DNA adducts in hamster and rat tracheas exposed to benzo(a)pyrene in vitro

Rob Roggeband*, André P.M. Wolterbeekb, Paula T.M. van den Berga, Robert A. Baana

aTNO Medical Biological Laboratory, Department of Genetic Toxicology, PO Box 5815, 2280 HV Rijswijk, The Netherlands
bTNO Toxicology and Nutrition Institute, Department of Biological Toxicology, PO Box 360, 3700 AJ Zeist, The Netherlands

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Abstract

Syrian golden hamsters are much more susceptible than Wistar rats to the induction of tracheal tumors by benzo(a)pyrene (BP). In order to investigate whether this difference is reflected in the pattern of DNA-adduct induction and removal, tracheas from either species were isolated and exposed to BP (5 μg/ml) in organ culture. At various time-points BP-DNA adducts in the epithelial cells were quantified by 32P-postlabeling; unscheduled DNA synthesis (UDS) was determined by [3H]thymidine incorporation. In an induction-repair experiment tracheas were exposed to BP for 2 days, and cultured for another 4 days without BP. After 2 days of exposure total BP-DNA adduct levels were 10 times higher in hamster compared to rat tracheas. In hamster tracheas one major adduct was formed (95%), vs. the adduct between (+)-anti-BP-diolepoxide and deoxyguanosine (BPDE-N’dG). In rat tracheas BPDE-N’dG comprised about 60% of the total adduct level. During exposure to BP the adduct level in hamster trachea increased to 36 ± 19 adducts/10^6 nucleotides (add/10^6n) on day 2. Two days after removal of BP the BP-DNA adduct level had decreased to 60% of that on day 2; there was no further decrease in the BP-DNA adduct level. UDS increased during exposure to BP and decreased after removal of BP. In rats, removal of BP did not lead to a decrease in the BP-DNA adduct level, which agreed with the observed absence of UDS. In a second experiment tracheas were exposed to BP continuously for 15 days. In hamster tracheas the total BP-DNA adduct level increased from 11 ± 0.7 add/10^6n after 1 day of exposure to 105 ± 2 add/10^6n after 15 days; also UDS increased with increasing exposure until day 11. In rat tracheas no progressive increase in the BP-DNA adduct level was seen. It was concluded

* Corresponding author.
that the difference in trachea tumor susceptibility between hamsters and rats exposed to BP correlates with the difference between the 2 species in BP-DNA adduct kinetics in the trachea epithelial cells.

**Key words:** Benzo(a)pyrene; DNA adduct; $^{32}$P-postlabeling; UDS

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1. **Introduction**

The lung is a major site of tumor formation in humans. Epidemiological studies show a higher relative risk of lung cancer for the smoking population compared to non-smoking controls [1]. With the $^{32}$P-postlabeling method tobacco-smoke specific DNA adducts could be detected in lung tissue from cigarette smokers [2–5]. Morphologically, the type of tumors observed in the lungs of humans shows a close similarity to benzo(a)pyrene (BP)-induced respiratory tract tumors in hamsters [6–8]. Contrary to the rat, the Syrian golden hamster is susceptible to tumor formation in the trachea after exposure to BP [9]. Several groups [10–12] already reported a difference in total BP-DNA adduct levels between hamster and rat tracheas, exposed to BP in vitro for 24 h. The objective of the experiments described in the present paper was to study differences in DNA-adduct formation and DNA repair between BP-exposed hamster and rat tracheas in organ culture. A more extensive description of these results will be published elsewhere.

