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微塑料对斑马鱼的毒性效应及机制研究进展*

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摘要 塑料污染已成为全球性重大环境问题, 受到国际的广泛关注. 2022年, 我国将微塑料(microplastics, MPs)列为四大新污染物之一. MPs是指直径小于5 mm的塑料颗粒, 具有生物毒性、环境持久性、生物累积性等特征, 其生产和使用与人类生活息息相关, 对生态环境和人体健康存在较大风险. 在水环境中, MPs易被水生动物摄取产生毒性效应, 并沿食物链富集和放大. 斑马鱼是被广泛使用的毒理学模型, 具有成本低、产卵率高、易饲养等特点, 与人类的基因序列有很高的同源性. 本文综述了国内外文章, 阐述微塑料在斑马鱼体内的富集情况, 归纳了微塑料对斑马鱼的生物毒性效应, 总结了微塑料对斑马鱼的毒性作用机制, 并从三个方面展望了未来的研究方向, 为进一步开展微塑料的生物毒性效应、机制研究以及生态风险提供理论支撑和参考.

关键词 微塑料, 斑马鱼, 毒性效应, 毒性机制.

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Research status of microplastics for zebrafish on the toxicity and mechanism

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Abstract Plastic pollution has grown into an important worldwide environmental issue, attracting broad international attention. In 2022, Microplastics (MPs) were classified as new pollutants in China. MPs are plastic particles with a diameter of less than 5 mm that are biotoxic, ecologically persistent, and bioaccumulative, and their manufacturing and usage are inextricably linked to human existence, providing a larger risk to the natural environment and human health. MPs are hazardous to aquatic creatures and are enhanced and amplified along the food chain in the aquatic environment. The zebrafish is a popular toxicological model due to its cheap expense, high spawning rates, ease of raising, and high degree of similarity with human DNA sequences. This paper reviews domestic and international articles, describes the enrichment of microplastics in zebrafish, summarizes the biotoxic

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effects of microplastics on zebrafish, concludes the mechanism of toxic effects of microplastics on zebrafish, and outlines future research directions from three perspectives, providing theoretical support and reference for further research on the biotoxic effects, mechanism, and ecological risks of microplastics.

Keywords microplastics, zebrafish, toxicity, mechanism.

塑料被广泛应用于工业、建筑、医疗和农业生产等领域,根据组分的不同,塑料可分为聚氯乙烯(polyvinyl chloride, PVC)、聚丙烯(polypropylene, PP)、聚苯乙烯(polystyrene, PS)、聚酯纤维(polyester, PET)、聚酰胺(polyamide, PA)或聚乙烯(polyethylene, PE)等。2020年,塑料在全世界总产量约为3.6亿吨(1950年仅为150万吨),并以每年3%的速度增长^[1]。全球每年有超过8万吨的塑料垃圾漂浮至海洋^[2]。这些塑料在环境中被物理、化学和生物作用降解成更小的塑料微粒或纤维,称为微塑料。微塑料已经成为一类普遍的水环境污染物质,分布广泛,不仅能存在淡水湖和海洋等人类活动频繁的地区,甚至是被科研人员称为地球上“最干净”的地方——南极洲^[3]、以及青藏高原也有所检出^[4-5]。

目前,国内广泛关注的新污染物有4大类,MPs便是其中之一。MPs通常被界定为平均粒径小于5 mm的塑料微粒,也有不同研究表明,MPs被归类为1 μm 至1000 μm ^[6]。MPs具有体积小、疏水性强、生物难降解等特征,易被生物体摄取并累积,危害生物体的健康甚至生态安全。MPs还可吸附其他污染物,造成更严重的毒理效应和生态危害^[7-9]。水环境中的微塑料可通过食物链进入到水生动物中,且具有生物富集和生物放大的作用。研究表明,MPs可能会引起水生生物的氧化应激、免疫反应、代谢变化及细胞坏死等生物毒性效应,并可能会通过食物链富集放大,到更高营养级的生物,如海龟、水生哺乳动物和海鸟等动物^[10]。

斑马鱼是环境污染物毒性测试的普遍生物模型,不同发育阶段的斑马鱼经常被用于研究MPs的健康风险。斑马鱼幼体全身透明,可通过荧光标记的MPs进行实时成像定位研究^[11-12],便于观察各个组织中的MPs摄取情况。鉴于利用斑马鱼研究MPs生物毒性效应的趋势日益显著,本文综述了现有文献的研究成果,阐述了微塑料在斑马鱼体内的累积效应,总结了MPs对斑马鱼的生态毒理学效应及毒性机制,包括生长发育毒性、运动神经与生殖系统代谢紊乱和肝脏变化等,并从三个方面对未来的研究方向提出建议。

1 微塑料在斑马鱼中的累积(Accumulation of microplastics in zebrafish)

