Experimental research into the pathogenesis of cobalt/hard metal lung disease

D. Lison*, R. Lauwerys*, M. Demedts**, B. Nemery**

ABSTRACT: In recent years clinical, epidemiological and experimental evidence has accumulated indicating that cobalt metal particles, when inhaled in association with other agents such as metallic carbides (hard metals) or diamond dust, may produce an interstitial lung disease termed "hard metal disease" or "cobalt lung". This article summarizes the progress accomplished in our two laboratories to understand the pathogenesis of this disease. Gaps and weaknesses in our current knowledge have also been highlighted in order to suggest potential avenues for further research. Whilst animal models have proved useful for the demonstration of the toxic synergy between cobalt and carbides (e.g., tungsten carbide), most animal models have remained descriptive and have not provided information on the mechanism for this synergy. In particular, the bizarre multinucleated giant cells which are an important hallmark of the human disease, have not been reproduced consistently in experimental animals. Since cobalt is a known sensitizer, there may also be a need to develop experimental models to test the possible involvement of immunological mechanisms in the pathogenesis of the interstitial disease. In vitro systems including macrophage cell cultures and physico-chemical tests have been useful to investigate the mechanism underlying the toxic synergy.

The recent finding that, in vitro, cobalt and metallic carbides interact with oxygen to produce toxic activated oxygen species opens a new avenue of research and may offer an alternative interpretation of the fact that only a limited proportion of exposed workers develop interstitial disease. Besides the possible involvement of immunological mechanisms, it may be speculated that individuals with a lower antioxidant defence are more susceptible to the toxic effect of activated oxygen species produced by cobalt-containing dusts from hard metal.

In the past few years, our two laboratories have, more or less independently, carried out experimental research into the pathogenesis of hard metal lung disease. Why should two Belgian teams be particularly interested in what is, after all, a relatively rare condition? The origin of the interest of the K.U. Leuven team goes back to their long association with other agents such as metallic carbides (hard metals) or diamond dust, may produce an interstitial lung disease termed "hard metal disease" or "cobalt lung". The interest of the UCL team goes back to their long tradition of research into the toxicity of various metals, which itself is possibly a reflection of Belgium's prominent position in the nonferrous metal industry. In this "Point of View", we will summarize our present knowledge, as well as the questions that are still unanswered, regarding the pathophysiology of hard metal lung disease, with an obvious emphasis on the possible mechanisms for the pulmonary toxicity of cobalt/hard metals.

The inhalation of excessive amounts of cobalt-containing dust may produce various respiratory manifestations including upper respiratory tract irritation, asthma and a peculiar form of interstitial lung disease, originally called hard metal pneumoconiosis [4–6]. This interstitial lung disease, the clinical presentation of which may vary from subacute alveolitis to progressive fibrosis, is characterized, particularly in the early stages, by the presence of numerous "bizarre" multinucleated giant cells in the lung interstitium and the alveolar lumen, as well as in the bronchoalveolar lavage (BAL) (fig. 1). It has been suggested that giant cell interstitial pneumonitis (GIP) is almost pathognomonic for hard metal-induced lung disease [7]. Whilst there is no doubt that exposure to cobalt...
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duce cellular toxicity, and we have recently clarified the concept of a synergy between cobalt and carbides to produce the enhanced toxicity of the mixture was attributed to the higher solubility of cobalt in the presence of tungsten. It is, however, not clear whether tungsten or tungsten carbide was used in this study.

Using state of the art methods for the evaluation of pulmonary toxicity (including cellular and biochemical analysis of BAL fluid, as well as pathology), we have compared, in the rat, the pulmonary response after intratracheal administration of tungsten carbide particles, hard metal powder (a mixture of tungsten carbide with 6% of cobalt metal particles) or an equivalent dose of cobalt metal particles alone. After a single administration, the acute and delayed lung responses to hard metal powder were much more pronounced than those produced by each individual component administered separately. As found by Kitamura et al. [13], the alveolar reaction had completely regressed by 4 months and no fibrosis developed. In a protocol involving repeated intratracheal administrations of the different particles, no effect on the parenchymal architecture was found 1 month after the last dose in the groups treated with tungsten carbide or cobalt alone, whereas clear fibrotic lesions were observed in the group instilled with hard metal [15]. Again, as noted by Kitamura et al. [13], this fibrotic reaction was not accompanied by inflammation, suggesting a scarring process rather than active fibrosing alveolitis. We did not detect giant multinucleated cells in BAL or lung tissue in any experimental group.

Animal studies

Most in vivo investigations to assess the lung toxicity of cobalt and related powders have been performed using intratracheal instillation. Different species have been used with varying results.

