XXXVII Congreso Nacional FSED
Fundación Sociedad Española de Diabetes
15 a 17 abril 2026
Barceló Sevilla Convention Center
FSED | FUNDACIÓN DE LA SOCIEDAD ESPAÑOLA DE DIABETES
La diabetes nos conecta

ENHANCED HYDROGEN SULFIDE PRODUCTION DECREASES HIGH-FAT DIET-INDUCED HEPATOCELLULAR CARCINOMA PROGRESSION IN MICE.

Inmaculada Pino-Pérez[1*], Raúl López-Fernández-Sobrino[1], María Ángeles Cáliz-Molina[1], María Camacho-Cabrera[1], Paula-Solla[1], Leopoldo Pérez-Rosendo[1], Almudena García-Ruiz[1], Alejandro Martín-Montalvo[1,2].
[1] Andalusian Center for Molecular Biology and Regenerative Medicine-CABIMER, Junta de Andalucía-University of Pablo de Olavide-University of Seville-CSIC, Seville, Spain.
[2] Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Madrid, 28029 Spain. *Contact: inmaculada.pino@cabimer.es

Introduction

Hydrogen sulfide (H_2S) is a gasotransmitter that has the ability to freely diffuse through cell membranes to induce intracellular signaling responses[1]. H_2S is produced through enzymatic and non-enzymatic pathways from sulfur donors[2]. Among its functions, H_2S is consider a potent antioxidant, is able to modulate electron transport chain, and regulates the activity of proteins mainly by cysteine persulfidation[3]. Interestingly, in rodent liver, between 10% and 25% of proteins are persulfidated.
Liver cancer is a major cause of cancer-related death. In experimental settings, the combination of HFD with diethylnitrosamine (DEN) constitutes a well-established hepatocellular carcinoma (HCC) model. Increasing evidence describes an emerging role of H_2S in cancer as H_2S production affects tumor development in a tissue- and dose-dependent manner[4,5]. This study examined the effects of increased H_2S production on liver cancer carcinogenesis and progression.

Materials and methods

Short-term study: Male mice were fed with the sulfur donor DAS (150 mg/kg of body weight (bw)) for 1 week and then injected DEN (100 mg/kg bw) to induce carcinogenesis. After 48h mice were euthanized. DNA damage, apoptosis and inflammatory/detoxification markers were analyzed.

Long-term study: Male mice receive an intraperitoneal injection of DEN (5 mg/kg bw) to initiate hepatocarcinogenesis and were fed a STD supplemented with DAS, which was maintained the remaining of their life. Mice were fed with a HFD starting at postnatal day 21. Body weight and caloric intake were monitored weekly. Two cohorts were sacrificed at defined time points to assess tumor progression; remaining mice were followed longitudinally for physical performance and lifespan. Transcriptomic and persulfidomic analyses assessed global gene expression and protein persulfidation.

Results

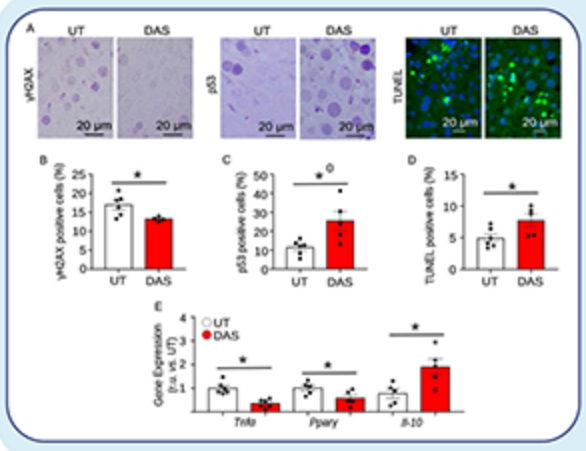

Figure 1. Acute effects of DEN in mice treated with DAS. (A) Representative images of γH2AX, p53, and TUNEL staining. (B) Quantification γH2AX-positive cells. n = 6 UT, n = 4 DAS. (C) Quantification of p53-positive cells. n = 6 UT, n = 5 DAS. (D) Quantification of TUNEL-positive cells n= 6 UT, n=5 DAS. (E) mRNA expression levels of genes associated with inflammatory processes. n = 6 for *Tnfa*. n = 5 for *Ppary*. n = 5 for *Il-10*. Data are presented as mean ± SEM. p < 0.05, DAS vs. UT. t-test.