呼吸和摄食是斑马鱼对MPs的主要摄入途径,不同发育阶段的斑马鱼可以通过不同的方式摄取MPs。斑马鱼对MPs的摄取能力主要受斑马鱼的发育程度和MPs粒径的影响。粒径较大的塑料颗粒通常只能滞留于胃肠道,粒径较小的塑料颗粒更容易累积,且容易从胃肠道转运到周边其他组织^[13]。胚胎可通过表皮接触MPs,成鱼或幼鱼可通过真皮、眼黏膜和口腔接触MPs^[14]。而Zitouni等指出,无论吸收途径如何,MPs的累积主要取决于粒径大小,较小的MPs更容易累积^[15]。Kim等的研究也表明,MPs的尺寸是决定其在鱼体内累积和毒性效应最大的决定性因素,而不是MPs的类型或吸收途径^[16]。斑马鱼的绒毛膜有用于氧气和营养物质运输的孔隙,较小的MPs可以在胚胎期进入这些孔隙,大于孔隙尺寸的MPs附着在绒毛膜上,随水流进入或被幼鱼误食^[17]。Duan等发现,直径100 nm的MPs可以有效地被胚胎绒毛膜阻断,通过口摄取、皮肤摄取与血液迁移等方式进入斑马鱼幼鱼,并在胚胎孵化时分布在肝脏和脑^[18]。

MPs可在斑马鱼胚胎的绒毛膜^[19-21]、卵黄囊^[22]、肌肉纤维、眼睛和脊髓^[19,23]中累积,甚至在仔鱼和成鱼的嘴^[21]、肠道^[24-30]、脑^[20]、血液^[31]、肝脏^[27,29]、心脏^[31]、鳃^[11,27,29]和肌肉^[25]等部位累积。MPs通常累积在斑马鱼的胃肠道中,部分会转移到循环系统和淋巴系统,甚至分布到肝脏和其他组织器官^[32-33]。MPs的大小决定了它们在循环系统中的运输效率,造成不同组织的累积差异。Lu等发现MPs在组织中的生物蓄积量与MPs的大小有关,直径为5 μm 在组织中的MPs的生物蓄积量是直径为20 μm 的2倍,且直径5 μm 的MPs能在肝组织中累积,而20 μm 则不能^[27]。这与Qiao等的研究结果相似,0.1 μm MPs可累积在斑马鱼肠道、肝脏、鳃,而20 μm 的MPs仅累积在斑马鱼的肠道^[29]。Chen等的研究也表

明,随着塑料颗粒尺寸的减小,塑料颗粒会转运到更多组织中^[25,34].MPs在组织中的累积会对斑马鱼生长发育^[35]、免疫系统^[36]、氧化应激^[37]、血糖水平^[38]、能量代谢^[37]和其他生物反应标记物^[27]产生不良影响.

MPs的粒径大小也决定能否通过斑马鱼的血脑屏障累积在脑组织,对中枢神经系统起到直接毒性作用.斑马鱼的血脑屏障大约建立在受精后72 h^[39],MPs可以进入红细胞^[40-41],穿透血脑屏障^[42],随血流进入脑组织.MPs暴露后首先在斑马鱼的卵黄囊和头部累积,随后在心包、胆囊、胰腺、肝脏和胃肠道等其他组织中累积^[20].目前,关于MPs对不同阶段斑马鱼的毒性研究很多,但还未有系统的总结,本文梳理了微塑料对斑马鱼的累积部位及毒性效应(表1).

表1 微塑料对斑马鱼的累积及毒性效应研究情况

Table 1 Study on the cumulative and toxic effects of microplastics on zebrafish

阶段 Stage	类型 Type of MPs	粒径/ μm Shape and size	暴露浓度/ ($\text{mg}\cdot\text{L}^{-1}$) Exposure concentration	累积部位 Site of accumulation	暴露时间/d Duration of exposure	毒性效应 Effects	参考文献 References
胚胎	PS	20×10^{-3}		脑	5	死亡率上升 DNA损伤	[43]
胚胎	PS	25×10^{-3} , 50×10^{-3} , 250×10^{-3} , 700×10^{-3}	5, 25, 50	肠道, 表皮, 眼睛	2	PS在肠道、表皮、眼睛等器官中的累积	[23]
胚胎	PE				3	核苷酸切除修复(NER)和转化生长因子 β (TGF- β)信号通路改变 存在天然酸性有机物的情况	[44]
胚胎	PS	50×10^{-3} — 100×10^{-3}	1×10^{-3} , 1		4	下,MPs的活性氧(ROS)水平协同增加	[45]
胚胎	PS	50×10^{-3} , 200×10^{-3} , 500×10^{-3}	10×10^{-3}	绒毛膜	1	PS在富含脂质的区域生物累积 MPs和Au协同加剧发育异常、存活率、孵化率以及ROS增加	[19]
胚胎	PS	0.5	1	肠道	2	环氧化酶(COX)活性和超氧化物歧化酶(SOD)的诱导显著降低	[46]
胚胎,幼鱼	PS	51×10^{-3}	0.1, 1, 10	胚胎-绒毛膜 幼鱼-卵黄,脑, 心包,胃肠道	5	心率降低、游泳行为改变	[20]
胚胎,幼鱼	PS	0.7	5		5	补体途径基因(cfh13、cfhl4、cfb和c9)的上调	[31]
胚胎,幼鱼	PS	1	0.1, 1	胚胎-绒毛膜 幼鱼-口腔, 胃,肠道	5	游泳能力下降 il1b、cat的表达上调	[21]
胚胎,幼鱼	PE	38.26 ± 15.64	6.2, 12.5, 25, 50, 100		6	胚胎早期孵化 幼鱼存活率低 形态学参数发生显著变化	[35]
幼鱼	PS	25×10^{-3}	0.2, 2, 20	肠,胰腺,胆囊	2	葡萄糖水平降低,皮质醇分泌增加 运动活动异常 微生物组的改变	[47]
幼鱼	PS	5—50	0.1, 1	肠道	7	CAT和GSH含量显著降低 糖脂和能量代谢紊乱	[48]
幼鱼	PE	10—45	5, 20	肠道	14	对幼鱼发育和生长没有影响 幼鱼基因表达发生短暂而广泛的变化	[49]
成鱼	PS	50×10^{-3}	1	头部,内脏, 鳃,肌肉	3	增加头部和内脏对BPA的摄取 抑制乙酰胆碱酯酶(AChE)活性 髓鞘、微管蛋白/基因表达、多巴胺含量、manf mRNA表达上调 脂质和能量代谢紊乱	[25]
成鱼	PS	70×10^{-3}	0.5, 1.5	性腺,肠道, 肝脏,大脑	7	ROS增加和ATP水平降低 抑制乙酰胆碱酯酶、多巴胺、褪黑素、褪黑素、氨基丁酸、5-羟色胺、加压素、kisspeptin和催产素	[37]
成鱼	PS	70×10^{-3} , 5, 20	20	5 μm -鳃,肝,肠 道 20 μm -鳃,肠道	7	肝脏炎症和脂质堆积 超氧化物歧化酶和过氧化氢酶活性增加 肝脏代谢改变	[27]

