Acta Biochimica Polonica

Vol. 47 No. 4/2000

1197–1198

QUARTERLY

*Letter to the Editor*

# Reactive oxygen species are formed in cell culture media

Agnieszka Grzelak, Błażej Rychlik and Grzegorz Bartosz✉

*Department of Molecular Biophysics, University of Łódź, St. Banacha 12/16, 90-237 Łódź, Poland*

Received: 23 August, 2000

When studying the effects of oxidative stress on mammalian and yeast cells, we observed that the media without cells generated reactive oxygen species. Mammalian cell media such as RPMI 1640 medium with Glutamax-I (GibcoBRL, Cat. No. 31996) and Dulbecco's Modified Eagle Medium with Glutamax-I, sodium pyruvate and pyridoxine (GibcoBRL, Cat. No. 31966; DMEM), as well as the yeast extract-peptone-glucose medium containing 1% yeast extract, 1% peptone and 2% glucose were found to oxidize 5 $\mu$M dichlorodihydrofluorescein diacetate ($H_2$DCFDA) and dihydrorhodamine 123 ($H_2$R123), and to generate an $^{\cdot}$OH-type adduct of 5,5′-dimethyl-1-pyrroline *N*-oxide (DMPO) under aerobic conditions. Spin trapping demonstrated formation of an $^{\cdot}$OH-type adduct. Electron spin resonance spectra originated from decomposition of $O_2^{-\cdot}$ adducts of DMPO rather than from trapping of $^{\cdot}$OH, since their intensity was significantly reduced in the presence of 50 $\mu$g/ml superoxide dismutase.

The rate of oxidation of $H_2$DCFDA and DHR123 as well as of DMPO adduct formation was higher for DMEM medium than for RPMI 1640 medium and was significantly attenuated in complete media containing 10% fetal calf serum (FCS). Horseradish peroxidase (HRP) considerably potentiated the rate of $H_2$DCFDA and $H_2$R123 oxidation, indicating that hydrogen peroxide was the main agent responsible for the oxidation of these fluorogens.

---

★Presented at the 5th Symposium on "Free Radicals in Biology and Medicine", Łódź, Poland, 2000.

✪This study was supported by grant No. 4P05E06215 by the State Committee for Scientific Research (KBN, Poland).

✉Fax (48 42) 635 4476; e-mail: gbartosz@biol.uni.lodz.pl

Reactive oxygen species (ROS) formation was light-dependent as it was much lower when the media were protected against light. $H_2R123$ oxidation in the presence of 1 U/ml HRP in DMEM was 1.35 ± 0.01 nM/h in a typical Herasafe HS12 (Heraeus) laminar flow cabinet illuminated at ≥ 800 lux and only 0.06 ± 0.004 nM/h in light-protected medium (mean ± S.D. for 3 experiments). In the presence of 10% FCS the oxidation rate was reduced down to 0.054 ± 0.006 and 0.004 nM/h when exposed to daylight and in the darkness, respectively. Under similar conditions, $H_2R123$ oxidation in the RPMI medium was lower (0.54 ± 0.03 and 0.016 ± 0.02 nM/h, respectively) and was less reduced by the presence of 10% FCS (down to 0.35 ± 0.002 and 0.018 nM/h, respectively). ROS formation was not changed significantly in DMEM devoid of Phenol Red (1.35 ± 0.005 and 0.06 ± 0.004 nM/h, respectively).

Measurements of $H_2R123$ oxidation in phosphate-buffered saline supplemented with various components of cell culture media demonstrated that riboflavin is the component responsible for the generation of ROS in the media. Additionally, tryptophan, tyrosine, folate and pyridoxal phosphate augmented the oxidation of the probe in the presence of riboflavin but were unable to so it in the absence of riboflavin.

These observations confirm previous data on the generation of superoxide and hydrogen peroxide in microbiological media exposed to air [1], on the interference of riboflavin in the cytotoxicity assays [2] and on the unfavourable effects of this compound on cultured neurons [3]. More importantly, they point to the possibility that cultured cells may be exposed to photoinduced oxidative stress during every manipulation with the cultures. This stress is unavoidable, since riboflavin is an obligatory component of cell culture media but can be controlled and limited, and should be taken into account when interpreting experiments with cells cultured *in vitro*.

## REFERENCES

**1.** Carlsson, J., Nyberg, G. & Wrethen, J. (1978) *Appl. Environ. Microbiol.* **36**, 223–229.

**2.** Granzow, C., Kopun, M. & Krober, T. (1995) *Cancer Res.* **55**, 4837–4843.

**3.** Lucius, R., Mentlein, R. & Sievers, J. (1998) *Free Radical Biol. Med.* **24**, 798–808.