT rats

A rat model of HT was successfully established via injections of porcine Tg and maintained with high iodine water (500 mg/L) feeding. As shown in **Table 1**, serum levels of FT3, FT4, Tg Ab, and TPO Ab increased, whereas serum levels of TSH decreased in EAT rats compared with normal rats at 10 weeks ($P<0.05$). After treatment, levels of FT3, FT4, Tg Ab, and TPO Ab

Table 1. Serum levels of FT3, FT4, TSH, TG-ab, TPO-ab and selenium of rats in five groups after treatment

	Control group	HT control group	AMSCs group	AMSCs+Na_2SeO_3 group	Na_2SeO_3 group
FT3 (pmol/L)	4.93±1.56	46.31±20.68*	28.37±15.24#	23.46±14.21$,^	34.85±18.97
FT4 (pmol/L)	16.12±8.35	98.32±52.46*	56.32±30.54#	40.97±25.14$,^	83.54±45.87
TSH (mIU/L)	0.01±0.93	<0.001*	0.001±0.054#	0.001±0.061$	<0.001
TG-ab (IU/mL)	16.89±9.35	79.95±43.65*	53.78±27.51#	48.93±26.47$	63.45±38.41
TPO-ab (IU/mL)	10.61±6.21	82.64±45.31*	53.21±25.14#	40.49±22.41$,^	60.83±34.17Δ
Selenium (μg/mL)	0.29±0.17	0.22±0.15	0.26 ± 0.14	0.33 ±0.19$	0.32±0.17Δ

*P<0.05, compared with Control group; #P<0.05, compared with HT control group; $P<0.01, compared with HT control group; ΔP<0.05, compared with HT control group; ^P<0.01, compared with AMSCs group and Na_2SeO_3 group.