续表 1

阶段 Stage	类型 Type of MPs	粒径/ μm Shape and size	暴露浓度/ ($\text{mg}\cdot\text{L}^{-1}$) Exposure concentration	累积部位 Site of accumulation	暴露时间/d Duration of exposure	毒性效应 Effects	参考文献 References
成鱼	PS	0.1, 5, 200	0.5	肠道	21	肠道免疫细胞功能障碍 吞噬细胞产生的ROS相关基因表达改变,增加分泌细胞的黏液分泌 特异性改变巨噬细胞溶酶体和细胞表面受体信号转导过程 类型依赖性致死效应	[50]
成鱼	PA, PE, PP, PVC, PS	0.1, 1, 5 (PS), 70 (PA, PE, PP, PVC)	0.001, 0.01, 0.1, 1.0, 10.0		10	小肠损伤伴绒毛断裂和肠细胞分裂 在鳃、肝和肾中未观察到组织学损伤	[51]
成鱼	PS	0.1, 20	0.2	肠道>肝脏>鳃	3	MPs和天然有机物(NOM)加剧肝脏和内脏中铜的累积 丙二醛(MDA)、金属硫蛋白(MT)水平升高,SOD水平降低 类杆菌和变形杆菌减少,厚壁菌增加	[29]
成鱼	PS	0.5, 50	0.1, 1		14	肠道IL1 α 、IL1 β 和IFN的蛋白水平显著升高	[52]
成鱼	PS	5	20×10^{-3} , 100×10^{-3}		21	葡萄糖、丙酮酸、 α -酮戊二酸和异柠檬酸脱氢酶下降 肠壁变薄和充血性炎症、绒毛损伤和上皮损伤.	[38]
成鱼	PS	5	50×10^{-3} , 500×10^{-3}	肠道>肝脏>鳃	21	肠道微生物群落的变化 CAT、SOD活性和D-乳酸水平升高	[53]
成鱼	PE	10—22, 45—53, 90—106, 212—250, 500—600	2	肠,鳃	4	癫痫发作和形态学变化(尾巴向下弯曲) cyp1a和vtg1上调	[33]
成鱼	PS	微珠(15),碎片, 纤维(25)	10×10^{-3}	肠道	21	形状依赖性积聚——纤维、碎片、珠子 肠黏膜损伤(D-Lac降低)和炎症(IL-1 α 升高) 氧化应激(SOD活性升高) 肠道通透性增加和肠道菌群失调 免疫反应或细胞增殖的一般激活 基因上调	[28]
成鱼	PS	90% < 90; 50% < 50; 10% < 25	0.1, 1		20		[36]
成鱼	PE	125—250			21	<i>prdx1</i> 、 <i>gstp1</i> 、 <i>chrna</i> 、 <i>ngn1</i> 、 <i>cyp1a1</i> 的过度表达	[54]
成鱼、幼鱼、胚胎	PS	42×10^{-3}	4.5、9、12			大脑、肌肉和睾丸的GR活性降低	[22]
成鱼、幼鱼、胚胎	PS	1—5, 10—20		成鱼-鳃 幼鱼-肠道, 胚胎,绒毛膜	4	通过附着到上皮细胞转移POPs cyp1a诱导	[11]

注:PE为聚乙烯, PVC为聚氯乙烯,PA为聚酰胺,PP为聚丙烯.

Note: PE (Polyethylene), PVC (Polyvinyl chloride), PA (Polyamide), and PP (Polypropylene).

