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Polycyclic Aromatic Hydrocarbons: Role of Apoptosis in Dermatotoxic and Carcinogenic Effect in Asphalt Road Paving Workers

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Abstract

Asphalt contains a complex mixture of polycyclic aromatic hydrocarbons (PAHs). This work aimed firstly to assess the dermatotoxic and carcinogenic risk associated with chronic PAHs exposure, and secondly to investigate the causal relationship between PAHs exposure and cancer through studying the effect of PAHs on apoptosis. This effect was studied by examining the expression pattern of P53, Bax and Bcl-2 apoptotic proteins in skin specimens from road paving workers. The study was conducted on one hundred and fifty two male subjects classified into 122 asphalt fumes exposed workers (tested group) and 30 non exposed workers (control group); careful skin examination and skin biopsies were obtained from all participants after written consent. Biopsies were examined histopathologically and immunohistochemically. SPSS version 15.0 was used for statistical analysis. Results showed that 71 (58.19%) PAHs exposed workers had erythema, itching, excoriations, chronic dermatitis, chemical keratosis, keratoacanthoma (KA) and nine (7.38%) exposed workers had squamous cell carcinoma (SCC). Imunohistochemically, wild type P53 was significantly higher in epidermal keratinocytes of PAHs exposed normal uninvolved (non lesional) skin (t=0.51, P=0.04) and mutant type P53 was significantly higher in SCC cases (t=4.79, p=0.003) when compared with control. A significant increase in Bax expression was observed in all asphalt exposed workers when compared with the control (t=2.73, P=0.03). A significant decrease in Bcl-2 expression was noted in PAHs exposed un-involved skin (t=2.49, P=0.047) while none of the 9 tested SCC cases were positive for Bcl-2. It could be concluded that chronic exposure to PAHs fumes in asphalt workers may increase the risk for developing dermatotoxic and/or cancer through disturbing apoptosis. Although PAHs exposure can disturb P53, Bax and Bcl-2 apoptotic protein, more extensive researches on other factors involved in controlling apoptosis as Bcl-xl, Caspase family and Fas are to be undertaken.

Keywords: Polycyclic aromatic hydrocarbons (PAHs); Dermatotoxicity; Apoptosis; Skin cancer; Asphalt workers

Introduction

Asphalts are complex hydrocarbon mixtures with molecular weights ranging from 500 to 2000, high boiling temperature (above 500°C) and carbon numbers predominantly higher than C25. Asphalts produced from the atmospheric and vacuum distillation of crude oil are used primarily in paving applications [1]. Asphalt typically contains about 80% by weight of carbon; around 10% hydrogen; up to 6% sulfur; small amounts of oxygen and nitrogen; and trace amounts of metals such as iron, nickel, and vanadium [2].

Asphalt fumes produced during heating of asphalts to facilitate their use commonly encountered by workers in road paving operations. They are complex mixtures of aerosols and vapors that contain various organic compounds including polycyclic aromatic hydrocarbons [3].

Polycyclic aromatic hydrocarbons "PAHs" are vapors result from incomplete combustion or pyrolysis of coal tar, pitch, coke and asphalt. They precipitate as particles or condense onto soot [4]. These fumes may be inhaled or deposited on skin or clothing [1].

The adverse effects of PAHs on humans are diverse [5]. PAHs have been reported to cause many skin disorders [6]; they were reported to be phototoxic [7] and verrucae inducer [8]. Numerous studies have described an excess risk of cancer among asphalt-exposed workers [9-12]. PAHs have been shown to be mutagenic in rat lung [13], reproductive and developmental toxicant [14] as well as genotoxic in humans [15].

Apoptosis is a natural, genetically controlled series of steps that occurs when a cell is old, unhealthy, or severely damaged. The end result is cell destruction. Apoptosis in the skin kills off harmed cells that they do not turn cancerous. In some cases, genetic mutations or other factors derail apoptosis. If this occurs; the cells can become "immortal" and continue to proliferate, resulting in skin cancer [16].

