6. Dose-response and potential thresholds in tumour development

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The development of a tumour is a multi-step process including genetic and epi-genetic events. In experimental models, the process of tumourigenesis is divided into different phases which are referred to as tumour initiation and tumour promotion. Accordingly, carcinogens are classified by their mode of action in either genotoxic or non-genotoxic compounds. Depending on their application schedule, genotoxic compounds serve as tumour initiators and/or promoters, whereas non-genotoxic compounds solely act as tumour promoters.

There is a highly controversial debate over the shape of dose-response curves and the existence of thresholds for genotoxic and non-genotoxic carcinogens. Defining the shape of a dose response curve and thresholds has high impact on risk assessment concerning carcinogenic compounds and human health, especially in the low-dose zone. Assuming dose-linearity or deviation from dose-linearity for tumour induction is of great importance in the context of extrapolation from experimentally available high dose data to the low dose range of in reality occurring exposure of humans.

Considering their (presumed) fundamentally different mode of actions, dose-linearity is often proposed for the carcinogenic effect of a DNA damaging genotoxic tumour initiator in contrast to dose-nonlinearity for this effect of non-genotoxic tumour promoters. Theoretically, dose-linearity of the effect of a genotoxic carcinogen implies per definition the lack of a threshold. However, this assumption is seemingly an over-simplification disregarding the possibility of practically instantaneous detoxification of the ultimately DNA damaging species as well as regulatory mechanisms downstream of the primary DNA-adduct formation. Therefore, we and others recently proposed to challenge the classical dogma of dose-linearity for genotoxic carcinogens [1,2]. The existence of a threshold for tumour promoting agents is also discussed highly controversially [reviewed in 3, 4].

In this chapter, we review data on dose-response relationships in animal models of carcinogenesis. For the sake of clarity, we will focus on two classes of model compounds, i.e. genotoxic PAHs and non-genotoxic “dioxins”.

Genotoxic PAHs

A linear dose-response relationship without the existence of a threshold is often assumed for the tumour initiating activities of DNA damaging genotoxic compounds, such as PAHs. This is based on the amount of primary DNA-lesions (below saturation of the respective carcinogen-activating enzymes) increasing with increasing concentrations of the compound. However, this assumption disregards regulatory mechanisms downstream of the primary DNA-adduct formation, such as DNA repair and cell cycle checkpoints. Such regulatory mechanisms are extremely relevant, especially at lower doses of PAHs. Hence, mutations are manifested when intracellular regulatory mechanisms are saturated resulting in linear dose-response curves above a certain threshold [5].

Another aspect which determines the shape of the dose response curve is that the correlation between DNA adducts, mutagenesis and carcinogenesis is not necessarily stringent. When comparing DNA adduct levels and tumour development in mice that have been treated orally or by intraperitoneal injection with benzo[*a*]pyrene, similar amounts have been observed in tumour target and non-target tissues [6]. For instance, injection of a single dose of 375 μg of B[*a*]P induces 1023 fmol adducts/mg DNA in liver, 840 fmol adducts/mg DNA in forestomach and 1851 fmol adducts/mg DNA in lung. However, tumour development is only detected in liver [6]. When given orally, a total dose of 6510 μg of B*a*P induces 905 fmol adducts/mg DNA in liver and 446 fmol adducts/mg DNA in forestomach, but tumors only occur in forestomach. In accordance, high levels of benzo[*a*]pyrene DNA adducts were detected in organs without any sign of tumour development (e.g. kidneys) [7]. In an oral feeding study with B*a*P (125 mg/kg/day for 5 consecutive days) using the Muta™ Mouse, similar amounts of mutations were found in the target organs forestomach and spleen and the non-target organs colon and glandular stomach [8]. However, an obvious link between tumour formation and increased cell proliferation was seen in the development of several tumours, such as forestomach and liver. Accordingly, work by Culp and coworkers [9,10] indicates that coal tar-induced cytotoxicity and cell proliferation were the final determinants — in addition to DNA binding — for the tumour induction in the small intestine of the mice. In a 4 week feeding study of a coal tar mixture the authors show that DNA adduct levels increase up to a concentration of 0.6% (13.4 ppm B[*a*]P), but then decrease, so that the adduct level in the mice fed with 1% coal tar mixture (22 ppm B*a*P) is similar to those found with the 0.3% dose (6.6 ppm B*a*P). However, tumours of the small intestine are only observed when feeding the mice with 0.6% or 1% of coal tar mixture. These concentrations of coal tar mixture, but not 0.3%, induce a nearly 50% increase in cell proliferation. Hence, additional factors to DNA adduct formation are essential for benzo[*a*]pyrene-induced tumour development (and also other PAHs-dependent tumours). Moreover, even the situation exists where DNA lesions (investigated lesion: strand breaks/alkali labile sites) are produced by reactive genotoxins (investigated genotoxin: styrene 7,8-oxide) to an observable extent only after a practical threshold caused by practically immediate detoxication is exceeded [11].

So far, no data are available defining precise threshold levels for PAHs in animal models. However, computational modeling of *in vitro* data sets would predict nonmonotonic dose-response relationships also *in vivo* [12,13]. Indeed, nonmonotonic dose-response relationships have been detected for tumour induction by the genotoxic compounds 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline and diethylnitrosamine [14,15].