2. **Induction-repair experiment**

On day -1 10-week-old male Syrian golden hamsters ($n = 40$) and Wistar rats ($n = 16$) were sacrificed, tracheas were isolated, and placed in culture. For a detailed description of the culture conditions, see [13]. From day 0 on, tracheal rings were exposed to BP (5 µg/ml) for 2 days. BP was dissolved in DMSO; the final concentration of DMSO in the medium was 0.1% (v/v). At the end of these 2 days, just after sampling, all remaining tracheal rings were washed, transferred to medium without BP, and cultured for another 4 days. Sampling comprised scraping off the epithelial cells for DNA isolation and adduct analysis by $^{32}$P-postlabeling [14–16], and preparation of sections for the assay of unscheduled DNA synthesis (UDS) [13].

In DNA isolated from hamster trachea epithelial cells, an increase in the total BP-DNA adduct level, from day 0 (start of exposure to BP) to day 2 (end of exposure to BP) was observed (Fig. 1A). The adduct level on day 2 corresponded to 36 ± 19 adducts per $10^6$ nucleotides (add/$10^6$n). One day after washing out BP the adduct level had decreased to 60% of the level on day 2. There was no further decrease in adduct level during culture. A typical adduct pattern obtained with DNA from the hamster trachea is depicted in Fig. 2. At all time-points the predominant adduct in the hamster trachea (adduct 1) co-migrated with the adduct formed between (+)-anti-BPDE and deoxyguanosine. It accounted for 95% of the total adduct level.

In DNA of rat trachea epithelial cells, the BP-DNA adduct level was 3.7 ± 1.5 add/$10^6$n after 1-day exposure to BP. However, contrary to the hamster, there was
Fig. 1. (A) Relative BP-DNA adduct level in hamster (□) and rat (○) tracheas exposed to BP (5 µg/ml) for 2 days. The 100% level at 48 h corresponds to 36 ± 19 add/10^6 in case of hamster tracheas and 3.5 ± 1.7 add/10^6 in case of rat tracheas. The horizontal bar indicates the period of BP-exposure. Error bars represent range of values of 2 postlabeling assays. (B) BP-DNA adduct level in hamster (□) and rat (○) tracheas exposed to BP (5 µg/ml) continuously for 15 days. Error bars represent range of values of 2 postlabeling assays.
Fig. 2. Typical adduct profile on TLC of postlabeled DNA from hamster (left) and rat (right) tracheas exposed to BP (5 μg/ml) for 5 days. The films were exposed for 6 h (hamster) and 26 h (rat) at -70°C. Adduct 1 was identified by co-chromatography as the reaction product of (+)-anti-BPDE and dG.
no further increase in the BP-DNA adduct level after 2 days exposure. Also in the rat trachea BP-DNA adducts could still be detected 4 days after removing BP (Fig. 1A). Actually, this is almost 90% of the level found on day 2. After 48 h of exposure to BP the total BP-DNA adduct level in the rat trachea was 10-fold lower than in the hamster trachea. As depicted in Fig. 2, the adduct pattern on TLC of rat trachea epithelial cells differed considerably from that of hamster; while adduct 1 was almost the only adduct in the hamster, the relative amount in rat tracheas was about 60%. The other substantial adduct in the rat trachea, adduct 3, comprised about 30%. Adduct 2 was a minor adduct (10%). During culture there were no significant changes in relative levels of adducts 1, 2 and 3.

In hamster trachea epithelial cells, the exposure to BP led to a well-measurable induction of UDS. The control level on day 0 of 0.22 ± 0.09 increased to 0.54 ± 0.03 net grains per nucleus on day 1 (statistically significant; Student's t-test; \( P = 0.0056 \)). On day 2 (48-h exposure to BP) and day 3 (one day after removal of BP) the UDS was also significantly higher than on day 0 (Student's \( t \)-test; \( P = 0.0003 \) and \( P = 0.0017 \), respectively). UDS on days 4, 5 and 6 was not increased compared to that on day 0. In contrast, in rat trachea epithelial cells UDS was not increased during or after exposure to BP at any time-point.