The process of apoptosis is controlled by important genes as P53 gene (tumour suppressor gene) and proteins such as Bcl-2 family i.e. several pro-apoptotic (Bax, Bak, Bad) and anti-apoptotic (Bcl-2, Bcl-xl) proteins and Caspases [17].

PAHs compounds have been shown to induce apoptosis in Hepa1c1c7 hepatoma cells [18], in human B cells [19], in macrophages [20] and in rat lungs [21]. Though numerous studies have described an excess cancer risk among asphalt workers, a causal relationship has not been established. Accordingly, the aim of this work firstly was to search for the dermatotoxic and carcinogenic risk associated with chronic PAHs exposure in asphalt workers and secondly to evaluate this cancer causal relationship through studying the PAHs effect on apoptosis by examining the expression of P53, Bax and Bcl-2 apoptotic proteins in skin specimens from road paving workers using immunohistochemical techniques.

Subjects and Methods

The study was conducted on 152 male subjects classified into two groups: 122 road paving workers who were exposed nearly daily to PAHs asphalt fumes "PAHs exposed workers" and 30 age-matched non-paving outdoor construction workers with no history of occupational PAHs exposure "control group". The age of the road paving workers

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ranged from 30-55 years with a mean of 42 years and the exposure period to PAHs fumes ranged from 9–25 years with a mean of 11.5 years.

Prior to sampling; written consent was obtained from each participant after discussing the purpose and the protocol of the study. All procedures were conducted in accordance with a standardized human subject's protocol that was approved by the ethics committee of Faculty of Medicine, Minia University.

Over 10 month period, for all subjects, a record of complete past medical history, occupational history and drug usage was compiled. Smokers were excluded. Complete general, local examination and photographing for any observed skin lesions were undertaken. 4 mm punch biopsies were taken from SCC lesions "if found" and from normal uninvolved forearm skin if no lesion were detected (non lesional skin). Patients with evident skin lesions were then referred to be properly managed. Biopsy specimens underwent routine haematoxylin and eosin light microscopic histopathological examination then evaluated immunohistochemically.

Immunohistochemistry was performed on paraffin wax embedded skin tissue according to Petrusz and Ordronneau [22]. 4 µm tissue sections were immunostained for P53, Bax and Bcl-2 proteins using a standard avidin-biotin-peroxidase method. Sections were dewaxed in two changes of xylene, hydrated in graded alcohol. Endogenous peroxidase was blocked with 0.3% H_2O_2 (v/v) in methanol for 30 minutes at room temperature. Samples were then incubated overnight with rabbit monoclonal antibody to human P53 antibody (code no.: RMPD 016, ready-to-use 1ry antibody, DBS, CA, USA) which reacts with both the wild and the mutant types of human P53 protein, Bax epitope specific rabbit antibody (E3381, Spring Bioscience, CA, USA) and mouse monoclonal antibody to human Bcl-2 oncoprotein (code no.: PDM 016, ready-to-use 1ry antibody, DBS, CA, USA) at 4°C. Sections were then immersed in a biotinylated secondary antibody diluted at 1:100 for 30 minutes and in avidin biotinylated peroxidase complex for 60 minutes (code no.: # Kp-50L, DBS, CA 94566, USA). Slides were then treated with 100 µL of freshly prepared DAB (3' 3' diaminobenzidine) solution for 10 min. Finally, slides were rinsed in running water, dehydrated in graded methanol, cleared in three changes of xylene and mounted in DPX then examined. Negative control sections were obtained by omitting the specific primary antibody from the staining process.

The level of expression was evaluated quantitatively according to scoring system made by Liang et al [23]. Application of this system gives a score for both the degree of positivity (the number of positively stained keratinocytes/100 examined cells) and the degree of intensity of staining (Table 1).