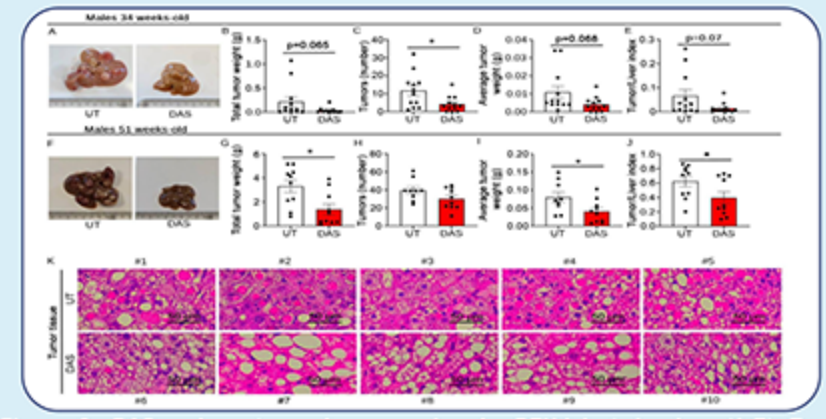

Figure 3. DAS reduce tumoral progression in DEN-injected mice. (A-E) Tumor characteristics of 34 weeks-old mice. (A) Representative pictures of livers. (B) Total tumor weight. n = 12. (C) Number of tumors per mice. n = 12. (D) Average tumor weight. n = 12. (E) Tumor weight to liver weight ratio. n = 12. (F-J) Tumor characteristics of 51 weeks-old male mice. (F) Representative pictures of livers. (G) Total tumor weight. n = 10. (H) Number of tumors per mice. (I) Average tumor weight. n = 10. (J) Tumor weight to liver weight ratio. n = 10. (K) Representative pictures of H&E staining of tumor tissue. n = 5. UT: untreated. Data shown are the means ± SEM. .* p < 0.05 DAS vs. UT.

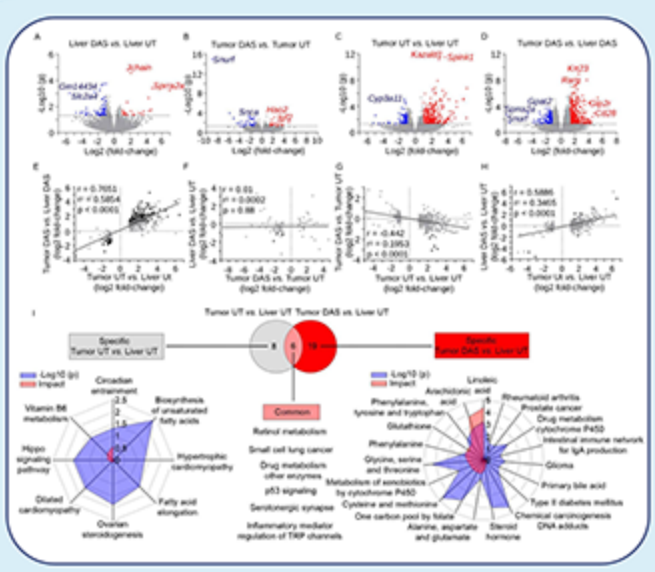

Figure 5. DAS promote transcriptional responses in liver tissue mimicking relevant aspects of tumor biology. (A-D) Volcano plots indicating DEGs. (A) Liver DAS vs. Liver UT. n = 4. (B) Tumor-DAS vs. Tumor UT. n = 4. (C) Tumor UT vs. Liver UT. n = 4. (D) Tumor DAS vs. Liver DAS. n = 4. (E) Scatter plot of mRNA changes found in Tumor UT vs. Liver UT and Tumor DAS vs. Liver DAS. n = 4. (F) Scatter plot of mRNA changes found in Tumor DAS vs. Tumor UT and Liver DAS vs. Liver UT. n = 4. (G) Scatter plot of mRNA changes found In Tumor UT vs. Liver UT and Tumor DAS vs. Tumor UT. n = 4. (H) Scatter plot found in Tumor UT vs. Liver UT and Liver DAS vs. Liver UT. n = 4. (I) Diagram indicating common and specific significant altered pathways found in tumors compared to NTL of mice treated or not with DAS. n = 4.

