氧化石墨烯和双酚A对斑马鱼早期发育氧化损伤的联合效应

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2019-04-18 收稿, 2019-05-23 修回, 2019-05-29 接受, 2019-07-17 网络版发表

国家自然科学基金(21737003)和国家重大水专项(2017ZX070301002)资助

摘要 探讨了氧化石墨烯(graphene oxide, GO)和双酚A(bisphenol A, BPA)复合暴露对斑马鱼机体氧化损伤的联合毒性效应. 将斑马鱼胚胎分别暴露于BPA(0, 50, 500 $\mu\text{g/L}$), GO(0, 0.1, 1 mg/L), BPA+GO(50 $\mu\text{g/L}$ +0.1 mg/L , 500 $\mu\text{g/L}$ +0.1 mg/L 和50 $\mu\text{g/L}$ +1 mg/L , 500 $\mu\text{g/L}$ +1 mg/L)溶液7 d后, 测定超氧化物歧化酶(superoxide dismutase, SOD)、丙二醛(malondialdehyde, MDA)、谷胱甘肽(glutathione, GSH)、过氧化氢酶(catalase, CAT)及活性氧(reactive oxygen species, ROS)等氧化应激相关指标. 结果表明, BPA单独暴露时对斑马鱼胚胎产生较强的氧化损伤效应, 而GO能缓解由此产生的各种氧化损伤指标. Bliss独立模型计算判断复合暴露组中GO和BPA具有拮抗效应, 表明本实验条件下GO可以降低BPA对斑马鱼胚胎的氧化损伤毒性.

关键词 双酚A(BPA), 氧化石墨烯(GO), 氧化应激, 联合毒性, Bliss独立模型

双酚A(bisphenol A, BPA)作为全球生产量最大的一种化工原料^[1], 当含有BPA的生活用品不完全聚合或暴露于高温、碱性等环境中时, BPA就会从产品中释放出来^[2], 并通过不同的途径排放到环境当中^[3]. BPA在地表水中广泛存在, 生产基地附近地表水中BPA的浓度甚至可高达28 $\mu\text{g/L}$ ^[4]. 相关研究发现一些垃圾处理厂BPA含量可高达17.2 mg/L , 平均含量也可达到269 $\mu\text{g/L}$ ^[5], 最终释放到水体环境中^[6], 并对水生生物产生发育、生殖、免疫等毒性效应^[7]. 氧化石墨烯(graphene oxide, GO)作为石墨烯的氧化产物^[8], 由于其独特的物化性质而被广泛应用于众多行业^[9,10], 并不可避免进入水体环境中, 对水体中共存污染物的环境行为和毒性效应产生一定的影响. 目前, 环境中GO的实际浓度还未见诸报道, 文献^[11,12]中体内和体外GO毒性实验的浓度通常设置在 $\mu\text{g/L}$ ~ mg/L 之间. 相关研究表明, GO可

以进入生物体并引起一定的氧化损伤、细胞毒性、基因毒性和发育毒性^[13~15].

斑马鱼早期发育阶段对活性氧自由基的伤害非常敏感, 使其在发育阶段更容易受到外界影响. 氧化应激是很多疾病的诱因, 污染物通过破坏机体氧化与抗氧化之间的平衡, 诱导机体产生过量活性氧(reactive oxygen species, ROS)并生成大量氧化中间产物, 进而导致机体发生氧化损伤^[16~18]. 胚胎是斑马鱼的敏感发育阶段, 该时期的不良效应将对后期的生长发育产生重要影响. 纳米材料如GO和有机污染物如BPA在环境中同时广泛存在, 而它们对斑马鱼胚胎的氧化损伤机理不同^[19,20], 因此有必要对GO和BPA在斑马鱼早期氧化损伤的联合效应及机制开展研究. 斑马鱼胚胎在早期发育时是透明的, 更容易观察和操作, 相对于离体培养细胞更接近于个体真实环境. 刚孵化出的仔鱼相对于斑

