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Hydroxyl Safflower Yellow-A Treatment Modulates Mitochondrial Autophagy in Knee Osteoarthritis through HIF-1α/BNIP3 Signaling

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SUMMARY. Knee osteoarthritis (KOA), prevalent among the middle-aged and elderly, correlates with reduced autophagy in cartilage, contributing to its degeneration. Hydroxyl safflower yellow A (HSYA) has demonstrated anti-inflammatory and autophagy-regulating properties, presenting a potential avenue for KOA treatment. This study aims to dissect the mechanistic underpinnings of HSYA in KOA intervention. Human KOA chondrocytes were isolated and categorized into groups: blank model, HSYA, hypoxia-inducible factor-1α (HIF-1α) inhibitor, autophagy inducer, HSYA + HIF-1α inhibitor, HSYA + autophagy inducer, and a normal control group consisting of normal chondrocytes. Enzyme-linked immunosorbent assays measured interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) expression. Proliferation and apoptosis were assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays and flow cytometry, respectively. Western blot examined HIF-1α, adenovirus E1B 19 kDa interacting protein 3 (BNIP3), and autophagic marker protein levels. Autophagic vesicles were visualized using monodanylcadaverin staining and transmission electron microscopy. In each model group compared to the normal, IL-1β, TNF-α, IL-6, and HIF-1α levels significantly increased, with elevated apoptosis rates and diminished proliferation ($p < 0.05$). The blank model group exhibited increased HIF-1α and reduced autophagic marker protein levels ($p < 0.05$). Autophagic marker protein expression in the HSYA group, HIF-1α inhibitor group, HSYA + HIF-1α inhibitor group, and HSYA + autophagy inducer group was upregulated. HSYA significantly elevated autophagy in KOA cartilage while mitigating apoptosis, potentially mediated through HIF-1α/BNIP3 pathway inhibition.

RESUMEN. La osteoartritis de rodilla (KOA), frecuente entre personas de mediana edad y ancianos, se correlaciona con una autofagia reducida en el cartílago, lo que contribuye a su degeneración. El hidroxilo de cártamo amarillo A (HSYA) ha demostrado propiedades antiinflamatorias y reguladoras de la autofagia, lo que presenta una vía potencial para el tratamiento de KOA. Este estudio tiene como objetivo analizar los fundamentos mecanicistas de HSYA en la intervención KOA. Los condrocitos KOA humanos se aislaron y clasificaron en grupos: modelo en blanco, HSYA, inhibidor del factor 1α inducible por hipoxia (HIF-1α), inductor de autofagia, HSYA + inhibidor de HIF-1α, HSYA + inductor de autofagia y un grupo de control normal que consta de condrocitos normales. Los ensayos inmunoabsorbentes ligados a enzimas midieron la expresión de interleucina-1β (IL-1β), factor de necrosis tumoral-α (TNF-α) e interleucina-6 (IL-6). La proliferación y la apoptosis se evaluaron mediante ensayos de bromuro de 3-(4,5-dime-

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tiltiazol-2-il)-2,5-difeniltetrazolio (MTT) y citometría de flujo, respectivamente. La transferencia Western examinó HIF-1α, la proteína 3 que interactúa con el adenovirus E1B de 19 kDa (BNIP3) y los niveles de proteína marcadora autofágica. Las vesículas autofágicas se visualizaron mediante tinción con monodanilcadaverina y microscopía electrónica de transmisión. En cada grupo modelo, en comparación con los niveles normales, los niveles de IL-1β, TNF-α, IL-6 y HIF-1α aumentaron significativamente, con tasas elevadas de apoptosis y proliferación disminuida ($p < 0,05$). El grupo del modelo en blanco exhibió un aumento de HIF-1α y niveles reducidos de proteína marcadora autofágica ($p < 0,05$). La expresión de la proteína marcadora autofágica en el grupo HSYA, el grupo inhibidor de HIF-1α, el grupo HSYA + inhibidor de HIF-1α y el grupo HSYA + inductor de autofagia estaba regulada positivamente. HSYA elevó significativamente la autofagia en el cartílago KOA al tiempo que mitiga la apoptosis, potencialmente mediada a través de la inhibición de la vía HIF-1α/BNIP3.