Non-genotoxic "dioxins"

There is good experimental evidence that the effects of dioxins, a prototype of which is TCDD, and dioxin-like compounds are receptor-mediated, i.e. require binding of the compound to the AhR. Although the binding of a compound to a given receptor is a bi-molecular reaction leading to a biological outcome, which is directly proportional to receptor occupancy [reviewed in 4], this simple assumption does not take into account that multiple downstream effects post-ligand binding generally occur in a living cell. For instance, it is still not clear whether nuclear translocation of the AhR requires a certain threshold concentration of the ligand. Furthermore, depending on the experimentally defined end-points, different dose response curves may exist. For instance, DeVito and coworkers compared different effects of repeated low level exposure to TCDD in mice and revealed different dose-response curves for the induction of Cyp1A1/1A2 and tyrosine phosphorylation in liver [16]. A significant increase in EROD-activity, a marker for CYP1A1 activity, was already detected at the lowest dose of 1.5 ng/kg/d. Even at the highest dose of 150 ng/kg/d the maximum of the dose response curve was not reached. While a significant increase in tyrosine phosphorylation in liver was also observed at the lowest dose of 1.5 ng/kg/d, maximal effects occurred at a dose of 4 ng/kg/d [16]. In several initiation-promotion studies, the promoting activity of TCDD has been analysed quantitatively. One of the first detailed dose-response studies was carried out by Kociba and coworkers [17]. They analysed the emergence of enzyme-altered foci, preneoplastic nodules and hepatocellular carcinomas in rats. Interestingly, enzyme-altered foci were observed at a dose of 0.01 μg/kg/d whereas higher doses were needed for the induction of preneoplastic nodules and hepatocellular carcinomas (0.1 μg/kg/d). In contrast to studies in mice, female rats appeared to be much more sensitive than male rats. Interestingly, an opposite gender specificity was described in mice [reviewed in 4].

Several studies on the dose-response relationship using an initiation-promotion protocol have followed using the appearance of enzyme altered foci as experimental endpoint [18–23] and have been modelled thereafter.

Portier and coworkers [24] provided a mathematical model of an experimental study in which four different TCDD doses (3.5, 10.7, 35.7, 125 ng/kg/d) were administered in DEN-initiated rats [20]. The data were consistent with dose-linearity at least for the smaller doses, and surprisingly suggested that TCDD enhanced the production of enzyme altered foci and hence acts as a tumour initiator, a conclusion recently supported also by Stinchcombe and coworkers [25]. However, based on the assumption of two differernt

types of initiated cells, Conolly and Andersen predicted a U-shaped dose-response curve in the low-dose range (0.1–10 ng/kg/d) [26,27], a model in line with the above mentioned Kociba-study.

A U-shaped dose-response curve indicates an inhibitory effect on tumourigenesis in the low dose range and might be explained by the existence of two different types of initiated cells (see above): cells of one type show a decrease in their proliferation in response to TCDD in the low dose range while cells of another type show an increase in proliferation in the high dose range. Indeed, Teeguarden and coworkers [23] have revealed an inhibition of proliferation of non-transformed liver cells in the rat at low TCDD concentrations (0.1 ng/kg/d) while proliferation increased at high doses (10 ng/kg/d) correlating with liver toxicity [summarised in 26]. In addition, Fox and coworkers have shown zonal differences in the TCDD-response with mitoinhibitory effects of TCDD in the centrilobular regions and proliferative effects in the periportal region in rat liver [28]: TCDD was administered by using a dose loading/maintenance regimen to achieve rapid quasi-steady-state TCDD liver concentrations of 0.03, 30 or 150 ng/g liver. At the dose of 150 ng/g liver, a significant elevation of BrdU labelling index was found in the periportal region, while a slight decrease in BrdU labelling index was observed in the centrilobular region. This raises the question if oval cells which are located periportally are also cellular targets of TCDD. Moreover, a number of studies performed in transformed rat hepatoma and oval cells and primary mouse thymocytes have demonstrated that TCDD, via acting through AhR, can dependent on the cell type either increase or decrease cell proliferation [29–31].

In summary, the shape of dose-response-relationship and the existence of thresholds for the tumourigenesis-related effects of TCDD and related compounds remain largely unresolved. Based on current understanding, receptor binding is not likely to be a threshold-related event. However, multiple intracellular signalling events downstream of the AhR result in complex biological responses as demonstrated by mito-inhibitory, proliferative and anti-apoptotic effects. These effects depend on the dose, zonal region and cell type. The molecular mechanisms underlying these pleiotropic effects remain to be elucidated.

Conclusion and perspectives

As outlined above, there is a great demand for elucidation of the cellular signalling pathways which are evoked by exposure to PAHs and TCDD in order to better understand dose-response relationships. Especially in the case of TCDD, the cellular effects in rodent liver are greatly dependent on the dose, zonal region and, as concluded from results with different liver cells in culture, also from the different intracellular equipment of different cell types. The same applies to disturbances by TCDD of developing tissues. Another open question is the role of the AhR, ARNT and the recently discovered AhRR (AhR repressor) as mediators of cellular effects of PAHs

and TCDD. Downregulating these mediators by siRNA-technology in cell culture and tissue specific conditional knock-out mice with individual or combined deletions in these genes will aid the clarification of the signalling pathways induced by PAHs and TCDD. Even more complexity is given by the fact that humans are generally exposed to mixtures of PAHs and TCDD. Almost no information is available about the cellular effects or signalling pathways that are induced by such mixtures.

To close this gap, the characterization of the intracellular signalling cascades induced by PAHs and TCDD is needed. In order to better understand possible cross-talk(s) between these two pathways, the signalling pathways have to be analysed, first independently. Establishing dose-response relationships by correlating gene expression profiles with the observed cellular effects, such as proliferation/survival and apoptosis — at different doses of the compounds — will be of great importance. Furthermore, comparison of gene expression profiles in different cell culture models (liver cells, cells from developing tissues including stem cells) will help to better understand and quantitatively relate dose responses to the in part already known cell type specificity of the effects of TCDD. Building on all of this the establishment of the mutual cross-talks of components of TCDD/PAH mixtures will be important.

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