引用格式: 杨晶, 陈朋宇, 钟文珏, 等. 氧化石墨烯和双酚A对斑马鱼早期发育氧化损伤的联合效应. 科学通报, 2019, 64: 2199–2206

Yang J, Chen P Y, Zhong W J, et al. The combined effects of graphene oxide and bisphenol A on oxidative damage in early development of zebrafish (in Chinese). Chin Sci Bull, 2019, 64: 2199–2206, doi: 10.1360/N972019-00340

马鱼成年个体也更为敏感,因此有很多研究^[21-23]都选择斑马鱼早期发育阶段进行暴露。

本文选取斑马鱼早期阶段进行研究,分别暴露在GO、BPA和GO+BPA的分散液中,研究不同暴露组中斑马鱼早期氧化损伤相关酶活性变化情况,探讨GO对BPA对斑马鱼早期阶段引起氧化损伤的影响与机制,对评估真实环境中二者的环境风险提供一定的科学依据。

1 材料与方法

(i) 实验材料. BPA(纯度>99.5%)购自德国Ehrenstorfer GmbH公司,助溶剂二甲亚砜(DMSO,色谱纯)购自美国Sigma Chemical公司. GO纳米片(纯度>99%)购于南京先丰纳米科技有限公司. 成年斑马鱼(*Danio rerio*)购于天津花鸟鱼虫市场. 斑马鱼饲养在恒温水箱,温度控制为(28±1)°C, 14 h/10 h明暗交替光照,饲养2周左右开始收集斑马鱼鱼卵. 饲养水中加入胚胎培养液(294 mg/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 123 mg/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 63.0 mg/L NaHCO_3 和5.5 mg/L KCl),保持pH7.0左右,每日喂食2次. 成鱼在光刺激下追尾、受精. 受精后立即收集胚胎,并用培养液冲掉粪便,剔除未受精的胚胎. 将胚胎转移到96孔板中,待发育6 h(hours post-fertilization, hpf)后在体视显微镜下(Olympus, SZX16, 日本)挑选出健康的胚胎,用于后续实验。

(ii) GO和BPA溶液的制备及GO表征. GO用Milli-Q水作为溶剂,超声4 h,配制成100 mg/L GO的母液,避光存放在4.0°C冰箱. 本实验GO暴露浓度设置为0.1和1 mg/L(分别标记为0.1G和1G). 采用透射电子显微镜(Transmission electron microscope, TEM, Hitachi HT7700, 日本)观察GO的形貌;可见光-紫外分光光度计(Purkinje General T90, 中国)、傅里叶转化红外光谱仪(FT-IR, Bruker Tensor 27, 德国)表征GO结构特征;粒径-Zeta电势分析仪(ZETAPALS/BI-200SM, Brookhaven, 美国)测定GO的动力学直径和Zeta电势. BPA用DMSO作为助溶剂配置成50 g/L的母液,再用Milli-Q水稀释至所需浓度,均避光储存. BPA暴露浓度设定为50和500 $\mu\text{g/L}$ (标记为50B和500B),其中DMSO浓度均为0.01%(体积比),并且设置0.01% DMSO(体积比)为对照组(SC).

(iii) 常规发育指标. 统计168 hpf的孵化率、存活率及畸形率,并在4倍显微镜(Olympus X71, 日本)下观察拍照记录其生长发育情况. 计算方法如下:

$$\text{孵化率} = \frac{\text{孵化数}}{\text{总数}}, \quad (1)$$

$$\text{存活率} = \frac{\text{存活数}}{\text{总数}}, \quad (2)$$

$$\text{畸形率} = \frac{\text{畸形数}}{\text{总数}}. \quad (3)$$

(iv) 生化指标测定. 每个暴露组取60条仔鱼,用去离子水清洗2次,加入3 mL预冷的0.1 mol/L PBS稀释液(pH 7.3),每组3个平行. 采用超声破碎仪进行匀浆,直至组织混合物匀质化,于4°C, 5000 g下离心15 min. 将3 mL上清液分别装在3个1 mL离心管中,于-80°C保存. 超氧化物歧化酶(SOD)、丙二醛(MDA)、谷胱甘肽(GSH)、过氧化氢酶(CAT)的含量均采用ELISA试剂盒(哈灵科技有限公司)测定,参照供应商提供的说明书进行相关操作. 参照文献^[24]方法,用考马斯亮蓝法测定总蛋白含量。