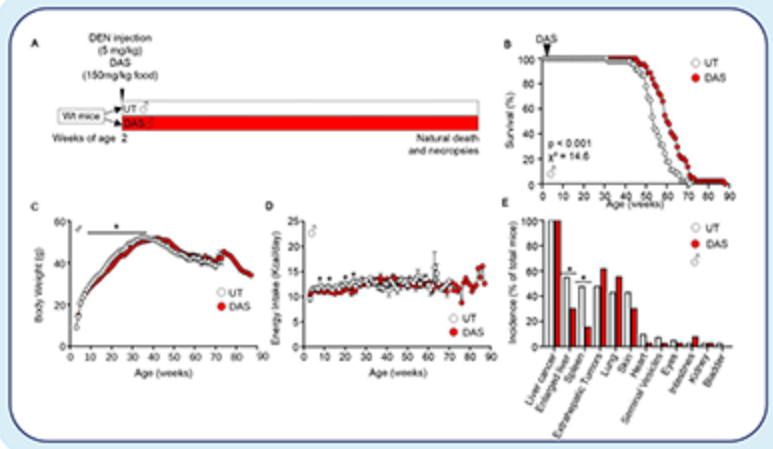

Figure 2. DAS extend lifespan in male mice harboring HCC. (A) Experimental design. (B) Kaplan–Meier survival curve of wild-type male mice treated or not with DAS. n = 45 Untreated. n = 44 DAS. (C) Body weight. n = 45 UT. n = 44 DAS. (D) Energy intake. n = 10 cages. (E) Major pathologies detected at necropsies. n = 42 UT. n = 40 DAS. Data shown are the means ± SEM.* p < 0.05 DAS vs. UT.

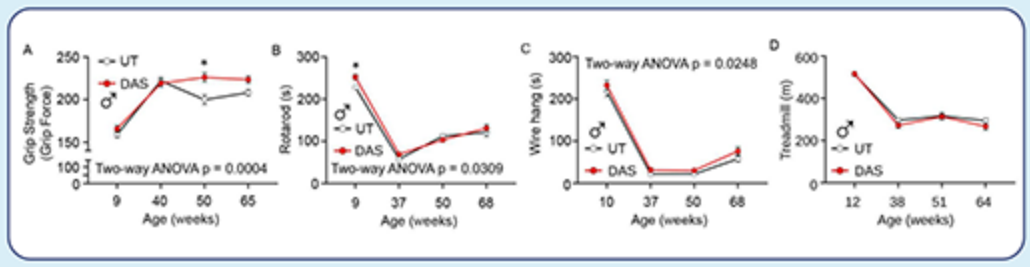

Figure 4. DAS improve physical condition in mice harboring HCC. (A) Longitudinal assessment of grip strength in mice. n = 25 at the beginning of the experiment. Two-way ANOVA Bonferroni. (B) Longitudinal assessment of rotarod performance in mice. n = 25 at the beginning of the experiment. (C) Longitudinal assessment of wire hang performance in mice. n = 25 at the beginning of the experiment. Two-way ANOVA Bonferroni. (D) Longitudinal assessment of treadmill performance in mice. n = 24 at the beginning of the experiment. Two-way ANOVA Bonferroni. Data shown are the means ± SEM.* p < 0.05 DAS *vs.* UT.

