唑来膦酸抗肿瘤作用的研究进展

刘俊彦 综述 王哲海 审校

摘要 唑来膦酸作为第三代双膦酸盐药物已广泛用于恶性实体瘤骨转移相关事件的防治。进一步研究发现，唑来膦酸可通过抑制甲羟戊酸途径中焦磷酸合酶的活性，使异戊烯焦磷酸大量蓄积，同时异戊烯焦磷酸与细胞内一磷酸腺苷结合形成三磷酸腺苷分解类似物蓄积于细胞中，从而改变细胞周期蛋白和凋亡蛋白水平，抑制肿瘤细胞生长并促进其凋亡，具有一定的直接和/或间接抗肿瘤作用。有关肺癌、乳腺癌、前列腺癌及肝癌等前期临床试验提示唑来膦酸与细胞毒药物、内分泌药物及靶向药物联合具有一定程度的协同抗肿瘤作用，并且在抗肿瘤药物之后序贯应用效果更好。一些相关的临床试验正在进行中。

关键词 唑来膦酸 抗肿瘤 联合治疗 异戊烯焦磷酸 骨转移
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Research Progress on Antitumor Effects of Zoledronic Acid

Junyan LIU, Zhehai WANG
Correspondence to: Zhehai WANG; E-mail: wzhai8778@sina.com
Department of Oncology, Shandong Tumor Hospital, Ji'nan 250117, China

Abstract Zoledronic acid (ZOL), a third-generation bisphosphonate, has been widely used for the treatment of skeletal-related events in malignant solid tumors. Previous studies have demonstrated that ZOL can induce direct and indirect antitumor activities through pyrophosphate synthase inhibition, which blocks the mevalonate pathway and causes isopentenyl pyrophosphate (IPP) accumulation. IPP becomes conjugated to AMP to form a novel ATP analog. The amount of IPP and ATP analog is correlated with cyclin and apoptotic protein levels, which are associated with cell line growth and apoptosis. Preclinical studies on lung cancer, breast cancer, prostate cancer, liver cancer, and so on, confirmed a synergistic effect between ZOL and cytotoxic, endocrine, and targeted drugs. The observed improvement in antitumor effects by using combination therapy with ZOL is currently being verified through additional clinical trials.

Keywords Zoledronic acid (ZOL); Antitumor; Combination therapy; Isopentenyl pyrophosphate (IPP); Skeletal-related events

唑来膦酸(zoledronic acid，ZA)是第三代双膦酸盐药物，已广泛用于恶性实体瘤骨转移事件的防治，随着研究的不断深入，ZA显示出一定的直接和/或间接抗肿瘤作用，同时还发现ZA与抗肿瘤药物联合应用具有一定的协同作用，本文对此作一综述。

1 抗肿瘤机制的研究

1.1 抑制甲羟戊酸途径

ZA能够抑制甲羟戊酸途径中的关键酶焦磷酸合酶(farnesyl pyrophosphate synthase，FPPS)的活性，使一些小G蛋白如Ras、Rap1、Rho和Rab等的异戊烯化受抑制，造成异戊烯焦磷酸(isopentenyl pyrophosphate，IPP)大量蓄积，IPP与细胞内AMP结合形成ATP分解类似物(ATP analog，Appp Ⅰ)，最终导致细胞内IPP/Appp Ⅰ蓄积，从而抑制肿瘤细胞的生长、粘附、播散及入侵[1]。

1.2 抑制破骨细胞的活性

ZA通过以下几方面抑制破骨细胞的活性发挥间接的抗肿瘤作用。第一，骨组织是人体内生长因子的储存库，这些因子主要包括生长转化因子β(transforming growth factor β，TGFβ)、成纤维细胞生长因子(fibroblast growth factor，FGF)、胰岛素样生长因子(insulin-like growth factors，IGFs)、血小板衍生生长因子(platelet derived growth factor，PDGF)和成骨蛋白(bone morphogenic proteins，BMP)等，当破骨细胞通过分泌蛋白酶等吸收骨质时，这些生长因子就会释放，从而为肿瘤细胞的生长提供了条件。而双膦酸盐类对于骨病灶中的钙化基质具有很强的亲和力，不仅减弱了破骨前体细胞的活化、增殖及分化能力，而且抑制了成熟破骨细胞对骨基质的吸附及吸收活性，加速了破骨细胞的凋亡，减少了相关生长因子的释放[2]。第二，修饰骨重塑蛋白。RANK和RANKL对于破骨细胞的产生具有重要作用。RANKL由成骨细胞产生，对应的RANK受体位于破骨细胞及其前体细胞表面，两者结合后能够激活破骨细胞的活性并抑制破骨细胞的凋亡。由多种组织和细胞合成的骨保护素作为RANKL的另一受体，能够抑制骨诱裂的发生。研究还发现癌细胞并不直接作用于破骨细胞，而是通过甲状旁腺激素受体激活成骨细胞或间质细胞，增加RANKL的含量，从而激活破骨细胞。ZA