(v) ROS测定. 参照文献^[25]方法,当胚胎发育至168 hpf时,用培养液冲洗数次,用含10 $\mu\text{mol/L}$ 2,7-二氯荧光素二乙酸盐(2,7-dichlorodihydrofluorescein diacetate, DCFH-HA)胚胎培养液避光孵育,然后再用培养液将其冲洗干净. DCFH-HA是非标记性氧化敏感荧光探针,自身不会发荧光,但可自由通过细胞膜. 通过细胞膜后,DCFH-HA被细胞内酯酶分解成为不能通过细胞膜的DCFH,使得探针被装载到细胞中. 细胞内ROS将DCFH氧化成能发荧光的DCF,荧光的强弱可反映细胞内ROS浓度. 用0.016 mol/L三卡因麻醉胚胎后,在倒置荧光显微镜(Olympus X71, 日本)下观察斑马鱼体内荧光产生的情况,激发波长和发射波长分别为485和535 nm. 及时拍照记录后用Image-J软件提取相关信息并确定其荧光强度,每组设3个平行。

(vi) 数据统计分析. 用SPSS 8.0软件进行统计学分析,多组间的比较使用方差齐性检验和单因素方差分析(one-way ANOVA). 对于组间两两比较,使用LSD检验,以 $P < 0.05$ 表示差异显著. 采用Pearson相关性分析研究ROS和MDA之间的相关性. 采用Bliss独立模型对GO和BPA的相互作用进行定量分析. 实验结果均以“平均值±标准误差”即(mean±SD)表示:

$$P_B = \frac{\text{BPA} - \text{Control}}{\text{Control}} \times 100\%, \quad (4)$$

$$P_G = \frac{|\text{GO} - \text{Control}|}{\text{Control}} \times 100\%, \quad (5)$$

$$\text{PE} = P_B + P_G - P_B P_G, \quad (6)$$

式中, P_B 和 P_G 分别表示BPA和GO单独暴露下的计算值, PE(predicted effects)表示复合暴露组的预测值. PE大于OE(observed effects, 复合暴露组的实测值), 为拮抗作用(antagonistic, ANT); PE小于OE, 为协同作用(synergistic, SYN); PE等于OE, 为相加作用(additive, ADD).

2 结果与讨论

2.1 GO纳米材料的表征

由紫外光谱可以看出GO水溶液在230 nm处具有一个典型特征峰, 它对应的是GO结构中苯环C=C的 π - π 跃迁^[26]. GO在~3340, ~1720, ~1620, ~1400及~1070 cm^{-1} 位置均出现了明显的特征峰, 说明GO 纳米片结构中含有-OH, -COOH, C=C, C-OH和C-O-C等基团^[27]. Zeta电势可指示GO在溶液中的稳定性, 当其绝对值大于30.0 mV时相对比较稳定^[28]. GO在去离子水溶液的 Zeta 电势为 (-32.7 ± 2.63) mV, 表明其在去离子水里具有较好的分散性. 当GO分散在胚胎培养液中时, 由于培养液中阳离子的静电吸附和压缩双电层作用, GO的Zeta电势上升为 (-14.4 ± 1.91) mV, 说明GO的稳定性有所下降. 但是溶液中并未观察到明显的沉降絮凝现象, 说明该培养液满足本研究静态实验的要求. GO在去离子水和培养液中的水合粒径分别为 (232 ± 14.3) 和 (725 ± 24.7) nm, 同样说明培养液中GO因团聚形成了大颗粒的团聚体. 图1中TEM图显示GO在水溶液中为半透明的二维片层结构, 而在胚胎培养液中则明显变厚, 进一步说明GO在培养液中有一定程度的团聚.