FIG. 1. – Cytospin of cells obtained by bronchoalveolar lavage in a 24-year-old female diamond polisher with severe interstitial lung disease. The cell distribution was: 84% macrophages, 3% lymphocytes, 9% polymorphonuclear neutrophils and 4% eosinophils. a) It can be seen that the cytospin contains numerous multinucleated giant cells (the darker and larger cells). b) A typical example of one of the multinucleated cells to illustrate their "cannibalistic features". Such "bizarre" cells are characteristically found in giant cell interstitial pneumonitis caused by hard metal/cobalt exposure. (May-Grünwald Giemsa stain; internal scale bar=5 μm).

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Harding [11] was first to describe the severe pulmonary oedema and haemorrhage found in rats administered cobalt metal powder by intratracheal instillation. Since tungsten metal or carbide alone did not produce the same effect, cobalt was deemed responsible for the parenchymal damage observed in the lung of hard metal workers.

Also in the rat, Kaplun and Mezencewa [12] found that the interstitial lung damage produced by cobalt metal was exacerbated by the simultaneous addition of tungsten or titanium. The morphological changes induced by the mixtures were identical in nature to that produced by cobalt alone but they were more marked. The enhanced toxicity of the mixture was attributed to the higher solubility of cobalt in the presence of tungsten. It is, however, not clear whether tungsten or tungsten carbide was used in this study.

Kitamura et al. [13] examined the pulmonary response to a single intratracheal administration of a high dose of hard metal powder in the rat. Six months later, the surviving animals presented pulmonary lesions of patchy fibrosis in the vicinity of deposited dust, but there was no evidence of an active inflammatory process in the lung. At 12 months, the lesions had apparently regressed. The toxic effect of these particles on the lung was attributed, without experimental support, to the cytotoxic action of cobalt released from the particles. Unfortunately, these investigators did not test cobalt or tungsten carbide alone.

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In a guinea-pig model, Schepers [16] and Delahant [17] found that intratracheal instillation of high doses of cobalt metal led to the development of acute pneumonia. The subchronic response assessed 8–12 months later was characterized by the presence of multinucleated cells, but a lack of fibrotic reaction within the alveolar walls. These investigators concluded that cobalt metal was not fibrogenic. In contrast, the instillation of cobalt metal mixed with tungsten carbide induced a transient inflammatory reaction, with residual fibrosis in the vicinity of retained particles, and multinucleated giant cells were present in some instances. In inhalation experiments, a similar mixture caused severe inflammation but no fibrosis.
Unfortunately, tungsten carbide alone was not tested. It should also be emphasized that these investigators observed multinucleated giant cells in animals treated with a combination of tungsten carbide and carbon (without cobalt), raising some doubt about the specificity of this observation and its relevance for the human disease. It is also unclear whether the multinuclear giant cells found in these animal experiments were ordinary foreign body cells or whether they were similar to the "bizarre" cannibalistic giant cells found in the human disease. Scheppers [16] in fact seemed to attach more importance to the eosinophil response, which he interpreted as suggestive in fact seemed to attach more importance to the eosinophil response, which he interpreted as suggestive of an allergic mechanism, and to the hyperplastic and metaplastic changes observed in the alveolar and bronchial epithelium.

Kerfoot et al. [18] exposed mini-pigs for 3 months to cobalt concentrations of 0.1 and 1.0 mg·m⁻³. In view of a possible involvement of an immunological mechanism (a type I allergic reaction has been suggested in cobalt asthma), they first submitted the animals to a "sensitization" period by inhalation. Immediately after exposure, lung function studies demonstrated a dose-dependent and reversible reduction of the lung compliance in cobalt-exposed animals. These compliance changes were interpreted as demonstrating functional impairment but they were not associated with any radiological or histological signs of fibrosis, except for some increased collagen deposition, which was only evident at the electron microscopic level. These authors did not present a quantitative analysis of this collagen excess, nor did they mention their methodology for selecting the sites for electron microscopic examination, which is critical, since there was no apparent fibrosis at the light microscopic level. The absence of an "innocuous substance" control group is also a major weakness of this study.

Overall, as already suggested by early studies, the higher lung toxicity of cobalt when combined with tungsten carbide has been clearly confirmed by the proper comparison of cobalt alone, tungsten carbide alone, and the combination thereof under the form of hard metal particles. Limited studies in hamsters have also shown that the intratracheal administration of cobalt (5 mg·kg⁻¹) and diamond particles (50 mg·kg⁻¹), caused more acute lung damage, as assessed by lactate dehydrogenase (LDH) activity in BAL fluid 2 days after intratracheal administration, than when these particles were given alone. However, this was also the case for a similar combination of cobalt and iron particles, and no long-term (up to 21 days) synergism was found [19].