2 微塑料对斑马鱼的毒性效应(Toxicity of microplastics on zebrafish)

微塑料对斑马鱼的毒性效应表现在多个方面,主要包括生长发育毒性、神经毒性、生殖毒性、肠道毒性以及心脏毒性等.

2.1 生长发育毒性

微塑料会影响斑马鱼的生长发育及繁殖,降低胚胎的孵化率或延迟孵化,从而影响斑马鱼的存活率. Malafaia 等的研究证实斑马鱼胚胎暴露于 MPs 后胚胎孵化率降低,幼鱼存活率也降低^[35]. 斑马鱼胚胎绒毛膜可以有效屏障直径为 100 nm 的 MPs,因此大于 100 nm 的 MPs 可吸附在绒毛膜外表面,改变绒毛膜的力学性质,导致胚胎处于低氧微环境中^[18]. Ong 等认为胚胎延迟孵化是 MPs 包裹在胚胎绒毛膜上引起,进而导致孵化酶被抑制、环境低氧和胚胎自主运动减弱等^[55].

MPs 对斑马鱼幼鱼具有致畸性,可引起幼鱼形态的改变. 斑马鱼幼鱼暴露于 MPs 可出现卵黄囊面

积增加、头部高度增加,心包/卵黄囊水肿,体轴弯曲,尾部畸形,视泡面积增大^[35,43]等形态改变.斑马鱼摄取 MPs 后会产生饱腹感,减少营养物质的摄入,成年斑马鱼暴露于 MPs(5 μm) 21 d 后,发现生长缓慢^[38].在环境中,MPs 与其他污染物共同作用时,其毒性效应可能发生改变.Trevisan 等的研究表明 MPs 和多环芳烃(phenanthrene, PAHs)共同暴露会减少斑马鱼幼体对 PAHs 的吸收,并减少其引起的发育畸形^[12].

2.2 神经毒性

斑马鱼被广泛用于发育神经毒理学研究,斑马鱼与人类在脑模式以及多个神经和生理系统的结构和功能方面具有广泛的同源性^[56],许多发育中的人类大脑分区在斑马鱼可以识别出对应部分^[57].中枢神经系统的功能表现为复杂的行为和认知功能改变,斑马鱼神经运动行为的研究主要包括斑马鱼的光暗刺激实验、敲击实验、旷场实验、新型水槽实验等.斑马鱼具有空间记忆和学习能力,对应激性刺激具有高度的敏感性,表现为焦虑行为、择偶行为、侵略行为、社会行为等^[58].Sarasamma 等的研究表明斑马鱼暴露于 MPs 后昼夜节律活动紊乱,其运动活动、攻击性、浅滩行为和捕食者回避行为均发生明显改变^[37].MPs 可导致斑马鱼脑组织产生氧化应激,神经元受损,造成行为障碍.研究表明直径 20 nm 的 MPs 可进入斑马鱼的大脑并在生物累积的脑区产生过量的活性氧(reactive oxygen species, ROS),导致行为障碍和脑损伤^[43].斑马鱼暴露于 MPs 可出现癫痫发作行为^[33],运动活动量减少^[20,43]以及运动能力下降^[21].MPs 能够穿透斑马鱼绒毛膜并被胚胎摄取,在组织中累积,显著降低幼鱼的活动能力,产生神经毒性^[20].LeMoine 等对斑马鱼的神经毒性研究表明,暴露于 MPs 后鱼体内与神经元功能相关的基因以及神经元分化和轴突形成基因表达下调^[49].

MPs 可通过破坏斑马鱼体内的脂质过氧化和干扰神经相关酶而导致神经毒性.斑马鱼暴露于 MPs,可干扰鱼体内多种神经递质的传递,如乙酰胆碱、多巴胺、褪黑素、氨基丁酸、后叶加压素、催产素、5-羟色胺和 Kispeptin 等^[59].在各种神经递质的传递作用中,乙酰胆碱酯酶(acetylcholinesterase, AChE)特别被用作神经毒性的主要指标,指示体内胆碱能神经的受损状况.乙酰胆碱酯酶(AChE)对维持神经肌肉系统的正常功能很重要,它能导致乙酰胆碱(acetylcholine, ACh)的失活,而 ACh 对神经肌肉接头和脑突触的胆碱能神经信号传递至关重要.有研究表明,MPs 可抑制斑马鱼的 AChE 活性,对胆碱能神经传递产生不利影响,并导致潜在的神经和神经肌肉功能障碍^[25,37].抑制 AChE 能显著增加大脑中的 ACh 水平,扰乱神经系统的功能.ACh 在突触裂隙中的蓄积会对受体产生过度刺激,阻碍神经信号的传递,导致斑马鱼运动功能丧失和死亡^[25].