2.2 斑马鱼胚胎的死亡、孵化与畸形

孵化是鱼类生命过程中的一个重要阶段, 在相应的发育阶段鱼卵脱膜孵化是幼鱼存活、觅食及进一步发育的基础. 斑马鱼胚胎孵化168 h后, 所有暴露组的孵化率和存活率均>95.5%, 说明实验设计的暴露浓度均不会对斑马鱼产生明显的急性毒性. 单独0.1和1 mg/L的GO暴露时, 胚胎的孵化率与对照组无显著性差异, 与Chen等人^[28]的研究结果类似. 500 $\mu\text{g/L}$ 的BPA单独暴露时, 斑马鱼在54 hpf的孵化率 $(74.3\% \pm 2.41\%)$ 明显高于空白组 $(59.6\% \pm 3.37\%)$, 说明中低浓度BPA能促使胚胎孵化提前^[29]. GO和BPA单独暴露均可以造成斑马鱼仔鱼畸形(图2). 然而, 500B+1G的复合暴露组的畸形率 $(5.80\% \pm 2.64\%)$ 相对于500B单独暴露组 $(13.4\% \pm 3.33\%)$ 显著降低($P < 0.05$). 50B和500B单独暴露组畸

图1 GO的TEM表征图. (a) 去离子水; (b) 胚胎培养液
Figure 1 TEM images of GO. (a) In deionized water; (b) in embryo culture medium

图2 (网络版彩色)早期斑马鱼畸形图. (a) 正常发育对照组; (b) 1, 心包囊水肿; 2, 卵黄囊水肿; 3, 尾畸; (c) 背畸; (d) 没有眼睛
Figure 2 (Color online) Malformations of early zebrafish. (a) Control group; (b) 1, pericardial edema; 2, yolk sac edema; 3, tail deformity; (c) spinal curvature; (d) no eyes

形率分别为 $(9.60\% \pm 2.23\%)$ 和 $(13.4\% \pm 3.33\%)$, 均高于0.1G $(3.30\% \pm 3.25\%)$ 和1G $(5.20\% \pm 3.21\%)$ 单一暴露组. 这些结果说明本实验条件下BPA对斑马鱼早期发育畸形影响要强于GO, 当二者共同作用时, GO在一定程度上缓解了由BPA主导引起的孵化率提前, 畸形率也有一定程度的降低.

2.3 单独和复合暴露对活性氧生成的影响

ROS是广泛存在于生物体内的一种活性物质, 线粒体是大多数细胞产生ROS的主要来源. 通常细胞内ROS 浓度可以在抗氧化系统调节下保持在正常的生理水平. 研究报道GO对生物体的主要毒性机制之一是通过诱导生物体产生活性氧, 导致生物体内氧化胁迫损伤^[28]. 图3显示培养溶液中较大的GO团聚体附着在胚

图3 不同浓度GO对斑马鱼胚胎绒毛膜包覆图. (a) 0 mg/L; (b) 0.1 mg/L; (c) 1 mg/L

Figure 3 Chorionic coating of zebrafish embryos at different concentrations of GO. (a) 0 mg/L; (b) 0.1 mg/L; (c) 1 mg/L

胎绒毛膜表面, 该现象随着GO浓度的升高逐渐明显. GO可以附着在胚胎绒毛膜表面, 阻碍胚胎体内外气体交换, 容易导致胚胎内部环境低氧. 因此在较高浓度GO (1 mg/L)存在时, 斑马鱼胚胎内产生ROS并引发氧化损伤(图4, 5), 这与Chen等人^[28]之前的研究结果一致. BPA也可以造成斑马鱼胚胎氧化损伤(图4, 5). 与GO不同的是, BPA通过胚胎绒毛膜主动吸收进入胚胎内部, 导致机体产生氧化损伤现象^[29]. 在50B和500B的单独暴露组中, 斑马鱼胚胎内产生的ROS要高于0.1G和1.0G的单独暴露组, 而复合暴露组中ROS显著低于BPA单独暴露, 但显著高于GO单独暴露组. Bliss模型结果说明BPA和GO之间对ROS的产生存在拮抗作用(表1), 说明GO降低了BPA所导致的斑马鱼早期发育的氧化损伤毒性.