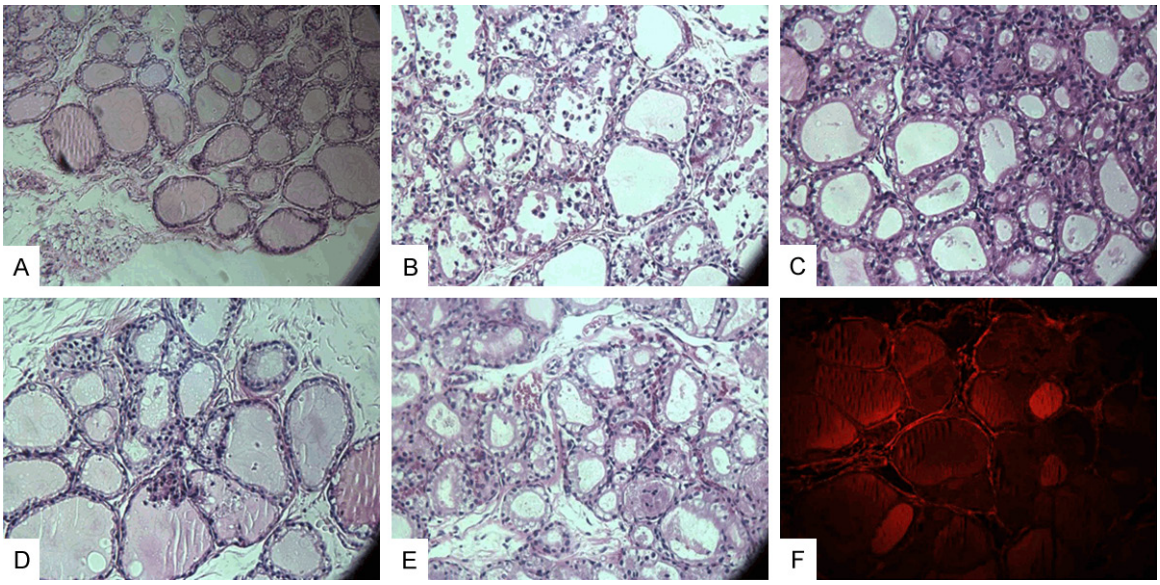

Figure 2. Pathological results of thyroid tissues. A: Pathological results of thyroid follicular structure in normal control group. B: The follicular structure of the thyroid in the HT control group exhibited moderate to severe damage with obvious infiltration of lymphocytes. C: In the AMSC group, the structure of the thyroid was almost completely restored with little lymphocyte infiltration and additional blood vessels observed around the lobules. D: In the AMSC + Na_2SeO_3 group, the structure of the thyroid was restored to that seen in normal controls after treatment. E: In the Na_2SeO_3 group, the structure of the thyroid was partially restored with little lymphocyte infiltration. F: AMSCs labeled with CM-DiI were observed in the thyroid using fluorescence microscopy.

in the AMSC and AMSC + Na_2SeO_3 groups significantly decreased, while in these same groups, the levels of TSH increased compared with the HT control group (P<0.05). The levels of FT3, FT4, and TPO Ab in the AMSC + Na_2SeO_3 group were significantly lower than in the AMSC and Na_2SeO_3 groups (P<0.01). Levels of selenium in the AMSC + Na_2SeO_3 and Na_2SeO_3 groups were higher than in the other 3 groups (P<0.05).

As shown in **Figure 2**, compared with the normal control group, the follicular structure of the thyroid in the HT control group exhibited moderate to severe damage with obvious infiltration of lymphocytes. In the Na_2SeO_3 group, the structure of the thyroid was partially restored with little lymphocyte infiltration. In the AMSC group, the structure of the thyroid was almost completely restored with little lymphocyte infiltration and additional blood vessels observed around the lobules. However, in the AMSC + Na_2SeO_3 group, the structure of the thyroid was restored to that seen in normal controls after treatment. AMSCs labeled with CM-DiI were observed in the thyroid using fluorescence microscopy, indicating that AMSCs injected into the venous circulation were translocated to the thyroid.