2.3 生殖毒性

性腺的遗传完整性是生殖成功的重要条件^[60].MPs 可影响鱼类精子和卵子发生过程^[61],导致生殖细胞早期死亡和 DNA 损伤,并将受损的基因作为缺陷基因遗传给下一代^[62],产生毒性.Ma 等发现 MPs 可以通过血睾屏障在性腺组织中累积,诱导性腺组织中的生殖细胞凋亡影响生殖活动^[63].Sarasamma 等将斑马鱼暴露于 MPs,发现 MPs 可损害卵泡的生长和功能^[37].塑料制造行业为了增加材料的可塑性和耐用性经常使用增塑剂,一些增塑剂如邻苯二甲酸酯和双酚 A,具有内分泌干扰作用,影响雌激素活性^[64].MPs 在迁移转化过程中会释放出有机单体和有毒添加剂,海洋生物摄入 MPs 后,增塑剂在肠道中被释放^[65],产生雌激素内分泌干扰作用^[66].

卵黄蛋白原(vitellogenin, Vtg)在肝脏中合成分泌入血,进入发育中的卵母细胞参与卵黄囊形成,在斑马鱼的繁殖中起着重要作用^[70].Vtg 可以促进 MPs 转移到卵母细胞,最终转移到胚胎卵黄囊^[22].MPs 可干扰细胞色素 P4501A1(*CYP 1A1*)和卵黄蛋白原相关基因(*vtg 1*)表达水平,影响卵子产生.*CYP1A1* 表达下调会引起卵泡发育障碍和雌二醇(E2)合成抑制^[67-69].*Vtg 1* 对水环境中外源性雌激素或抗雌激素物质暴露具有响应性,常用作鱼类雌激素效应的生物标志物.当雄性斑马鱼暴露在雌激素物质中时也可以合成和分泌 Vtg^[71-72].Mak 等对雄性斑马鱼暴露于 MPs 的急性毒性效应研究表明,MPs 使斑马鱼肝脏中 *Vtg1* 表达显著上调,即雄性斑马鱼发生了雌化^[33].

2.4 肠道毒性

肠腔表面的黏液层能分泌黏蛋白,保护肠黏膜抵御肠道菌群侵袭^[73].MPs 在鱼类肠道中累积可导致黏膜上皮损伤、通透性增加、充血性炎症等组织学改变,并引起肠道微生物菌群失调和代谢紊

乱^[28,53]。Lei 等发现,MPs 暴露会导致斑马鱼的绒毛损伤和肠细胞分裂,改变肠细胞中钙离子等离子水平,导致肠道功能障碍^[51]。通过肠道被摄入的 MPs 进入鱼类血液,引起局部组织炎症或过敏反应^[74]。MPs 可以破坏肠道黏膜细胞,影响肠道黏液的生成^[50]。Jin 等在关于 MPs 诱导斑马鱼成鱼肠道菌群失调和炎症反应的研究中,发现斑马鱼暴露于 MPs 后,肠道黏液体积显著增加^[52]。也有一些研究结果与之相反^[75]。MPs 还能减少斑马鱼杯状细胞的数量,致使黏蛋白的生成降低,导致条件致病菌生长繁殖^[76]。因此,MPs 对斑马鱼肠道黏液的影响有待进一步探究。幼鱼暴露于 MPs 后,体内脂肪储量的减少表明其营养不足^[14]。纤维状的微塑料暴露可引起肠道形态变化,包括肠黏膜和肌层厚度减少和异常空泡化,这些通常与营养缺乏和饮食改变有关^[77-78]。

MPs 通过摄入累积在肠道,并通过粪便排出^[75],其肠道累积和充血程度与 MPs 的暴露浓度呈正相关。肠道的损伤程度主要受 MPs 大小的影响,而不是化学成分^[51]。微塑料纤维对斑马鱼幼鱼和成鱼的肠道损伤具有浓度和长度依赖性,可产生氧化应激、炎症和脂质耗竭等反应。与短的微塑料纤维相比,长的微塑料纤维可以显著减少斑马鱼的摄食量。纤维越长,浓度越高,造成的肠壁细胞液泡化和物理损伤越严重^[14]。MPs 的形状也影响斑马鱼肠道的组织学损伤程度,微塑料纤维导致的肠道毒性比微塑料碎片和微珠更严重^[28]。

2.5 心脏毒性

MPs 可在斑马鱼心包内高度累积,导致心率改变,引起心脏毒性。MPs 通过扩散或黏附作用进入细胞内,直接接触细胞内的蛋白质、细胞器和 DNA^[41],可通过与心脏肌节相互作用或氧化应激机制影响心率^[20]。Duan 等发现斑马鱼胚胎暴露于 MPs 24 hpf 后,心率显著加快,可能由于聚苯乙烯微塑料颗粒附着在胚胎绒毛膜上导致胚胎内部的缺氧微环境^[18]。Pitt 等发现暴露于 MPs 的斑马鱼幼鱼心率显著降低,浓度为 0.1、1、10 mg·L⁻¹ 的 MPs 暴露组心率分别下降 5%、8% 和 10%,呈剂量依赖关系^[20]。研究表明心动过缓可能由于聚苯乙烯微塑料颗粒进入心肌细胞与肌节相互作用导致心功能改变^[41]。

3 微塑料的毒性效应机制(Toxic effect mechanisms of microplastics)

微塑料可诱导斑马鱼肠道炎症、肠道菌群失调、氧化应激以及代谢紊乱^[53]。微塑料和其他有机污染物的共同暴露会导致斑马鱼对有机污染物的积累增多,导致更严重的氧化损伤,从而刺激免疫功能,改变肠道微生物组成,对斑马鱼的健康造成危害。研究表明微塑料和部分有机污染物之间具有协同毒性作用^[79]。