So far, all these in vivo studies have remained purely descriptive and they have failed to offer any definitive information on the mechanism of the human disease. In particular, an important hallmark of the human disease, i.e. the bizarre multinucleated cells has not been properly reproduced in an animal model. This represents a serious limitation to the further study of the pathogenesis of hard metal disease and there is a need for investigation of other experimental systems. It is also evident that intratracheal instillation does not adequately reproduce the exposure pattern in the industrial setting, and it would still be useful to perform inhalation studies in the rat or in other species.

Another important aspect which deserves attention is the possible involvement of immune mechanisms in the pathogenesis of hard metal disease. Cobalt is a known sensitizer, and cobalt exposure may lead to allergic contact dermatitis or to bronchial asthma. In both these conditions, evidence for humoral or cellular immune mechanisms has been provided but, so far, "allergy to cobalt" has only been demonstrated conclusively in very few patients with the parenchymal form of hard metal lung disease, in spite of considerable attempts to verify this very plausible hypothesis. Although, in general, immunologically-mediated interstitial lung disease has proved rather difficult to reproduce experimentally (e.g. berylliosis and hypersensitivity pneumonitis), it appears relevant to try and develop an animal model which would combine the toxic properties of hard metal dust and the known allergenic potential of cobalt. This is an avenue of research in which one of our teams is now engaged.

**In vitro studies**

During recent years, the use of in vitro cell culture systems has played an important role in studying the toxicology of mineral dusts and in providing insight into their mechanism of action. In particular, such studies have contributed to a better understanding of the basic biochemical and molecular mechanisms of how mineral dusts interact with cells to elicit inflammation. Numerous studies on mineral dust cytotoxicity have focused on the macrophage because it is generally accepted that interactions with this cell type constitute a first critical step in the initiation of the inflammatory process. Many studies have examined cytotoxicity end-points in peritoneal and alveolar macrophages, with similar responses. However, for investigating functional capabilities (e.g. cytokine secretion), peritoneal and alveolar macrophages differ.

**Cytotoxicity studies**

The different biological reactivities of cobalt and hard metal particles have been reproduced in a cytotoxicity macrophage model [20]. Using LDH release as an index of cytotoxicity, it was found that hard metal powder was almost as reactive as crystalline silica; whereas, when tested separately, tungsten carbide had no effect and pure cobalt only moderately impaired cell viability. Similar cytotoxic responses were observed with peritoneal and alveolar macrophages. Since these responses were consistent with the in vivo findings, peritoneal macrophages were used to investigate the mechanisms of toxicity of hard metal powder. Thus, it was found that, under certain conditions of specific surface area and chemical nature, cobalt metal particles also interact with carbohydrates other than that of tungsten to produce cytotoxicity [21]. These studies have also revealed that the unique toxicity of cobalt-carbide mixtures could not be ascribed to a non-specific carrier effect of carbide particles [22]. As mentioned above, it has long been suggested that the enhanced solubility of cobalt and, hence, its increased bioavailability in the presence of a carbide was responsible for the high toxicity of hard metal particles [11, 12]. However, we have recently demonstrated, with the in vitro model, that cobalt solubilization and bioavailability are not the critical factors for explaining this toxicity [22, 23].
Research is still required to verify whether the concepts derived from these experiments using tungsten carbide and a number of other metal carbides also apply to the situation encountered with the diamond polishers. The latter were certainly not exposed to tungsten carbide or any of the other well-known metal carbides used for making hard metal, but it has to be admitted that we still do not know the exact physicochemical characteristics (i.e. the speciation) of the cobalt, carbon and iron which made up the bulk of the dusts from these diamond polishing workshops. The testing of the toxicity of mixtures of cobalt and diamond particles should help to elucidate this issue.

Cytokines and growth factors

Cytokines and growth factors have been widely investigated as mediators and markers of pulmonary toxicity. In particular, attention has been focused on their role in the lung response to inhaled inorganic dust. In particular, the potential involvement of cytokines in the pathogenesis of hard metal disease has been suggested by the demonstration of antigenic tumour necrosis factor-α (TNF-α) in one case of this disease [24]. In our laboratory, experimental exposure (in vitro or ex-vivo) to cobalt alone or to hard metal particles did not stimulate the production of interleukin-1 (IL-1), TNF-α or fibronectin by rat alveolar macrophages [25]. While these results indicate that the production of these cytokines by alveolar phagocytes probably does not contribute to the acute lung toxicity of hard metal powder, it cannot be excluded that other cytokines (e.g. platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β)), possibly produced by other cell types (e.g. epithelial cells), contribute to the inflammatory process and this issue deserves further investigation.

