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FORMATION OF REACTIVE OXYGEN SPECIES IN CELLULAR MEDIA

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A potential complication of studies concerning the production of free radicals and other reactive oxygen species (ROS) by cells is the formation of ROS by media used for cell culture. One source of ROS formation are photoreactions involving riboflavin, present in all cellular media, augmented by some component of the media like tryptophan, tyrosine and HEPES. Another source of ROS are reactions of autoxidation of polyphenols, thiols and other compounds.

INTRODUCTION

When studying the generation of free radicals by xenobiotics in cultured cells, we found with some surprise that hydralazine, a hypotensive drug, and some other xenobiotics produce significant amount of free radicals and other reactive oxygen species (ROS) also in the media used for cell culture, in the absence of cells (Weglarz & Bartosz, 1991). More recently, generation of large amounts of hydrogen peroxide upon interaction of considerable amounts (micromolar concentrations) of hydrogen peroxide in Dulbecco's modified Eagle medium (DMEM) and RPMI 1640 cell culture media added with plant polyphenols (quercetin, gallic acid, epigallocatechin gallate and epigallocatechin) to cellular media has been reported (Long, Clement & Halliwell, 2000). A similar phenomenon has been observed in MEM, DMEM and RPMI media upon addition of thiol compounds (cysteine, N-acetylcysteine, γ-glutamylcysteine, cysteinylglycine, cysteamine, homocysteine, glutathione and ascorbate, due to interaction of these compounds with various components of the media and autoxidation (Hua, Long & Halliwell, 2001). Generation of ROS by autoxidation of antioxidants in cellular media may complicate their effects on cells, including toxicity of ascorbate (>0.3 mM) for 3T6 fibroblasts, attenuated by catalase (Arakawa, Nemoto, Suzuki & Otsuka, 1994). Interestingly, CuZn-superoxide dismutase, showing an activity of thiol oxidase (Winterbourn, Peskin & Parsons-Mair, 2002), can catalyze rather than inhibit this prooxidative effects of antioxidants. Other antioxidants may also show similar effects, including butylated hydroxytoluene which produces superoxide upon autoxidation in aqueous solutions (Smirnova, Lyubimov, Malinina, Lyubimova, Alexandrushkina, Vanyushin, Kolesova & Yaguzhinsky, 2002). Using spin trapping, we confirmed the production of superoxide during interaction of quercetin, menadione and BHT with cellular media used for culture of mammalian cells (DMEM and RPMI) and YPD (yeast extract-peptone-glucose) medium used for yeast culture (in preparation). Superoxide and hydrogen peroxide were found to be formed upon autoxidation of broth media autoclaved under anaerobic conditions and then exposed to atmospheric oxygen (Carlsson, Nyberg & Wrethen, 1978).

We revealed an apparently spontaneous formation of ROS in DMEM, RPMI-and YPD cell culture media. In the media used for mammalian cell culture, the effect was much more pronounced in the absence of serum which attenuated it significantly. Results of the assays, based on the EPR detection of superoxide formation with 5,5,-dimethyl-1-pyrrolidine-N-oxide (DMPO) and oxidation of fluorogenic probes, 2',7'-dichlorofluorescin diacetate and dihydrorhodamine 123, were quite irreproducible suggesting the critical involvement of another factor. Light turned out to be this factor; very little ROS formation took place in the darkness. Phenol Red present in the media was not responsible for the photoreaction since ROS formation in Phenol Red-free medium was not lower (Fig. 1). Checking various components of the media we found that riboflavin was the component responsible for the light-dependent generation of ROS in the media (Grzelak, Rychlik &