Statistical Analysis

Statistical analysis of data was performed by using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) for windows. Results were expressed as mean values (M) and standard deviation (± SD). Unpaired two-tailed

Score	Positivity	Score	Intensity
0	< 1.0%	0	No staining (negative)
1	1 – 10%	1	Faint brown (mild-weak)
2	10 – 50%	2	Brown (moderate)
3	> 50%	3	Deep brown (strong)

Table 1: Scoring system for quantifying the level of P53, Bax and Bcl-2 expression in epidermal keratinocytes (Liang et al. 1999).

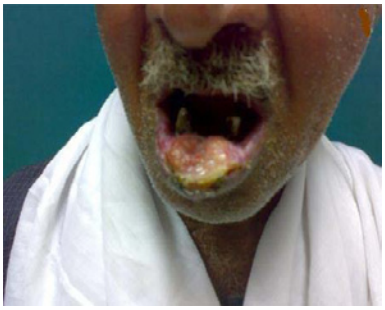

Figure 1: SCC of lower lip.

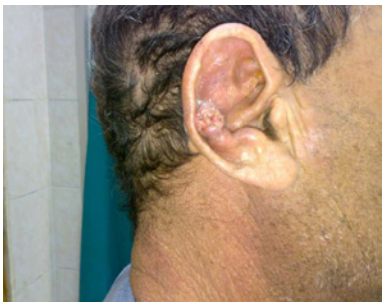

Figure 2: SCC of ear.

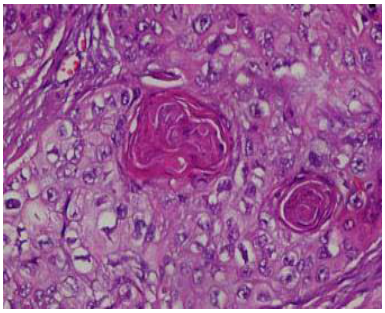

Figure 3: Moderately differentiated SCC showing an increased number of atypical keratinocytes and fewer horn pearls (H&E, x 400).

Students' t-test was performed to compare results. P Values of less than <0.05 was considered statistically significant.

Results

Many dermatologic toxic symptoms and signs were observed in 71 PAHs exposed workers out of 122 examined (58.19%) as erythema (8 cases), itching (19 cases), excoriations (13 cases), chronic dermatitis (16 cases), chemical keratosis (9 cases) and keratoacanthoma (KA) (6 cases).

Nine out of 122 (7.38%) workers examined were found to have skin lesions in the form of 3 skin ulcers, 2 hyperpigmented lesions and 4 fungating masses. They gave history of working in asphalt paving for more than 15 years before noting skin lesions. They were exposed to PAHs fumes on a nearly daily basis. Lesions were limited to lower lip (Figure 1), ear pinna (Figure 2), back of scalp, knee and the nostrils entrance. Clinical as well as histopathological examination revealed squamous cell carcinoma (SCC) (Figures 3 and 4).

The level of P53 expression in the epidermal keratinocytes of

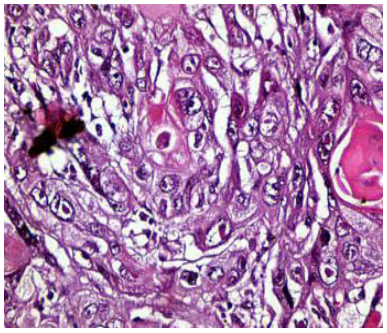

Figure 4: Poorly differentiated SCC with numerous atypical keratinocytes Occasional horn pearl can be seen within atypical keratinocytes (H&E, x 400).

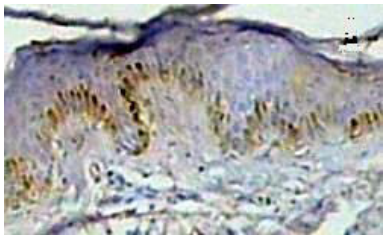

Figure 5: positive nuclear P53 expression in basal layer of epidermal keratinocytes of control group (Immunoperoxidase; x 200).