Activated oxygen species

The production of activated oxygen species (AOS) is another attractive and fashionable hypothesis often invoked to explain the toxicity of inhaled inorganic particles. With regard to cobalt, some recent publications have suggested that its toxicity might be mediated by the production of hydroxyl radicals generated through a Fenton-like reaction, where cobalt replaces ferrous ions [26]. The possible contribution of AOS in the pathogenesis of the lung disease observed in diamond polishers was suggested in the discussion of a case report describing a rapid deterioration of cobalt lung after oxygen administration [27]. Different approaches have been used in our respective laboratories to test this hypothesis.

The intratracheal instillation of cobalt chloride (1–1000 μg·kg⁻¹) into hamster lungs induced biochemical changes compatible with the development of an oxidative stress: decreased levels of reduced glutathione, increased levels of oxidized glutathione, as well as stimulation of the pentose phosphate pathway. These changes occurred early and following the administration of amounts of cobalt well below those causing any lung injury. Similar biochemical changes were observed in vitro after incubating hamster lung slices with cobalt chloride (0.1–10 mM); here again, these manifestations preceded the detection of cellular toxicity, indicating their possible early involvement in the pulmonary toxicity of Co (II) ions [28]. In a later in vitro study on lung slices [29], it was found, however, that, even at a dose which induced cell dysfunction, the extent of glutathione oxidation by Co (II) was not of a sufficient magnitude to result in oxidation of protein thiol groups, an event which is likely to constitute the critical consequence of glutathione oxidation in the toxic process. We, therefore, concluded that although cobalt definitely caused oxidant stress, the oxidation of glutathione was, in itself, probably not the direct cause of the cellular damage induced by cobalt ions.

With the deoxyribose assay to monitor hydroxyl radical production it was not possible to properly compare cobalt and hard metal particles due to an interfering reaction of the carbide with the substrate used. Among the different antioxidants tested for their ability to reduce the toxicity of WC-Co particles towards macrophages, only butylated hydroxytoluene (BHT) which is a highly lipophilic compound was effective, suggesting that peroxidation of lipidic membranes may constitute a critical step in the toxicity of these particles [30, 31]. In hamster lung slices, we also found that BHT was able to mitigate the cellular toxicity of cobalt chloride (Lewis et al., unpublished experiments). Recently, using electron spin resonance (ESR) and electrochemical techniques, we have shown that the unique toxicity of WC-Co particles is due to the production of AOS resulting from the interaction between cobalt metal and tungsten carbide particles (presumably hydroxyl radicals) [31]. The precise mechanism of this interaction is as follows (fig. 2): 1) cobalt metal is thermodynamically able to reduce ambient oxygen but, due to the surface characteristics of the Co particles, the rate of this reaction is very low (cobalt metal is resistant to oxidation); 2) tungsten carbide is an inert material unable to react with oxygen by itself, but it is a fairly good electron conductor and possesses unique surface properties, which are used in numerous catalysis processes (e.g. to replace platinum in combustion systems and in petroleum chemistry); and 3) when both particles are associated, electrons provided by cobalt metal are easily transferred to the surface of carbide particles, where reduction of oxygen can occur at a greatly increased rate.

It is important to emphasize that in this system, soluble cobalt ions constitute the product and not the source of the critical reaction and this is very different from the situation occurring in a Fenton-like reaction. This provides a good explanation for the higher solubility and bioavailability of cobalt when it is associated with a carbide; the latter is not the cause of the unique toxicity but rather a consequence of the reaction which generates the toxic species. It now remains to be demonstrated that AOS are also produced in vivo and effectively cause the toxicity. If this could be confirmed, it may offer a reasonable explanation for the fact that only a small proportion of workers exposed to hard metal powders (1–5%) develop interstitial disease. It may be speculated that individuals with lower antioxidant defence are more susceptible to the toxic effect of AOS produced by inhaled hard metal particles. However, other hypotheses, including immune reactions, are not excluded (see above).

In conclusion, starting from various clinical and epidemiological observations, our research teams have carried out experimental work, both in animals and in vitro,
to understand the mechanisms of hard metal or cobalt lung, which is a fascinating and fairly unique type of interstitial lung disease. Apart from the possible direct implications of our research with regard to occupational health, such as the setting of acceptable air concentrations for cobalt and cobalt-containing dusts, our research has also led, or will lead, to interesting spin-offs in the wider fields of metal toxicity, oxidant cell injury, inflammatory lung disease, and pulmonary immunology.

References