3. Induction-accumulation experiment

Hamster (\( n = 30 \)) and rat (\( n = 12 \)) tracheas were isolated and placed in culture on day -1. On day 0 trachea rings were exposed to BP (5 \( \mu \)g/ml) which continued for 15 days. Samples were taken on days 1, 3, 5, 7, 11 and 15. Fig. 1B shows the DNA adduct postlabeling data of the tracheas that had been exposed to BP continuously for 15 days. In hamster trachea epithelial cells, a steady increase in the total BP-DNA adduct level from 11 ± 0.7 add/10⁶n on day 1 to 105 ± 2 add/10⁶n on day 15 was observed. Adduct 1 comprised about 95% of the total adducts, comparable with the results of the induction-repair experiment.

In rat trachea epithelial cells, the total adduct level on day 1 was 6.1 ± 1.5 add/10⁶n. Contrary to the hamster, in the rat trachea no steady increase in the total adduct level was found; on day 15 the total adduct level was 8.1 ± 0.3 add/10⁶n. The difference in total adduct level between the hamster and rat trachea increased from about 2-fold on day 1 to about 13-fold on day 15. The adduct patterns of both the induction-repair and the induction-accumulation experiment were comparable, with the exception of adduct 4, a minor adduct (\(< 10\%\)), which was only observed in the induction-accumulation experiment.

In the hamster trachea epithelial cells UDS was significantly increased on days 1, 3, 5, 7 and 11 (Student's \( t \)-test; \( P < 0.025 \) at all time-points) compared to the unexposed control in the induction-repair experiment. In rat trachea epithelial cells UDS was at background level.

4. Conclusions

In view of the difference between the Syrian golden hamster and the Wistar rat with respect to the susceptibility to BP-induced tracheal tumor formation, it is of in-
terest to find out whether a similar difference can be observed with respect to DNA-adduct kinetics. This paper deals with experiments in which tracheas from hamsters and rats were exposed to BP in vitro during various periods of time.

In the induction-repair experiment tracheas from the 2 species were exposed to BP for 2 days, followed by post-treatment incubation for 4 days. In the hamster trachea the amount of BP-DNA adducts increased during BP treatment and reached a maximum on day 2. Postlabeling analysis revealed that one adduct (adduct 1) was formed almost exclusively (95% of the total amount). One day after removal of BP the adduct-level decreased to about 60% of the maximum value. There was no further change on days 4, 5 and 6. Upon exposure to BP, UDS increased significantly. Despite the fact that 60% of the adducts were still present on days 5 and 6, UDS gradually decreased to control levels. It seems unlikely that part of the adducts are not susceptible to repair, as the adduct patterns at various time-points were similar. Another explanation may be a gradual loss of repair activity during the in vitro incubation. In the rat trachea the maximum adduct level was reached already on day 1 and remained approximately the same during days 2 to 6. This agrees with the absence of a detectable level of UDS. Apart from adduct 1, in rat tracheas another major adduct could be detected, namely adduct 3, which is probably derived from interaction of syn-BPDE with deoxyadenosine [17]. Most strikingly, the total level of BP-DNA adducts on day 2 was about 10 times higher in hamster than in rat tracheas.

In the induction-accumulation experiment, tracheas were exposed to BP continuously for 15 days. In the hamster a steady increase in adduct level could be observed, whereas in rat tracheas a constant, low adduct level was found. The adduct patterns, as determined by postlabeling analysis, were comparable to those found in the induction-repair experiment at all time-points. In hamster tracheas, an increased UDS level was observed up to day 7. Thereafter the UDS gradually decreased, despite a steady accumulation of DNA adducts. This may be due to a saturation of the repair system. Again, in rat tracheas no significant increase in UDS was observed.

It can be concluded that the difference in BP-DNA-adduct induction in hamster and rat tracheas is in line with the difference in susceptibility of the 2 species to BP-induced tracheal tumor formation. Further experiments are ongoing to investigate induction and removal of DNA adducts in specific cell types of the trachea epithelium and the effects on cell proliferation.

5. References