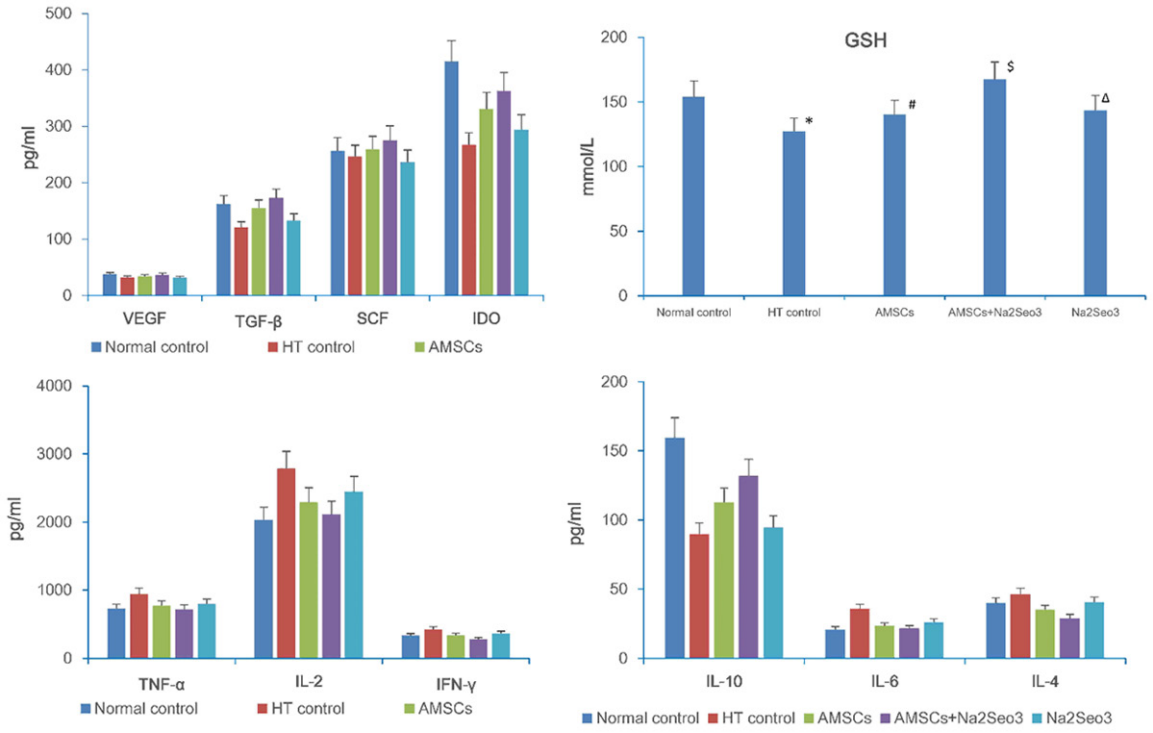

Figure 3. Cytokines analysis in thyroid tissues. Levels of VEGF, TGF-β, SCF, IDO and GSH in HT control group decreased compared to the normal control group. After treatment, levels of these cytokines increased in the AMSC + Na_2SeO_3 group compared to the other three groups. *P<0.01, #P<0.05, $P< 0.05, ΔP<0.05 and ^P<0.01. The regulation of immune cytokine levels in AMSC + Na_2SeO_3 group, including TNF-α, IL-2, IFN-γ, IL-4, IL-6 and IL-10, appeared better than in the AMSC and Na_2SeO_3 groups. *P<0.01, #P<0.05, $P<0.05, ΔP<0.05 and ^P<0.01.

AMSCs combined with Na_2SeO_3 improve the antioxidant and immunoregulatory capacities of HT rats

We further examined the levels of antioxidant and inflammatory cytokines released by AMSCs in thyroid tissues. Levels of VEGF, TGF-β, SCF, and IDO were decreased in the HT control group compared to the normal control group ($P<0.05$); after treatment, the levels of VEGF, TGF-β, and SCF in the AMSC and AMSC + Na_2SeO_3 groups increased significantly ($P<0.05$, **Figure 3**). Levels of these cytokines were higher in the AMSC + Na_2SeO_3 group compared to the AMSC group ($P<0.01$).

The levels of GSH were lower in the HT control group than in the normal control group ($P<0.05$); after treatment, these levels were higher in the AMSC, Na_2SeO_3, and AMSC + Na_2SeO_3 groups than in the HT control group ($P<0.05$), especially in the AMSC + Na_2SeO_3 group ($P<0.01$, **Figure 3**). The levels of GSH were significantly higher in the AMSC + Na_2SeO_3 group compared to the AMSC and Na_2SeO_3 groups ($P<0.01$), indicating that the antioxidant ability of HT rats was significantly improved in the AMSC + Na_2SeO_3 group after treatment compared to the AMSC and Na_2SeO_3 groups.

The levels of TNF-α, IL-2, IFN-γ, IL-4, and IL-6 were increased in the HT control group compared to the normal control group ($P<0.01$); after treatment, these levels were lower in the AMSC, Na_2SeO_3, and AMSC + Na_2SeO_3 groups than in the HT control group ($P<0.05$), especially in the AMSC + Na_2SeO_3 group (**Figure 3**). While IL-10 levels in the HT control group were lower than those in the normal control group ($P<0.01$), after treatment, IL-10 levels were higher in the AMSC and AMSC + Na_2SeO_3 groups than in the HT control and Na_2SeO_3 groups ($P<0.05$), especially in the AMSC + Na_2SeO_3 group. The regulation of immune cytokine levels in the AMSC + Na_2SeO_3 group appeared better than in the AMSC and Na_2SeO_3 groups ($P<0.01$).