2.4 单独和复合暴露对生化指标的影响

生物体受到外界刺激时会生成大量ROS, 生物体依靠改变体内抗氧化酶的活性进行氧化应激, 清除ROS来降低机体氧化损伤^[30]. SOD是抗氧化酶中最开始起作用的特异性酶, 主要在生物体胞质及线粒体中存在. SOD可以将超氧阴离子(O_2^-)催化氧化为 O_2 和

H_2O , 使ROS维持在生理水平, 保护机体免于损伤^[30]. CAT是过氧化物酶体中的含铁血蛋白抗氧化酶, 会使生物体内 H_2O_2 转化为 O_2 和 H_2O ^[31], 防止毒性更强的 $\cdot OH$ 生成, 从而保护生物体免受更大的损伤. ROS过量会攻击生物膜系统的多不饱和脂肪酸, 从而导致脂质过氧化. MDA是发生不可逆脂质过氧化的产物, MDA的变化可反映生物体内脂质过氧化的水平和损伤程度^[31]. GSH作为生物体内一种重要的非酶抗氧化剂, 生物体在受到外界刺激后诱导产生GSH, 以有效清除氧自由基调控氧化应激^[32]. 当GSH逐渐减少到一定水平时, 生物体将发生中毒效应. 由图6可以看出, 在BPA单独暴露时, 斑马鱼仔鱼体内SOD, CAT, GSH均受到抑制($P<0.05$), 且MDA含量大幅上升($P<0.05$), 这

图4 斑马鱼体内ROS的荧光强度. (a) 空白对照组; (b) GO(1 mg/L); (c) BPA(500 µg/L); (d) 500 µg/L BPA+1 mg/L GO

Figure 4 The fluorescence intensity of ROS in zebrafish. (a) Control group; (b) GO (1 mg/L); (c) BPA (500 µg/L); (d) 500 µg/L BPA+1 mg/L GO

图5 相对荧光强度. a代表BPA单独处理组与复合处理组有显著差异, $P<0.05$. b代表GO单独处理组与复合处理组有显著差异, $P<0.05$. *代表与对照组有显著差异, $P<0.05$

Figure 5 Relative fluorescence levels in each treatment and control group. a indicates that there is a significant difference between the BPA treatment group alone and the mixed treatment group, $P<0.05$. b indicates that there is a significant difference between the GO treatment group alone and the mixed treatment group, $P<0.05$. * indicates there is a significant difference between treatment and control groups, $P<0.05$

表 1 GO和BPA联合毒性的相互作用^{a)}

Table 1 The interaction between GO and BPA combined toxicity^{a)}

	GO(mg/L)	0.1		1	
		BPA(μ g/L)	50	500	500
ROS	PE(%)		107	179	104
	OE(%)		76.3	118	82.5
	结论		ANT	ANT	ANT
SOD	PE(%)		90.5	90.7	64.0
	OE(%)		30.7	21.5	50.6
	结论		ANT	ANT	ANT
MDA	PE(%)		34.1	45.7	122
	OE(%)		14.6	20.4	40.3
	结论		ANT	ANT	ANT
CAT	PE(%)		28.1	31.3	50.4
	OE(%)		17.5	14.6	32.1
	结论		ANT	ANT	ANT
GSH	PE(%)		40.2	40.4	61.6
	OE(%)		22.9	20.5	42.2
	结论		ANT	ANT	ANT

a) PE, 复合暴露预测值; OE, 复合暴露实际值; ANT, 拮抗作用

图 6 GO(0.1G, 1G)和BPA(50B, 500B)不同暴露处理对斑马鱼SOD(a), MDA(b), CAT(c)和GSH(d)的影响. a代表BPA单独处理组与复合处理组有显著差异, $P<0.05$; b代表GO单独处理组与复合处理组有显著差异, $P<0.05$. *代表与对照组有显著差异, $P<0.05$