作者单位：山东省肿瘤医院内三科（济南市250117）
通信作者：王哲海 wzhai8778@sina.com

则通过刺激骨保护素的生成，中和RANK-RANKL介导的破骨细胞活性来发挥其抗肿瘤作用[2]。

1.3 诱导γδT细胞的增殖

γδT细胞是外周血中含量较少的T细胞亚型，具有免疫防御功能。外周单核细胞或者癌细胞内吞ZA分子后，细胞内的FPPS受到抑制，造成IPP积聚，促使T细胞不断增殖，表达γδT细胞受体[3-4]。同时，ZA能够诱导细胞产生ApppI，驱使γδT细胞向癌细胞移动。IL-18与唑来膦酸和IL-2共同作用后，促使T细胞具有更强的细胞杀伤能力[5]。另外，外周单核细胞吞噬ZA分子后促进了TNF-α和IFN-γ的释放[6]，这也是唑来膦酸免疫调节作用的重要方面。体外研究中唑来膦酸同样依靠IPP/ApppⅠ对癌细胞系的诱导作用，然而不同的细胞系中IPP/ApppⅠ的表达水平有所不同，表达水平高的细胞系在γδT细胞的作用下可以全部死亡，而表达水平低的细胞系在γδT细胞作用下似乎无细胞毒效应。

1.4 抑制血管生成

ZA能够抑制软组织血管生成。体外试验结果显示低剂量的ZA能够抑制脐静脉血管内皮细胞的增殖，而高剂量的ZA直接诱导内皮细胞凋亡[7-8]。在体内试验中，ZA能够减少$CD11b^+$巨噬细胞的浸润并降低血管内皮细胞生长因子（vascular endothelial growth factor，VEGF）水平，因此ZA被认为能够改变血管生成的微环境，具有潜在的抗血管生成活性[9]。

2 唑来膦酸与抗肿瘤药物联合

2.1 唑来膦酸与细胞毒药物联合应用的基础研究

多柔比星与唑来膦酸联合能够诱导乳腺癌和前列腺癌细胞凋亡，随后的一些研究支持这一发现，并且发现细胞毒药物之后应用ZA效果最好。有学者报道[10]选择前列腺癌细胞系PC3，DU145和LNCaP进行体外观察研究，结果显示对PC3细胞系，多柔比星序贯ZA组、单药ZA组和多柔比星单药组的凋亡率分别为8.87%、1.73%、3.60%，提示两者联合应用具有一定的协同作用。观察其余两种细胞系也得出相同结论。同时研究还发现，LNCaP细胞系在多柔比星序贯ZA组的凋亡率明显高于ZA序贯多柔比星组（4.77%：2.50%；$P<0.05$），DU145细胞系的凋亡率也符合上述规律，提示细胞毒药物序贯应用ZA效果最好。ZA与多柔比星联合应用可诱导乳腺癌和前列腺癌细胞凋亡，同时与异环磷酰胺、多西紫杉醇等多种细胞毒药物也具有协同作用，可使多种癌细胞阻滞于$S/G_2/M$期，也可将纤维肉瘤细胞阻滞于G_1期，促进细胞凋亡[11-12]。

动物模型研究结果显示ZA与多柔比星、紫杉醇、异环磷酰胺、顺铂、伊立替康等多种细胞毒药物具有协同抗肿瘤作用，并且在最佳的用药方式方面，得出与体外研究相一致的结论。Ottewell等[13]以乳腺癌小鼠模型对唑来膦酸进行了研究，结果显示多柔比星序贯ZA组、多柔比星组、ZA组、ZA序贯多柔比星组的平均肿瘤体积分别为122 mm^3、328 mm^3、447 mm^3、418 mm^3；四组中凋亡蛋白酶阳性细胞的平均数分别为605.0 /mm^2、82.19 /mm^2、98.44 /mm^2、103.1 /mm^2，多柔比星序贯唑来膦酸对肿瘤细胞增殖的抑制及促凋亡作用显著高于其他用药方式。

2.2 唑来膦酸与靶向药物联合的基础研究

近期研究结果显示唑来膦酸与索拉非尼、吉非替尼等分子靶向药物在小鼠模型中同样具有一定的协同抗肿瘤作用[14-15]。Chang等[14]报道，对于小分子靶向药物吉非替尼，唑来膦酸能够协同抑制ERK1/2和Akt磷酸化，使非小细胞肺癌（non-small cell lung cancer，NSCLC）细胞阻滞于$S/G_2/M$期，促进其凋亡。在肝癌小鼠模型中，ZA联合索拉非尼使LM3-R、SMMC7721小鼠的肿瘤体积分别缩小67%和39%，与索拉非尼单药相比具有显著的统计学差异。该试验还通过集合荧光吸收度比较了肺转移情况，吸收度越高，表明转移越严重。LM3-R模型中联合组与单药组的集合荧光吸收度分别为3.67±2.28和58.0±3.6（$P<0.05$），提示ZA联合索拉非尼抑制肝癌细胞肺转移效果更强[15]。

2.3 相关临床研究

有学者对于唑来膦酸与化疗联合应用是否具有协同作用进行了临床研究。AZURE试验[16]将205例乳腺癌患者随机分为新辅助化疗组（103例）和新辅助化疗联合ZA组（102例）。结果发现，术后残留的肿瘤大小两组分别为27.4 mm和5.5 mm，病理完全缓解率分别为6.9%和11.7%，均存在显著的统计学差异，提示化疗联合唑来膦酸比单纯化疗具有更强的抗肿瘤作用。

3 小结

虽然临床前研究显示唑来膦酸与抗肿瘤药物具有一定的协同作用，但目前的临床试验尚未得出一致结论，因此仍需要进一步研究。

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