Sodium selenite promotes AMSC growth

The combination of AMSCs and Na_2SeO_3 achieved better *recovery of thyroid and antioxi-*

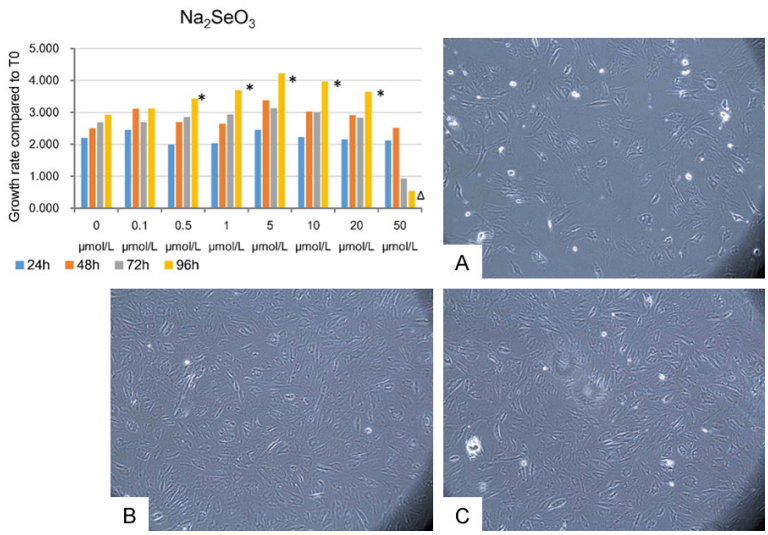

Figure 4. Sodium selenite promotes AMSC growth. Na_2SeO_3 accelerated AMSC growth (at passage 4) at concentrations from 0.5 μmol/L to 20 μmol/L, compared to control AMSCs. *P<0.05, ΔP<0.01. A: Control AMSCs at passage 4; B: AMSCs cultured with Na_2SeO_3 0.5 μmol/L; C: AMSCs cultured with Na_2SeO_3 5 μmol/L.

dant and immunoregulatory capacities on HT than treatment with either AMSCs or Na_2SeO_3 alone. To investigate the effects of Na_2SeO_3 on AMSCs, we added different concentrations of Na_2SeO_3 to the culture medium. As shown in **Figure 4**, Na_2SeO_3 accelerated AMSC growth (at passage 4) at concentrations from 0.5 μM to 20 μM, compared to control AMSCs (P<0.05), especially at 5 μM, whereas 50 μM Na_2SeO_3 inhibited AMSC growth (P<0.01). We selected concentrations of 0.5 μM and 5 μM Na_2SeO_3 to perform subsequent experiments. AMSCs at passages 12-14 that were cultured with Na_2SeO_3 were still more vigorous and grew more rapidly than control AMSCs.

Sodium selenite repairs oxidative damage and enhances cytokine secretion from AMSCs

Cells can undergo oxidative damage after culture in vitro for long periods of time, so we examined the levels of GSH and T-AOC to evaluate the extent of oxidative damage in AMSCs. The levels of GSH and T-AOC decreased with length of time in culture in vitro (P<0.001), whereas Na_2SeO_3 (both 0.5 μM and 5 μM) increased the levels of GSH and T-AOC in AMSCs (P<0.05, **Figure 5**).

The levels of secreted HGF, SCF, and TGF-β decreased in the cell supernatant (comparison of passages 4, 5, and 10, **Figure 6**) after culture in vitro (P<0.05), whereas Na_2SeO_3 caused an increase in these levels in the AMSC supernatant (P<0.05, data from cells in passage 10 are shown in **Figure 6**). These findings indicate that Na_2SeO_3 enhances the secretion of cytokines from AMSCs.

Gene microarray analysis

AMSCs were cultured in 5 μM Na_2SeO_3 for 60 days prior to gene microarray analysis to investigate the differences in gene expression between AMSCs cultured in Na_2SeO_3 and control AMSCs. As shown in **Figure 6** and **Table 2**, we found expression levels of 15 genes increased, whereas the expression levels of 14 genes decreased significantly. The expression differences in these genes indicate that Na_2SeO_3 is involved in mitosis, DNA replication and repair, synthesis and metabolism, ubiquitination, mitochondrial transport, cell growth, and migration in AMSCs. We further testified the results of *microarray analysis* by q*uantitative RT-PCR*. There was a good correlation in the fold change in gene expression levels between the quantitative RT-PCR and microarray data (**Table 3**).