3.1 氧化应激

MPs 暴露后在鱼类的组织中累积,通过物理和化学作用引起氧化应激,例如肠梗阻和内分泌紊乱^[80]。MPs 对鱼类细胞的毒性主要是由氧化应激引起的,包括氧化还原平衡紊乱、细胞器的受损和过多 ROS 的产生^[81]。当抗氧化防御系统紊乱时,过量的 ROS 参与多种细胞信号通路的激活,引起其他病理生理反应,如细胞自噬、DNA 氧化损伤、细胞凋亡^[82-83] 和炎症反应^[84-85] 等。ROS 可导致线粒体 DNA 突变,抑制线粒体氧化磷酸化,致使与能量代谢相关的 ATP 水平显著下降。Trevisan 等通过研究 MPs 暴露后斑马鱼线粒体的能量产生过程,发现斑马鱼胚胎或仔鱼 ATP/ADP 比值减小,即 NADH 产生增加(可能由于微塑料引起线粒体功能障碍减缓了电子传递链对 NADH 的消耗并随时间累积或机体的代偿机制),ATP 合成减少即机体能量产生减少,进而影响生长发育^[12]。

MPs 通过影响斑马鱼体内的抗氧化应激酶的活性,破坏 ROS 产生和抗氧化损伤之间的抗氧化平衡,导致氧化损伤。轻微的氧化应激会诱导抗氧化酶的活性^[86]。然而当氧化应激达到一定程度时,抗氧化酶的活性将受到抑制^[87]。抗氧化应激酶通常指超氧化物歧化酶(SOD)、过氧化氢酶(CAT)、谷胱甘肽-S-转移酶(GST)、还原型谷胱甘肽(GSH)、谷胱甘肽过氧化物酶(GPX)和谷胱甘肽还原酶(GR),这些酶是衡量生物体内氧化应激的重要生物标志物^[88]。SOD 是鱼类对氧化应激的主要防御机制,CAT 是将过氧化氢转化为水和氧气的酶^[89]。GST 参与多种细胞毒性物质的解毒、细胞免受氧化损伤的防御以及脂质、血红素和类固醇等物质的运输^[90]。GPX 和 CAT 一样都是通过分解活性物质保护细胞免受氧化的酶,GSH 是小分子抗氧化剂,可作为 GPX 的底物,阻止自由基和 ROS 的过量产生,并抑制它们与 DNA、蛋白质和脂类的反应^[91]。Umamaheswari 等的研究表明 MPs 暴露会导致斑马鱼中 GPX 活性

降低,阻止过氧化氢转化为无毒的羟基底物,导致组织中 ROS 的蓄积^[92].将斑马鱼 96 hpf 幼鱼暴露于 100 $\mu\text{g}\cdot\text{L}^{-1}$ 和 1000 $\mu\text{g}\cdot\text{L}^{-1}$ 的 MPs 后, CAT 的 mRNA 的表达显著上调, GPX 活性升高、GR 活性降低^[21].而 Parenti 等将 72 hpf 的斑马鱼胚胎暴露于浓度为 1 $\text{mg}\cdot\text{L}^{-1}$ 的 MPs 时,结果表明 SOD、CAT 和 GPX 活性无任何显著变化^[46].Lu 等研究发现直径 5 μm 的 MPs 导致斑马鱼肝脏中 SOD 和 CAT 的活性显著提高,并呈剂量依赖关系^[27],Wan 等则表明 MPs(5—50 μm)对斑马鱼幼鱼的 CAT 活性有明显的抑制作用, GSH 的含量显著下降^[48].

肠道损伤与肠壁细胞发生的氧化应激有关,慢性氧化应激可引起各种肠道损伤,如炎症反应和肠壁通透性改变等.鱼类组织中 ROS 水平的增加会引起氧化应激,导致炎症反应和细胞死亡^[93].研究表明 MPs 会增加肠道中 ROS 的产生,同时 SOD 和 IL-1 α 的水平会提高,诱导了明显的氧化应激和炎症反应^[14].Lu 等的研究发现微塑料和镉(Cd)的共同暴露会增加斑马鱼的氧化应激和炎症反应^[94].

3.2 代谢紊乱

斑马鱼暴露于 MPs 后,会引起血糖、胆固醇和总蛋白水平的变化,导致斑马鱼的血糖、脂肪代谢和氨基酸代谢功能紊乱^[49,53].MPs 可以与质膜、溶酶体、细胞骨架(肌动蛋白)和代谢相关蛋白(细胞色素 P450 酶)相互作用并破坏它们^[95].长期暴露于 MPs 可导致斑马鱼肝脏葡萄糖代谢受损,血糖显著升高,其糖代谢、脂肪代谢和氨基酸代谢途径相关基因的表达水平均下降^[38].Wan 等发现 MPs 暴露会引起斑马鱼幼体糖酵解和脂肪代谢相关基因的变化^[48].Veneman 等将 MPs 显微注射到斑马鱼卵黄囊后,发现参与脂质代谢的多个核受体表达上调^[31].Duan 等发现胚胎暴露于聚苯乙烯微塑料颗粒时,不饱和脂肪酸、亚油酸和丙氨酸的生物合成途径以及天冬氨酸和谷氨酸的代谢途径发生广泛变化^[18].有研究表明,因炎症反应和刺激,谷草转氨酶(AST)和碱性磷酸酶(ALP)由肝细胞入血,MPs 导致斑马鱼 AST 和 ALP 的水平显著升高^[96].