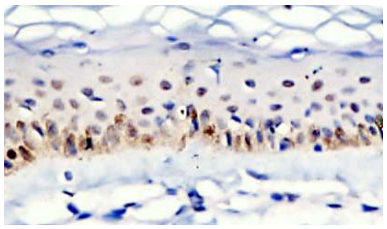

Figure 6: Increased nuclear P53 expression in epidermal keratinocyt- es of uninvolved skin of PAHs exposed group. The expression extended from basal to geanular cell layer (Immunoperoxidase; x; 400).

the control group ranged from 0.7-1.1 with a mean of 0.84 ± 0.16. Immunoreactive keratinocytes exhibited nuclear staining and were limited to the basal cell layer of the epidermis indicating wild type (diffuse pattern) P53 expression (Figure 5). While the level of wild P53 expression in the epidermal keratinocyte of the PAHs exposed uninvolved skin ranged from 0.62-1.9 with a mean of 1.3 ± 0.46 (Figure 6). Mutant type P53 (compact pattern) was over-expressed in SCC cases with a level ranged from 16.64-63.48 with a mean of 49.60 ± 32.7 (Figure 7). A significant increase in P53 expression level in epidermal keratinocytes of PAHs exposed normal uninvolved skin "non lesional" (t=2.51, P=0.04) and in SCC cases (t=4.79, p=0.003) was observed when compared with the control group (Histogram 1).

Bax was overexpressed in all PAHs exposed workers "either within PAHs exposed normal uninvolved skin or SCC lesions". The expression was cytoplasmic and its level in epidermal keratinocytes was 2.2-2.46 with a mean of 2.3 ± 0.099, 1.57-3.75 with a mean of 2.81 ± 0.59 and 2.3-3.91 with a mean of 2.9 ± 0.02 in the control group, PAHs exposed normal uninvolved skin and SCC cases respectively (Figures 8, 9 and 10). A significant increase in Bax expression in epidermal keratinocytes of the PAHs exposed workers and in SCC cases was observed when compared with the control group (t=2.73, P=0.03) and (t=2.98, P= 0.01) respectively (Histogram 1).

Bcl-2 expression of control group was confined to basal keratinocytes. They exhibited cytoplasmic staining with perinuclear enhancement (Figure 11) with mean expression level of 2.69 ± 1.7. In PAHs exposed normal uninvolved skin; Bcl- 2 was down regulated with a mean level of 1.3 ± 1.2 (Figure 12). On other hand; none of the 9 tested SCC cases were positive for Bcl-2 (Figure 13). A significant decrease in Bcl-2 expression in epidermal keratinocytes of PAHs exposed subjects was noted (t=2.49, P=0.047) (Histogram 1).

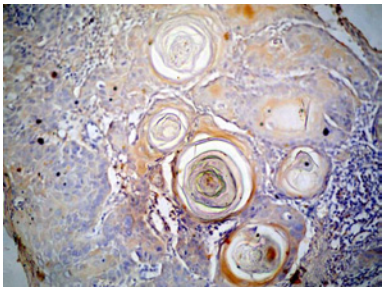

Figure 7: Positive nuclear P53 expression in SCC (Immunoperoxidase; x 200).

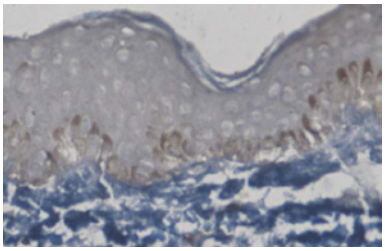

Figure 8: Cytoplasmic expression pattern of Bax in basal cell layer of the epidermal keratinocytes of control group (Immunoperoxidase x 400).

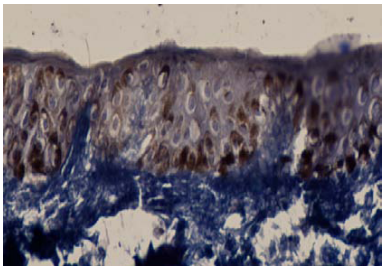

Figure 9: Bax expression in all layers of epidermal keratinocytes of PAHs exposed un-involved group (Immunoperoxidase; x 400).