Discussion

Recent research found that Na_2SeO_3 could ameliorate oxidative damage and decrease the serum levels of TPO Ab in patients with HT [17]. While the effects of AMSCs in an animal model of HT were also reported [11], the effects of AMSCs combined with Na_2SeO_3 on HT were unclear. In the present research, we investigated the effects of AMSCs combined with Na_2SeO_3 in a rat model of HT.

AMSCs combined with Na_2SeO_3 resulted in greater recovery of thyroid structure and function than treatment with AMSCs or Na_2SeO_3 alone. AMSCs are known to home to injured tissues [18]. The presence of CM-Dil in the thyroid indicated that AMSCs injected into the tail vein could be translocated to thyroid tissue, but we didn't find any thyroid cells containing CM-Dil, suggesting that AMSCs play a role in HT

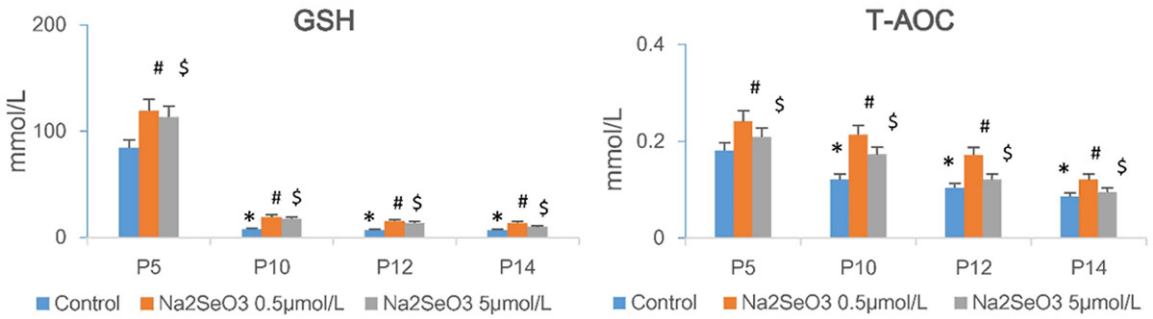

Figure 5. Sodium selenite repairs oxidative damage in AMSCs. The levels of GSH and T-AOC decreased with length of time in AMSCs culture in vitro, Na_2SeO_3 (both 0.5 μmol/L and 5 μmol/L) increased levels of GSH and T-AOC in AMSCs. *P<0.001, #P<0.05, $P<0.05, &P<0.05, ΔP<0.05.