有研究表明微塑料会造成斑马鱼多种组织学改变,如肝细胞坏死和脂肪浸润,并引起肝脏炎症反应和脂质堆积^[27].脂质很容易被氧化,长期的氧化应激和炎症可损害脂质代谢^[97].脂质过氧化物(LPO)升高表明发生了氧化应激和脂质损伤,暴露于 MPs 的斑马鱼体内 ROS 和 LPO 显著增加^[92].这表明肠道损伤可能由 MPs 暴露导致的脂质过氧化引起,这与斑马鱼暴露于 MPs 后,氧化应激诱导 LPO 的产生是组织中的显著特征的结论相一致^[98].丙二醛(MDA)被用作 LPO 的生物标记物,因为它是脂质氧化损害后的最终产物^[99].

斑马鱼暴露于不同浓度的 MPs 会产生肠道损伤^[27-28,36,51],肠道损伤通常与肠道炎症和细胞因子表达水平的变化有关.肠道炎症与肠道微生物区系失衡密切相关^[53],引发肠道通透性的变化.斑马鱼暴露于 MPs 后,与新陈代谢、疾病和炎症密切相关的细菌发生改变,拟杆菌门^[48,52]和变形菌门^[48,52-53]显著减少,梭杆菌门^[52-53]和厚壁菌门^[48,52]显著增加.许多研究评估了不同类型、大小和形状的微塑料的风险,强调肠道微生物区系失调与肠道毒性之间的联系^[36,50,100].暴露于 MPs 可增加斑马鱼肠道菌群的多样性,且不同浓度的 MPs 引起肠道菌群在门和属水平上的反应不同^[75].斑马鱼幼体暴露于 MPs(5 μm 和 50 μm , 1000 $\mu\text{g}\cdot\text{L}^{-1}$, 持续 7 d)导致肠道微生物丰度下降,并产生属水平丰度的显著变化^[48].另一项研究分析了低剂量 MPs 微球(5 μm 、50 $\mu\text{g}\cdot\text{L}^{-1}$ 和 500 $\mu\text{g}\cdot\text{L}^{-1}$, 持续 21 d)对斑马鱼肠道菌群的影响;结果显示菌群多样性减少,梭菌丰度增加,变形杆菌丰度降低^[53].也有研究评估了 MPs 纤维对斑马鱼肠道菌群的影响,暴露在 10 $\mu\text{g}\cdot\text{L}^{-1}$ 的微塑料纤维(20—100 μm)中 21 d,导致斑马鱼细菌多样性减少和某些菌群的变化(变形杆菌增多,放线杆菌减少)^[28].肠道菌群的失调会破坏肠黏膜屏障功能和免疫稳态之间的平衡,引发炎症反应^[53,101].

3.3 免疫反应

MPs 能跨越生物屏障进入循环系统,导致机械破坏和能量储备减少,从而影响免疫系统^[59].被鱼类摄入的 MPs 可能与肠道组织相互作用并转移到循环系统,导致免疫反应的调节受损^[102].研究表明,MPs 可以影响斑马鱼的先天免疫系统和获得性免疫系统功能. PEI 等证实了 MPs 可以改变斑马鱼肠道微生物区系的丰富度和多样性,引起强烈的氧化应激,破坏先天性免疫系统^[100].补体系统是先天性免疫系统的一部分,可攻击病原体的细胞膜以清除病原体,MPs 通过激活补体系统引起免疫反应.经 MPs 暴露的斑马鱼幼体,与免疫反应相关的信号通路被激活,补体替代途径基因(如 *CFHL3*、*CFHL4*、

Cfb 和 *C9*) 表达上调^[31]. 在 MPs 暴露后斑马鱼产生的免疫反应多种多样, 如溶菌酶、中性粒细胞、过氧化物酶、吞噬作用和免疫球蛋白等. Gu 等发现斑马鱼暴露于 MPs 后, 吞噬、趋化和整合素介导的信号通路的功能改变, 细胞色素 B-245 β 链 (*Cybb*)、组织蛋白酶 S (*Ctss*)、整合素亚单位 $\beta 2$ (*ITGB2*) 表达下调, 主要组织相容性复合体 (MHC) 表达上调^[50].