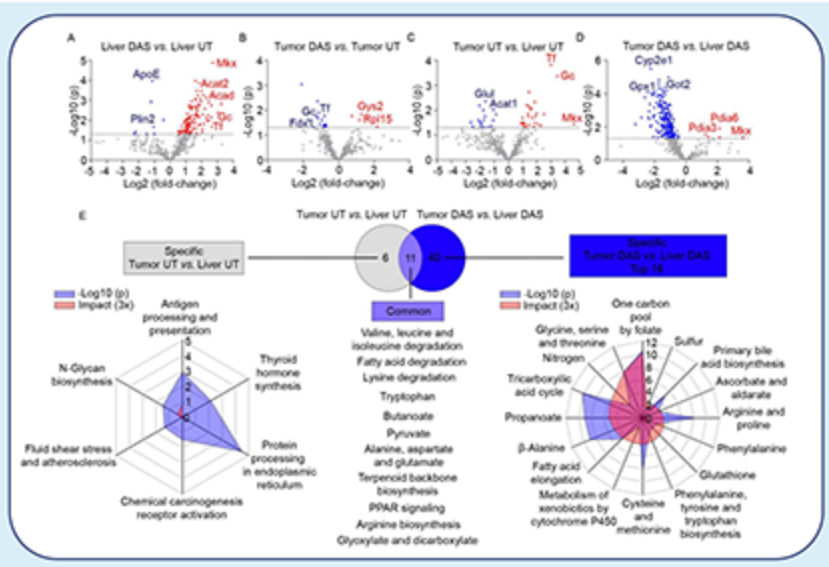

Figure 6. DAS enhances protein persulfidation in liver tissue of mice harboring HCC. (A-D) Volcano plots indicating significantly altered persulfidated proteins. Red indicates higher levels. Blue indicates lower levels. (A) Liver DAS vs. Liver UT. n = 5. (B) Tumor-DAS vs. Tumor UT. n = 5. (C) Tumor UT vs. Liver UT. n = 5. (D) Tumor DAS vs. Liver DAS. n = 5. (E) Diagram indicating common and specific significant altered pathways found in hepatic tumors compared to NTL liver. Differentially persulfidated proteins were included in the analyses using JPA form Metaboanalyist. Specific significantly altered pathways are included in radar plots indicating the p-value and impact. The top 16 of 40 significantly enriched pathways were included in Tumor DAS vs. Liver DAS. n = 5.

Discussion

Here, we show that short-term DAS treatment in mice reduced DNA damage and apoptosis, while decreasing inflammatory activity and enhancing anti-inflammatory responses. In parallel, in long-term DAS treatment significantly extended lifespan in a DEN-induced HCC model. At early stages of disease, mice treated with DAS exhibited reductions in both tumor number and weight. Tumor weight was lower at advanced stages. DAS improved physical performance. Transcriptomic analyses of liver and tumor tissues revealed that DAS induce transcriptional responses in liver tissue that mimic those observed in tumors. Persulfidomic profiling revealed that DAS affected central liver metabolic pathways (lipid, carboxylic acid, amino acid), with limited changes in tumors. Collectively, these findings underscore the therapeutic potential of organosulfur compounds in cancer suppression, supporting further investigation into their mechanistic roles and translational applications.

References and acknowledgements

1. Wang, R. Physiological reviews 92, 791-896. 2. Yang, J. et al. Commun Biol 2, 194. 3. Mustafa, A. K. et al. Science signaling 2, ra72. 4. Hellmich, M. R . et al. Antioxid Redox Signal 22, 424-448. 5. Singh, S. V. et al. Biochem Biophys Res Commun 225, 660-665.

This work has been possible thanks to the grant PID2021-123965OB-100 and PID2024-156080OB-I00, funded by MCIN/590214 and ERDF A way of making Europe Junta de Andalucía of public funding, in the 2024 call of the Consejería de Salud y Consumo for the financing of research, development and innovation (R&D&I) in Biomedicine and Health Sciences in Andalusia PI-003502024, Formación de Profesorado Universitario 2021 Grant (FPU21/01338) and RYC2023-043221-I. Acknowledgments to the support of the Basic research group of the Spanish Diabetes Association and the Foundation of the Spanish Diabetes Association, Conexión COMETA and the CIBERDEM.

Project PI-003502024
Projects PID2021-123965OB-I00 and PID2024-156080OB-I00 funded by