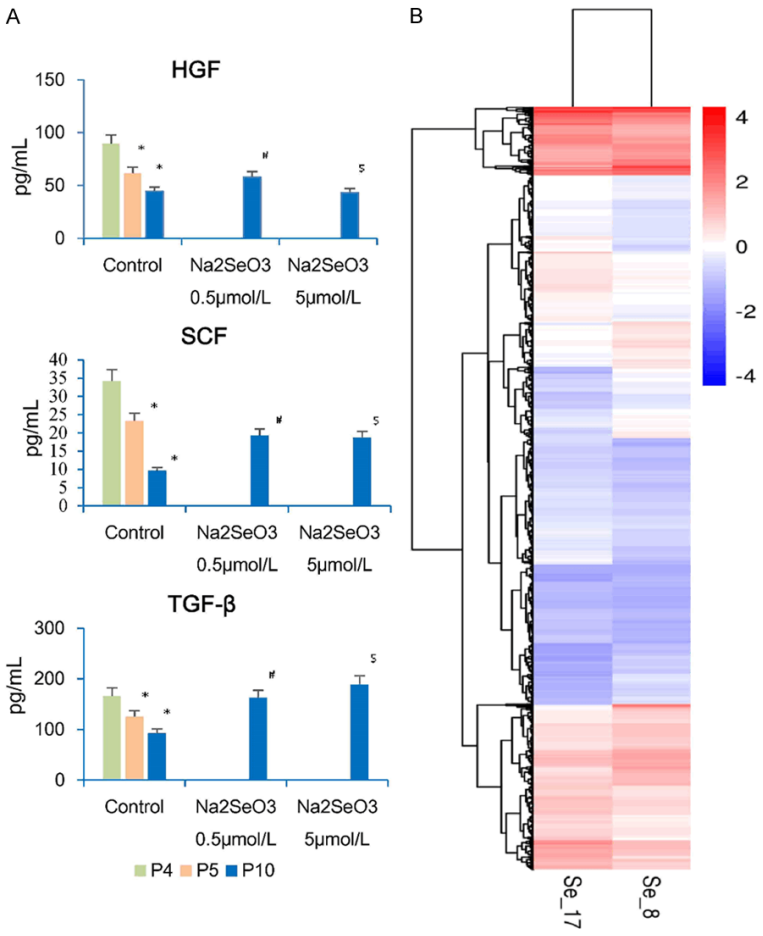

Figure 6. The effect and mechanisms of sodium selenite on AMSCs cytokine secretion. A: The levels of secreted HGF, SCF and TGF-β decreased in the cell supernatant (comparison of passage 4, 5 and 10) after culture *in vitro,* Na_2SeO_3 caused an increase in these levels in the AMSCs supernatant in passage 10. *P<0.05, #P<0.05, $P<0.05, &P<0.05, ΔP<0.05. B: Microarray analysis of AMSCs cultured with medium including 5 μmol/L Na_2SeO_3 in vitro for 60 days.

by secreting cytokines but not by transdifferentiating into thyroid cells, consistent with results in a previous report [19].

AMSCs secrete growth factors that modulate the immune system [20, 21]. TGF-β, VEGF, and SCF are multifunctional cytokines with anti-inflammatory and regenerative functions [22-24]. IDO has emerged as a key regulator of innate and adaptive immune responses in autoimmune diseases [25]. Increased levels of VEGF, TGF-β, SCF, and IDO in thyroid tissues appear to improve immune system function and promote tissue regeneration. These results are in accordance with a previous EAT study using human AMSCs [26]. AMSCs also can secrete cytokines to effectively regulate immune function [27]. After treatment, levels of TNF-α, IL-2, IFN-γ, IL-4, and IL-6 decreased, and levels of IL-10 increased in the AMSC and AMSC + Na_2SeO_3 groups. Treatment with AMSCs may ameliorate some autoimmune diseases by modulating IL-4 and IFN-γ levels in effector T-cells [27, 28]. Moreover, the effects of AMSCs combined with Na_2SeO_3 were greater than with AMSCs alone.

GSH is one of the most important intracellular antioxidants that is released in response to various noxious stimuli, including oxidative stress [29]. Levels of GSH in the Na_2SeO_3 and AMSCs + Na_2SeO_3

Table 2. The genes with changed expression in AMSCs cultured with Na_2SeO_3

Gene symbol	Na_2SeO_3/Ctl	Gene name
Ubiquitination		
Rlim	13.71	ring finger protein, LIM domain interacting
Rmnd5b	-6.08	required for meiotic nuclear division 5 homolog B
Synthesis and metablism		
Rasl10b	9	ras-like protein family member 10B
Ppcdc	4.75	phosphopantothenoylcysteine decarboxylase
Hprt1	4.22	hypoxanthine-guanine phosphoribosyltransferase
Hnrnph2	3.4	heterogeneous nuclear ribonucleoprotein A/B
Tyw1	-36.56	S-adenosyl-L-methionine-dependent tRNA 4-demethylwyosine synthase
Iars	-7.37	isoleucine--tRNA ligase, cytoplasmic-like
Cyp19a1	-5.67	cytochrome P450, family 19, subfamily a, polypeptide 1
Atp2b4	-4.21	ATPase plasma membrane Ca_2^+ transporting 4
Rps25	-3.78	40S ribosomal protein S25-like
Mitochondrial transport		
Selenbp1	8.8	Selenium binding protein 1
Ndufa1	8.37	NADH:ubiquinone oxidoreductase subunit A1
Mfsd14a	5.83	major facilitator superfamily domain containing 14A
Timm17b	-31.68	mitochondrial import inner membrane translocase subunit Tim17-B
Mitosis		
Ankrd12	8.71	ankyrin repeat domain-containing protein 12
Hira	5.74	protein HIRA-like
Fgd1	5.61	FYVE, RhoGEF and PH domain containing 1
Wbp1	4.98	WW domain binding protein 1
Cetn3	-6.2	centrin-3-like
Cell growth and migration		
Zbed6	14.67	PHD finger protein 6
Ppfia3	-6.33	PTPRF interacting protein alpha 3
Dgcr2/idd	-5.92	integral membrane protein DGCR2/IDD
Structural protein		
Chid1	9.2	chitinase domain containing 1
Ankra2	-9.38	ankyrin repeat family A member 2
Replication and repair		
Alkbh6	5.1	alkB homolog 6
Mmp12	-5.31	matrix metallopeptidase 12
Rsl1d1l1	-5.2	ribosomal L1 domain containing 1-like 1
Tnks2	-4.51	tankyrase 2