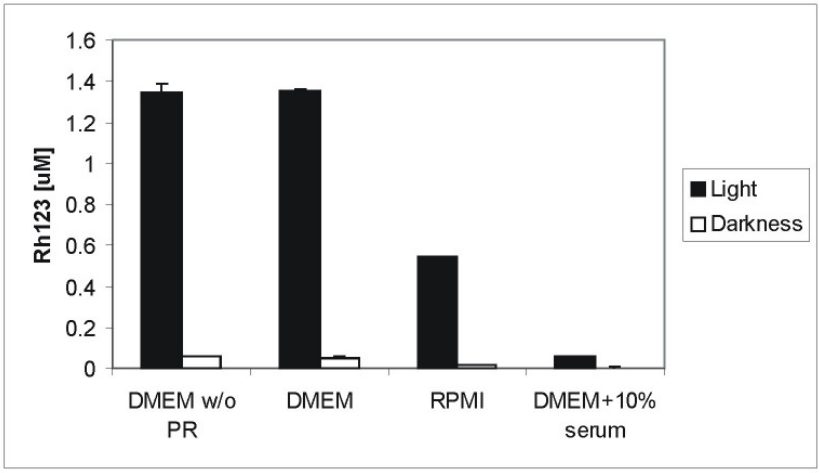

Fig. 1. Oxidation of dihydrorhodamine 123 in cell culture media exposed for 1 h to standard illumination of a Herasafe HS12 laminar flow cabinet (Heraeus' ≥ 800 lux) or incubated in the darkness, at room temperature, for 1 h. PR, Phenol Red.

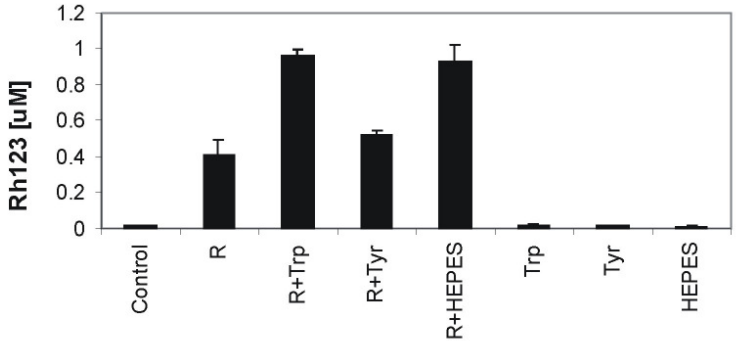

Fig. 2. Oxidation of dihydrorhodamine 123 in phosphate-buffered saline supplemented with riboflavin (R; 1.1 µmol dm^{-3}), tryptophan (Trp; 78 µmol dm^{-3}), tyrosine (Tyr; 397 µmol dm^{-3}) and HEPES (20 mmol dm^{-3}). The solutions were illuminated in a Herasafe HS12 laminar flow cabinet for 1 h at room temperature.

Bartosz, 2001). However, several components of the media potentiated the effect of riboflavin, including tryptophan, tyrosine and HEPES (Fig. 2).

Similar results were reported by Mahns et al. who studied the effect of UVA on cell culture media. These authors demonstrated that irradiation of DMEM medium with UVA (30 J/cm^2) generated 58-357 µM H_2O_2 (depending on the source and composition of the medium) and that the illuminated medium when added to the cells, was able to induce stress response involving the ERK1/2 pathway (Mahns, Melchheier, Suschek, Sies & Klotz, 2003).

Riboflavin is a necessary component of cell culture media, its concentration ranging from 0.0038 mg dm^{-3} in MCDB 131Medium up to 1 mg dm^{-3} in Waymouth's media. Photochemical generation of ROS by this compound may affect especially cell cytotoxicity assays (Granzow, Kopun & Krober, 1995) in a complex manner: decreasing cell survival on one hand, and affecting the compounds used for the assays on the other hand. E. g., riboflavin mediates photo-oxidation of adriamycin leading to its decreased cytotoxicity in cellualr media (Ramu, Mehta, Liu, Turyan & Aleksic, 2000).

The formation of ROS in media used for cell culture is hard to avoid. It has been estimated that ROS coming from the medium may constitute the main flux of ROS for bacterial cells cultured under aerobic conditions (Imlay, 2003). They may also contribute to the oxidative stress posed on cells by the in vitro cell culture conditions (Halliwell, 2003).

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