EFFECT OF COPPER ON CYTOCHROME P450 1A-CATALYSED XENOBIOTIC BIOTRANSFORMATION AND BARRIER PROPERTIES IN RAINBOW TROUT GILL CELLS

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EXTENDED ABSTRACT ONLY - DO NOT CITE

The gill is a site for uptake and toxic action of waterborne contaminants, including metals and organic pollutants. Metals, such as silver, cadmium and copper, disrupt ion regulation and cause histological damage, whilst dioxins, polychlorinated biphenyls (PCBs) and polychlorinated aromatic hydrocarbons (PAHs) induce cytochrome P4501A (CYP1A). Polluted bodies of water often contain a mixture of different chemicals, many of which are mutually interactive. For example, metals can reduce the CYP1A-activity, as is the case with copper in bass (*Dicenrarchus labrax*) liver and hepatocyte microsomes (Stien et al., 1997; Viarengo et al., 1997).

In this study, copper effects on the CYP1A-catalysed reaction, *7*-ethoxyresorufin *O*-deethylation, (EROD), were examined in rainbow trout (*Onchorhyncus mykiss*) gills *in vitro*. Gill cells were cultured as polarised tight

epithelia on permeable supports; *i.e.* with apical and basolateral compartments (Wood and Pärt, 1997). These cultures, which 'tolerate' water at the apical membrane (Wood and Pärt, 1997), were exposed (24 hours) to aqueous copper and β-naphthoflavone (BNF; to induce CYP1A), via the apical the basolateral compartments, respectively. This cell model was also compared with conventional gill cell monolayers, grown in 12-well tissue culture plates (*i.e.* contacting culture medium with only one side). Monolayers were exposed for 24 hours to copper and BNF in the medium.

EROD-activity was determined (in intact adherent cells) according to Carlsson et al. (1999); *i.e.* water and media were replaced with 1 μM *7*-ethoxyresorufin and, after 20 minutes, resorufin concentrations were determined by fluorescence (at 544 nm ex and 590 nm em). In polarised gill epithelia resorufin is selectively distributed to the basolateral compartment (Carlsson and Pärt, submitted), and therefore apical and basolateral resorufin concentrations were measured separately. EROD-activity was calculated from the total resorufin concentration.

In polarised epithelia, the effects of copper on barrier properties (conductivity, Na^+-efflux and polyethylene glycol (PEG)-permeability) were also investigated. Conductivity was obtained from the inverted transepithelial electrical resistance, measured with an epithelial voltohmmeter, connected to an Ag^+/AgCl 'chop-stick' electrode. Na^+-efflux is both transcellular and paracellular, whereas PEG-permeability is a paracellular marker (used to evaluate tight junction integrity). Na^+-efflux and PEG-permeability were measured after addition of $^{22}Na^+$ and ^{3}H-PEG-4000 to the basolateral medium.

The exposure groups (within a culture type) and apical / basolateral resorufin distribution (in polarised epithelia) were statistically compared using two-tailed and one-tailed Wilcoxon matched pairs tests, respectively. Significant differences are indicated in the tables by superscript letters ($p<0.05$) and stars (* = $p<0.05$ and ** = $p<0.01$ and *** $p < 0.001$).

EROD-activity in monolayers was reduced by exposure to 5–1000 μM copper, whilst EROD-activity in polarised epithelia was unaffected by copper exposure (0.5–100 μM; Table 1). However, the selective basolateral resorufin distribution levelled out following exposure to 75–100 μM copper (Table 1).

Table 1. Copper effects on EROD-activity in rainbow trout gill cell cultures

Copper	Pmoles resorufin mg protein^{-1} min^{-1}					
	Cultured epthelia				Monolayers	
	EROD-activity[1]		Basolateral-apical difference[2]		EROD-activity[1]	
µM	Median	Range	Median	Range	Median	Range
0	27^{A}	9–43	5*	(-11)–23	49^{a}	15–77
0.5	23^{A}	14–43	8*	(-1)–13		
1	17^{A}	9–41	7**	0–12	49^{a}	12–77
5	18^{A}	8–49	6**	(-1)–12	41^{b}	3–62
10	28^{A}	9–46	8**	(-6)–18	35^{b}	6–59
25	33^{A}	9–53	7*	(-9)–22		
50	31^{A}	10–53	7*	(-12)–12	35^{cd}	4–53
75	28^{A}	15–50	2	(-18)–15		
100	18^{A}	10–41	3	(-11)–12	40^{bc}	6–57
500					30^{d}	4–48
1000					7^{e}	2–35
0 (-BNF)	1^{B}	0–3	0	0–3	8^{e}	1–26

[1] Significant differences are indicated by superscript letters, [2] or by stars.

PEG-permeability and Na^+-efflux increased following exposure to 75 µM copper (to 140 and 127 % of control, respectively), whilst conductivity was unaffected by 25 and 75 µM copper (Table 2).

In monolayers EROD-activity was considerably more sensitive to copper than in polarised epithelia, but the reason for this is unknown. The low susceptibility of polarised epithelia, suggests that their capability to metabolise organic pollutants is retained during exposure to copper. However, the results indicate that changes of the barrier properties have occurred. We interpret the break-down of the apical / basolateral resorufin distribution pattern as being due to increases of epithelial permeability. This was supported by that Na^+-efflux increased following exposure to 75 µM copper. The effect on PEG-permeability indicates more specifically that the tight junctions were targeted.

Table 2. Copper effects on conductivity, PEG-permeability and Na^+-efflux in polarised gill epithelia.

Effect parameter	N	copper	Relative values % of control	
		μM	Median[1]	Range
Na^+-efflux	14	0	100	
		25	103	35–162
		75	140*	75–269
PEG-permeability	10	0	100	
		25	113	68–255
		75	127***	105–340
Conductivity	11	0	100	
		25	100	<43[2]–235
		75	122	43–299

[1] Significant differences are indicated by stars. [2] Beyond the epithelial volt-ohm-meter limit.

The gill has been suggested to function as a first-pass defence to organic pollutants (dioxins, PCBs and PAHs), by reducing their bioavailability (*i.e.* lipophilicity) before they enter the systemic circulation (Levine and Oris, 1999; Carlsson et al., 1999). The present findings also support the idea that since the outermost branchial cell layer is intimately exposed to any hazardous physico-chemical variations in the surrounding water, these cells would need to have a high resistance to toxic action. Injury of the respiratory cells would reduce their plasticity and impair their ability to maintain barrier integrity and thus be an acute danger for the animal.

In conclusion, the results of this study suggest that both the CYP1A enzyme activity and the barrier properties of intact polarised gill epithelia have a low susceptibility to aqueous copper exposure. This could have implications for the tolerance of sub-lethal waterborne pollutants *in vivo*.

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