groups increased after treatment, and the increase was greater in the AMSCs + Na_2SeO_3 group. Selenite plays a vital role in maintaining the activity of antioxidant enzymes, which reduce levels of hydrogen peroxide and lipid and phospholipid peroxides, thereby blocking the formation of free radicals and reactive oxygen species; such antioxidant activity has proven beneficial in cases of HT [30, 31]. These results reveal that the combination of AMSCs and Na_2SeO_3 significantly improve antioxidant activity in rats with HT.

Based on the above findings, we further explored the role of Na_2SeO_3 on AMSCs in vitro and found that it promoted cell growth and maintained the viability of AMSCs. In cultured AMSCs, the antioxidant capacity of AMSCs decreased, but after treatment with Na_2SeO_3, levels of GSH and T-AOC in the AMSCs

Table 3. Comparison of gene expression levels between microarray and quantitative RT-PCR in AMSCs cultured with Na_2SeO_3

Gene name	Fold change
Rlim	12.98/13.71
Rmnd5b	-6.75/-6.08
Rasl10b	9.58/9
Tyw1	-35.87/-36.56
Selenbp1	9.12/8.8
Timm17b	-31.21/-31.68
Ankrd12	8.13/8.71
Cetn3	-6.52/-6.2
Zbed6	14.1/14.67
Ppfia3	-6.54/-6.33
Chid1	9.87/9.2
Ankra2	-9.81/-9.38
Alkbh6	5.67/5.1

Data are expressed as $\log_2$ gene expression fold change from quantitative RT-PCR/M-value ($\log_2$ gene expression fold change) from microarray.

increased, indicating that Na_2SeO_3 was able to improve the antioxidant capacity of AMSCs. The observed increases in the levels of growth factors, including HGF, SCF, and TGF-β, indicate that the paracrine capacity of AMSCs was also enhanced after treatment with Na_2SeO_3.

The results of both the microarray analysis and quantitative RT-PCR experiments showed that the mRNA expression levels of many genes involved in mitosis, DNA replication and repair, ubiquitination, mitochondrial transport, synthesis and metabolism, cell growth, and migration were altered after supplementation with Na_2SeO_3, including *Rlim*, *Ankrd12*, *Chid1*, *Selenbp1*, *Timm17b*, *Ankra2*, *and Tars*. However, the specific mechanism by which Na_2SeO_3 involves these genes to impact AMSCs must be further investigated. Additionally, the mechanism by which AMSCs and Na_2SeO_3 affect immune cells in rats with HT is unclear and will be detailed in a separate report.

In conclusion, we found that AMSCs combined with Na_2SeO_3 significantly restored the function and structure of the thyroid, improving immunity in rats with HT. Na_2SeO_3 promoted cell growth and improved the secretory ability of AMSCs. These findings may provide a new approach for HT treatment. However, the specific effects and mechanisms must be further investigated.

The datasets used and/or analyzed during the current study are available from the corresponding author upon request.

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Disclosure of conflict of interest

None.

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