Figure 6 Effects of different exposure treatments of GO (0.1G, 1G) and BPA (50B, 500B) on zebrafish SOD (a), MDA (b), CAT (c) and GSH (d). a indicates that there is a significant difference between the BPA treatment group alone and the mixed treatment group, $P<0.05$. b indicates that there is a significant difference between the GO treatment group alone and the mixed treatment group, $P<0.05$. * indicates there is a significant difference between treatment and control groups, $P<0.05$

与Wu等人^[33]的研究结果一致。而在GO单独暴露组中, SOD, CAT与对照组相比没有显著性变化, 但GSH受到抑制($P<0.05$), 且MDA含量上升($P<0.05$)。这与Chen等人^[28]的研究结果略有不同, 可能是由于选用的胚胎培养液不同, 本实验条件下 Ca^{2+} 离子浓度相对更高, 使得GO更容易发生团聚。因此, 尽管GO暴露组中产生了活性氧, 但是本实验条件下SOD与空白对照相比没有显著性差异。由图7可见, 除了0.1G组外, 其他暴露组的MDA水平都显著高于空白组($P<0.05$), 且MDA与胚胎内ROS水平呈现正相关性($R^2=0.905$), 说明由于暴露引起的活性氧物质上升是导致机体内脂质过氧化的主要原因。与单独GO暴露相比, 复合暴露组与氧化损伤相关的指标均有所增强; 然而, 与单独BPA暴露相比, 复合暴露组氧化损伤相关的指标均有所缓解。Bliss模型结果说明GO和BPA对斑马鱼早期氧化酶活性均为拮抗作用(表1)。这些结果表明, 在此实验条件下, BPA单独暴露对于斑马鱼氧化损伤要强于GO单独暴露组; 而二者共同暴露时, GO能减轻BPA对斑马鱼氧化损伤。

2.5 复合暴露致毒机理分析

上述研究结果说明, 在斑马鱼早期发育阶段过程中, GO和BPA对斑马鱼胚胎体内活性氧的产生、氧化损伤及孵化率等存在拮抗效应。GO不仅会附着在胚胎绒毛膜或者细胞膜上, 阻止BPA通过胚胎绒毛主动吸收进入胚胎。而且GO对BPA具有一定的吸附作用^[34], 可能降低暴露溶液中自由态BPA的浓度, 也在一定程度上降低了BPA在胚胎中的富集, 从而缓解了由于BPA主导造成的氧化损伤。已有相关研究表明, 多壁碳纳米管(MCNWs)可以降低全氟辛烷磺酸(PFOS)对斑马鱼早期阶段氧化应激作用^[35]。富勒烯(C60)吸附17 α -乙炔基

图7 ROS与MDA之间的相关性

Figure 7 Correlation between ROS and MDA

雌二醇(EE2)后降低了EE2的生物可利用性, 缓解了EE2对斑马鱼的毒性^[36]。这些研究结果表明由于GO与有机物之间相互作用, 在一定程度上都有可能降低有机污染物对斑马鱼发育早期的毒性。

3 结论

BPA单独存在时, 会诱导斑马鱼胚胎产生活性氧ROS, 导致机体脂质过氧化、孵化提前等毒害效应。高浓度的GO组也会诱导斑马鱼胚胎产生少量活性氧物质和MDA。当二者共同作用时, GO和BPA对斑马鱼早期活性氧的产生及氧化酶活性均产生拮抗效应, 表现为GO缓解了BPA所导致的氧化损伤效应。这可能是由于包覆在绒毛膜表面的GO在一定程度上抑制了BPA的主动吸收, 同时GO通过吸附降低溶液中自由态BPA, 导致BPA在斑马鱼胚胎中富集量降低, 从而缓解了由BPA所主导的氧化损伤效应。研究结果为科学评估GO纳米材料的环境行为和环境风险提供理论依据。

