Tissue-specific TfR Expression in Organs of Immature Mice after Chronic Exposure to $CoCl_2$

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Abstract

Cobalt (Co) has significantly increased its concentration in the environment in the last years due to anthropogenic and industrial activities. The present study was designed to elucidate the effect of chronic cobalt chloride ($CoCl_2$) exposure on transferrin receptor 1 (TfR1) in various organs of immature mice. Pregnant ICR mice were subjected to daily dose of 75 mg/kg body weight $CoCl_2x6H_2O$ 2–3 days before they gave birth and treatment continued until day 25 after delivery. The compound was dissolved and administrated with drinking tap water. Age-matched mice obtaining regular tap water were used as a control group. On days 18 and 25 pups were sacrificed, blood plasma, livers, kidneys and spleens were obtained and processed for analysis. The results show altered tissue-specific TfR expression in the studied organs of $CoCl_2$-treated immature mice. A significant time-dependent increase in tissue Co levels was observed. Our data on the effects of chronic $CoCl_2$ exposure on TfR expression contribute for the elucidation of the complex regulatory mechanism of iron homeostasis.

Keywords: cobalt chloride, *in vivo* model, transferrin receptor 1, tissue specific expression, iron homeostasis

1. Introduction

Cell surface TfR expression and concentration reflect iron requirements of the cells and may be a useful marker for quantitative evaluation of erythropoiesis and iron deficiency [1]. During the process of recycling of transferrin receptors, parts of the extracellular domain of TfR are "shed" in the blood stream and appear as soluble transferrin receptors (sTfR). Measurement of sTfR is a marker of iron metabolism that reflects body iron stores and total erythropoiesis [3]. Rapidly proliferating cells express high levels of TfR1 due increased iron demands [2]. Hypoxia plays a key regulatory role in TfR1 expression through the induction of hypoxia inducible factors which bind to the hypoxia response elements located in the promoter of TfR gene [4]. Cobalt chloride ($CoCl_2$) is one of the most commonly used agents in experimental models for inducing chemical hypoxia [5]. The aim of the present study is to elucidate the effect of perinatal $CoCl_2$ exposure on transferrin receptor 1 (TfR1) in various organs of immature mice.

2. Material and Methods

Pregnant ICR mice were subjected to daily dose of 75 mg/kg body weight $CoCl_2x6H_2O$ 2–3 days before they gave birth and treatment continued until day 25 after delivery. The compound was dissolved and administrated with drinking tap water. Age-matched mice obtaining regular tap water were used as a control group. On days 18 and 25 pups were sacrificed. Blood plasma was collected and livers, kidneys and spleens were excised and processed by routine immunohistochemical (IHC) technique for TfR1 expression. Also, blood plasma and tissue homogenates were analysed using mouse TFR1 ELISA kit (Elabscience Biotechnology Co., Ltd, China). Assessment of Co levels in biosamples was performed using inductively-coupled plasma mass-spectrometry (ICP-MS) at NexION 300D (Perkin-Elmer, USA) after microwave digesion of samples in HNO_3 using SW-4 (Berghof, Germany). Data were processed using Statistica 10 (Statsoft, USA). Mean ± SD or Median (IQR) were used as descriptive statistics with Mann-Whitney U-test for paired group comparisons.

3. Results and Discussion

Perinatal exposure to cobalt chloride of day 18 and 25 mice resulted in a significant time-dependent increase in plasma, liver, spleen, and kidney Co levels by a factor of 149, 41, 4, 68 and 214, 253, 35, 101, respectively ($p< 0.001$) (Table 1). Co distribution in tissues generally corresponds to data on Co kinetics in the organism [6]

Table 1. Sample Co levels (μg/g) in $CoCl_2$-exposed mice

Sample	Control (n=5)	Co-treated (n=7)
Day 18		
Serum	0.002 (0.002-0.004)	0.28 (0.27-0.29) *
Liver	0.024 (0.008-0.328)	0.99 (0.93-1.24) *
Kidney	0.011 (0.007-0.053)	0.75 (0.64-0.91) *
Spleen	0.077 (0.070-0.123)	0.30 (0.25-0.46) *
Day 25		
Serum	0.003 (0.003-0.008)	0.56 (0.52-0.70) *
Liver	0.024 (0.012-0.053)	6.08 (5.29-7.67) *
Kidney	0.013 (0.011-0.02)	1.31 (0.20-1.58)*
Spleen	0.019 (0.005-0.087)	0.66 (0.52-0.80) *