鱼类中的免疫球蛋白 IGZ/IGT 的功能类似于哺乳动物中的 IgA, 可以快速有效地消除特定的病原体, 在黏膜免疫中发挥着重要作用^[103]. 黏膜免疫球蛋白 (哺乳动物的 IgA; 硬骨鱼的 IGZ 或 IGT) 的产生是由食物抗原和肠道微生物区系引起的, 有助于建立和维持与微生物区系的有益互动^[104-105]. 由于水环境的原因, 大量的微生物不断刺激鱼类的黏膜屏障. 免疫球蛋白在硬骨鱼 (IGT) 和哺乳动物 (主要是 IgA) 之间进化保守性高, 维持共生微生物群落以抵御病原体^[106]. 斑马鱼暴露在浓度为 $500 \mu\text{g}\cdot\text{L}^{-1}$ 的 MPs 后, 影响肠道免疫网络产生 IgZ, 这可能产生促炎细胞因子, 吞噬微生物区系, 并启动免疫反应^[50]. 溶菌酶是一种由白细胞释放的具有抗生素特性的酶, 是先天免疫系统的重要防御分子, 可介导阻止微生物的入侵, 并且经常被用作非特异性免疫功能的指标^[107]. Limonta 将斑马鱼暴露于 MPs (PS 和 PE) 后, 观察到斑马鱼的免疫反应显著增强, 溶菌酶和中性粒细胞含量增加^[36].

斑马鱼的免疫系统有许多细胞因子, 维持其体内的稳定环境, 是免疫系统的重要组成部分. 微塑料可以特异性改变巨噬细胞溶酶体和细胞表面受体信号转导过程^[50], 影响相关细胞因子的基因表达. MPs 是潜在的免疫刺激剂, 以粒径大小依赖和浓度依赖的方式诱导细胞因子和趋化因子的产生. 研究表明, 暴露于 $1000 \mu\text{g}\cdot\text{L}^{-1}$ 直径 $0.5 \mu\text{m}$ 聚苯乙烯的斑马鱼, 肠道中与先天性免疫系统相关的细胞因子 (*IL-1A*、*IL-1B* 和 *IFN*) 的 mRNA 水平和蛋白质含量显著增加, 产生炎症反应^[52]. 在正常情况下, 肠道微生物区系和宿主免疫系统之间保持着良好的平衡关系. 当肠道微生物区系失衡时, 内毒素可诱导 *IL-10*、*IL-15*、*CXCL12* 或 *CXCR4* 等炎症因子表达增加, 这些炎症因子可抑制革兰氏阴性杆菌的生长和扩散^[108-110]. 暴露于 MPs 后, 斑马鱼体内编码 *IL-1B* 的基因表达上调, 不同组织 (肝脏和肠道) 的炎症反应增加^[21,27-28,51,53]. 暴露于高密度 PE 和 PS 颗粒会降低免疫基因白三烯 B4 受体 (*ltb4r*) 和干扰素诱导的跨膜蛋白 (*ifitm1*) 的肝脏转录水平^[36]. 环氧合酶 (COX) 参与前列腺素的合成, 与炎症反应密切相关. 将斑马鱼胚胎暴露于 MPs 后, COX 活性显著降低^[46].

3.4 细胞凋亡

微塑料通过影响斑马鱼体内 ROS 代谢, 引起细胞凋亡, 进而导致胚胎或幼鱼畸形的增加^[111-112]. MPs 累积在斑马鱼幼体大脑区域, 存在 ROS 诱导的 DNA 氧化损伤和细胞凋亡^[43]. MPs 诱导斑马鱼产生剂量和时间依赖的 ROS 介导的细胞凋亡反应, *P53*、*Gadd45ba* 和 *casp3b* 基因表达上调^[100]. *P53* 基因通过 *Casp3b* 和 *Gadd45ba* 的激活^[113-114] 以及 *ptgs2a* 的表达 (斑马鱼中的一种炎症生物标志物) 传递信号来刺激细胞凋亡. *P53* 基因激活与细胞凋亡相关的分子 (如 *Bcl2/Bax* 或细胞色素 c) 触发 caspase 信号通路^[115], 诱导细胞凋亡. *Gadd45ba* 基因与对任何化学或环境应激源的应激反应有关, 可引起 DNA 损伤、细胞凋亡、甚至生长停滞^[116].

4 研究展望 (Research and prospect)

塑料污染问题日益突出, 近几年关于微塑料的研究爆炸性地增加, 主要集中在环境迁移转化及生态毒理效应. 在生态毒理方面的研究较多集中在工业微塑料标准品对生物的毒性影响, 仅有少数研究探究了环境微塑料对鱼类的毒性效应. 环境微塑料相比工业微塑料制品对生物体的毒性影响更为复杂, 微塑料的粒径大小、浓度、表面老化程度及添加剂的浸出都是影响生物毒性的重要因素. 此外, 水生态系统中存在多种环境污染物, 微塑料可以吸附持久性有机污染物、重金属以及微生物, 引起生物富集和生物放大, 对生物体本身及生态环境存在较大的危害性风险. 目前, 微塑料吸附污染物的研究大多数集中在重金属上, 且关于微塑料与 POPs 的吸附解析与复合毒性效应研究较为单一, 主要在抗生素及阻燃剂等方面有所涉及, 有待进一步探索; 塑料添加剂的浸出及生态毒性研究也较为匮乏, 因此今后应在以下三个方面有所侧重: (1) 环境微塑料及添加剂浸出的生态毒理效应及作用机制; (2) 模拟环境老化过程, 探讨环境老化及老化过程中添加剂的浸出及其相关毒性机制; (3) 微塑料与 POPs 的复合毒性及作用机制.

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