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Summary for “氧化石墨烯和双酚A对斑马鱼早期发育氧化损伤的联合效应”

The combined effects of graphene oxide and bisphenol A on oxidative damage in early development of zebrafish

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Bisphenol A (BPA) is widely present in natural surface water, and was found to be at the high level of $\mu\text{g/L}$ in the surface water around a BPA manufacturing plant. Mass production and application make BPA ubiquitous in the environment, biota and humans. As a two-dimensional carbon based nanomaterial, graphene oxide (GO) is used in many fields, and there is a great potential for GO to discharge to aquatic environment via various pathways. Thus GO has the potential to interact with organic pollutants, such as BPA, copresent in water system. GO could adsorb organic pollutants in water, and adhere on the surface of organisms, displaying different effects on the behaviors and toxicities of organic pollutants in organisms at different developmental stages. But the intrinsic mechanisms for the different effects remain unclear. To fill this gap, zebrafish (*Danio rerio*) was selected as a vertebrate model to investigate the toxicities of a variety of pollutants and nanomaterials in this study. Embryogenesis is an important growth phase in the zebrafish life cycle. Pollutants can induce excessive reactive oxygen species (ROS) and generate a large number of oxidative intermediate products by destroying the balance between oxidation and antioxidation, thus causing oxidative damage. Superoxide dismutase (SOD) can catalyze the oxidation of superoxide anion (O_2^-) to O_2 and H_2O , in an attempt to maintain ROS at a physiological level and protect the body from damage. Catalase (CAT) exists primarily within peroxisomes and removes the H_2O_2 generated by the long-chain fatty acid β -oxidation pathway. Excessive ROS can attack the polyunsaturated fatty acids in the biomembrane system, resulting in lipid peroxidation. Malondialdehyde (MDA) is the product of irreversible lipid peroxidation, and the change of MDA can reflect the level and damage degree of lipid peroxidation in organisms. Glutathione (GSH), as an important non-enzymatic antioxidant in organism, can be induced and effectively scavenged by external stimulation to regulate oxidative stress. Toxic effects occur in organisms when GSH is gradually reduced. This study focused on the joint effects of GO and BPA on the oxidative responses in zebrafish at early development stage. Zebrafish embryos were individually exposed to BPA (0, 50, 500 $\mu\text{g/L}$), GO (0, 0.1, 1 mg/L), BPA+GO (50 $\mu\text{g/L}$ +0.1 mg/L , 500 $\mu\text{g/L}$ +0.1 mg/L , and 50 $\mu\text{g/L}$ +1 mg/L , 500 $\mu\text{g/L}$ +1 mg/L) solutions for 7 d. The levels of SOD, MDA, GSH, CAT, and ROS in the exposed zebrafish larvae were determined. The results indicated that BPA displayed strong oxidative damage effects on zebrafish larvae. However, the presence of GO distinctly alleviated the various indicators of oxidative damages caused by BPA. Based on analyses using the Bliss independence model, GO and BPA exhibited antagonistic effects on the oxidative damage of zebrafish in the early development stage, which indicated that GO could reduce the oxidative damage toxicities of BPA on zebrafish embryos under the experimental conditions. These could be due to the formation of a GO layer on the zebrafish embryos, which depressed the absorption of BPA by zebrafish, leading to reduced BPA accumulation in zebrafish in early developmental stage.

bisphenol A (BPA), graphene oxide (GO), oxidative damages, joint effects, Bliss independence model

doi: [10.1360/N972019-00340](https://doi.org/10.1360/N972019-00340)