Data represent Median (IQR); * $p < 0.001$

IHC studies showed that TfR1 was well expressed in the target organs – liver, kidneys and spleen of both control and Co-exposed day 18 (Fig. 1) and day 25 mice. The perinatal $CoCl_2$ exposure of day 18 mice induced a significant 10-fold increase in plasma TfR1 (Table 2) which is common in cases of use of erythropoietic agents [7]. A 33% reduction though was observed in day 25 Co-exposed mice (Table 3). Co-exposure decreased TfR1 content in the liver and kidneys and increased it in the spleens of day 18 mice (Table 2). As seen from Table 1 liver and kidneys accumulate more Co than the spleen. We suggest that organs with initially high iron content are less sensitive to Co accumulation. The reduced TfR1 expression in day 18 livers is also possibly due Co-enhanced hepcidin production. In contrast, on day 25 TfR1 content was reduced in the spleen which is in agreement with the higher Co content after longer treatment.

The results suggest tissue and time-dependent TfR1 expression. The observed changes also suggest alterations in iron accumulation and distribution as TfR1 regulates cellular iron up-take.

Table 2. Alterations in TfR1 tissue content (ng/mL) in day 18 mice after $CoCl_2$ exposure

Sample	Control (n=5)	Co-treated (n=7)	Increase/Decrease
plasma	0.5±0.28	5.38±3.46	10-fold ↑**
liver	6.12±0.32	4.67±1.09	23 % ↓
kidney	2.21±0.62	2.03±0.55	8 % ↓
spleen	0.32±0.06	0.55±0.48	1.7-fold ↑

Data represent as mean ± SD. ** $p<0.01$

Table 3. Alterations in TfR1 tissue content (ng/mL) in day 25 mice after $CoCl_2$ exposure

Sample	Control (n=5)	Co-treated (n=4)	Increase/Decrease
plasma	4.64±1.97	3.11±1.19	33 % ↓
liver	5.20±0.33	5.33±0.43	15 % ↑
kidney	2.35±0.06	2.03±0.89	2 % ↓
spleen	0.33±0.03	0.28±0.03	16 % ↓

Data represent as mean ± SD.

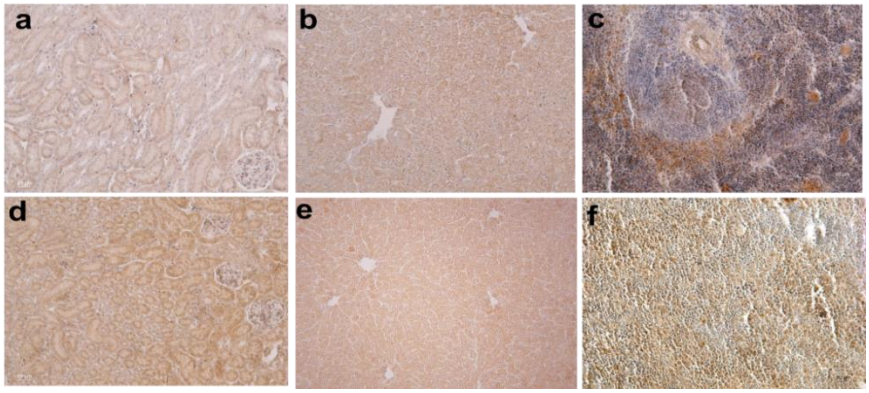

Figure 1. TfR1 expression (brown staining) in control kidneys (a), liver (b) and spleen (c) and $CoCl_2$-exposed kidneys (e), liver (f) and spleen (g) of day 18 mice. x 200.

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References

Gluhcheva Y. (2016), Transferrin receptors and hematopoiesis, *Acta morpholollica et anthropologica*, **23**,140-144.

Kawabata H. (2019), Transferrin and transferrin receptors update, *Free Radical Biology and Medicine*, **133**, 46-54.

Kohgo Y., Torimoto Y., Kato J. (2002), Transferrin receptor in tissue and serum: updated clinical significance of soluble receptor, International Journal of Hematology, **76**, 213-218.

Lok C.N., Ponka P., (1999), Identification of a hypoxia response element in the transferrin receptor gene, Journal of Biological Chemistry, **274**, 24147–24152.

Muñoz-Sánchez J. and Chánez-Cárdenas M.E. (2018), The use of cobalt chloride as a chemical hypoxia model, Journal of Applied Toxicology doi: 10.1002/jat.3749

Skalny A.V., Zaitseva I.P., Gluhcheva Y.G., Skalny A.A., Achkasov E.E., Skalnaya M.G., Tinkov A.A. (2019) Cobalt in athletes: hypoxia and doping - new crossroads, Journal of Applied Biomedicine, **17**, 28. doi: 10.32725/jab.2018.003.

Skikne B.S. (2008), Serum transferring receptor, *American Journal of Hematology*, **83**, 872-875.