Functional changes of mice Sertoli cells induced by Cr(V)

M. de Lourdes Pereira¹, T.M. Santos², F. Garcia e Costa³ and J. Pedrosa de Jesus²
¹Department of Biology; ²Department of Chemistry, CICECO, University of Aveiro, Aveiro, Portugal; ³Department of Morphology and Clinic, Pathology Section, Faculty of Veterinary Medicine, Technical University of Lisbon, Portugal

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Abstract

Transport of macromolecules from the interstitial testis tissue to cells at the adlumenal compartment of the seminiferous epithelium occurs naturally through Sertoli cells. In previous studies we have shown that Cr(V) intoxication disturbed spermatogenesis in mice. To test if Sertoli cells are affected by chromium, a well proved carcinogen, the uptake and the horseradish peroxidase transport ability of isolated seminiferous tubules of mice administered with a chromium(V) compound, have been studied. Male CD-R mice were exposed daily for 5 days to [Cr⁵⁺-BT]²⁻ through subcutaneous injection and comparisons were made with groups of vehicle-treated mice. Using an in vitro assay we demonstrated that the seminiferous tubules were able to uptake and transport the tracer, in a much faster way than controls, mainly via intercellular and transeellular pathways, providing evidence that this functional role of Sertoli cells is affected by the Cr(V) compound. These findings might improve the knowledge on the toxicity mechanisms of chromium.

Introduction

The increasing concern with hazardous chemicals within the environment and at the workplace points to their potential harmful effects on public health.

Chromium is a long-listed potent industrial and environmental carcinogen, mainly as Cr(VI) and Cr(V) (IARC, 1990; Cross, 1997; Pellerin, 2000; Levina, 2001, 2003), but is one of the most widely used elements in the workplace. Molecular damage caused by chromium has been attributed to the intracellular metabolism of Cr(VI) that ultimately leads to Cr-induced cancers. It is well known that from Cr(VI) reduction highly reactive species of different types are formed, such as intermediate species of Cr(V) and Cr(IV) (O’Brien, 1995; Dayan, 2001) and hydroxyl radicals (Lloyd, 1997; Liu, 2001). Nevertheless, the underlying mechanisms of toxicity of chromium are not totally understood and still under intense research (Levina, 2003). Concerning the present work, the in vivo intoxication studies with a Cr(V) compound may contribute to the understanding of the role of each species formed along the Cr(VI) reduction processes (Vasant, 2001; Codd, 2001; Levina, 2001, 2003; Dillon, 2002).

There is a small number of stable Cr(V) compounds under physiological experimental conditions, but the in situ prepared [Cr⁵⁺-BT]²⁻ (Fonkeng, 1998) has appeared to be adequate for in vivo studies (Pires das Neves, 2001, 2002; Pereira, 2002) with the additional fact that BT ligand is a very common biological buffer.
Several ongoing studies in our laboratory that address the target organs associated with Cr(V) toxicity have demonstrated that the liver was severely affected (Pires das Neves, 2002). These changes, characterised at the light microscopy level, included a disturbed lobular architecture and vacuolation of hepatocytes. This compound has also been described to be harmful to the testis (Pereira et al., 2002). In this paper the alterations of the integrity of the blood–testis barrier was the main mechanism proposed for Cr(V)-induced testis toxicity. In the study of the testis lesions produced by Cr(V), special attention was paid to the Sertoli cells owing to their key role during spermatogenesis, bearing in mind that this type of compound may negatively impact male fertility (Giwerzman, 1998; Li, 2001).

Sertoli cells, a fixed population of supporting columnar cells in the germinal epithelium, provide, select, and transport the required molecules for the development of spermatocytes and spermatids situated at the adluminal compartment of the seminiferous tubules. The presence of tight junctions between these somatic cells is the key for their selective barrier function. Macromolecules must pass through the Sertoli cell cytoplasm in order to access the spermatocytes and spermatids located at the adluminal compartment. Functional properties of Sertoli cells have been widely reported (for reviews see Carreau, 1994; Griswold, 1998; Grotegoed, 2000; Nakamichi and Shiratsuchi, 2004). Due to its critical role in supporting spermatogenesis, the Sertoli cell is one of the most common target cells for toxicity (Boekelheide, 2000; Monsees, 2000; Creasy, 2001). As a consequence, the dependent germ cells are also affected.

The goal of this work was to investigate if the functional role of the Sertoli cells in macro-molecular transport is affected in Cr(V)-injected mice. For this purpose we evaluated the capacity of Sertoli cells in the uptake and transport of horseradish peroxidase, using isolated seminiferous tubules cultured in vitro.

Materials and Methods

Chemicals and solutions

Horseradish peroxidase (HRP-type II, molecular weight 40 000) and 3,3’-diaminobenzidine (DAB) were obtained from Sigma-Aldrich (Spain) and Eagle’s medium from Biomérieux (France). Fetal calf serum was purchased from Gibco (USA).

Potassium chromate, K₂CrO₄, 2-ethyl-2-hydroxybutanoic acid (H₂ehba) and bis(hydroxyethyl)-aminotris(hydroxymethyl) methane (BT) buffer were purchased from Sigma-Aldrich (Spain) and used without any further purification. BT/buffer solutions (pH 7.4) were prepared adding the appropriate amounts of hydrochloric acid to the BT aqueous solutions.

[Cr⁶⁺-BT]²⁻, the Cr(V) complex, was prepared in situ from the precursor Na[Cr⁶⁺(ehba)₂] as previously described (Pires das Neves, 2002).

Animals and treatment

Male CD-R mice from Harlan Interfauna Iberica, Barcelone (Spain), aged two months and weighing 30 g, were used after one week of quarantine and acclimatisation under standard animal housing for small rodents (24°C; 50 ± 5% relative humidity; 12:12 h light–dark cycle), and provided with diet and water ad libitum. Two groups of animals were used (n = 10). One group was daily injected subcutaneously with 0.5 ml of [Cr⁶⁺-BT]²⁻ (8 μmol Cr/mouse), during 5 days. The other, used as control, was similarly dosed with BT buffer solution for an equal period of time. On the 6th day all the animals were sacrificed and testes were removed from the scrotum.