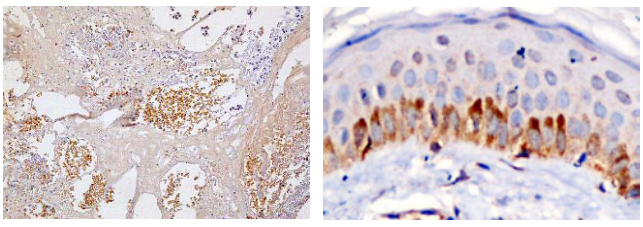

Figure 10: Higher Bax expression within SCC cells (Immunoperoxidase; x 200).

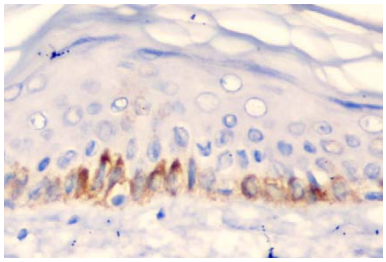

Figure 11: Cytoplasmic expression of Bcl-2 of basal cell layer in control skin (Immunoperoxidase; x 400).

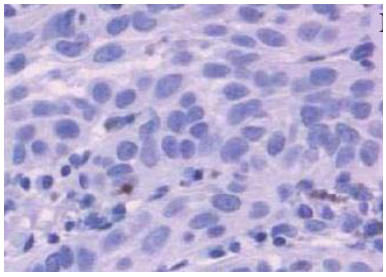

Figure 12: Dowenregulated Bcl-2 expression in PAHs exposed workers (Immunoperoxidase; x 400).

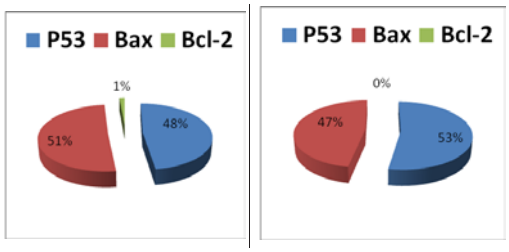

Figure 13: Negative Bcl-2 expression in SCC (Immunoperoxidase; x 400).

Number of positively immunostained cases, immunostaining intensity and the expression score for P53, Bax and Bcl-2 in both PAHs exposed normal uninvolved skin and SCC cases are illustrated in Tables 2 and 3 and Figures 14A and 14B.

Discussion

Occupational exposure to hot asphalt may increase the risk of cancer (lung, stomach, bladder, leukemia and non-melanoma skin cancer) among asphalt workers [24]. Changed expression of apoptosis related molecules could be detected during the process of malignant transformation and in malignant cells [25]. As dermatotoxic and carcinogenic potentials of PAHs are not fully clarified and mechanisms involved in PAHs-induced apoptosis are less characterized; so this work aimed to study chronic toxic effects of PAHs fumes on skin and to search for different cellular pathways of apoptosis underlying malignant transformation through studying P53, Bax and Bcl-2 apoptotic protein expression in skin of a population of road paving workers to assess the toxic role of PAHs on apoptosis using immunohistochemical techniques.

The observed increased toxic skin symptoms in PAHs exposed workers agree with what reported by [6-8]. This may be explained on the basis that asphalt alters cutaneous barrier integrity and induce skin disease. Results also proved an association between cancer risk and exposure to PAHs fumes where 9 chemical keratosis cases (with pre-cancerous potential) and 9 SCC cases were diagnosed which in line with [26,27].

Apoptosis which is equated to programmed cell death is an evolutionary conserved cell death process that plays a major role during normal development, homeostasis and elimination of potentially dangerous cells. The ordered execution of this internal death program leads to typical morphological and biochemical changes [28]. Dysregulation of the apoptotic process may result in a wide range of pathological conditions as cancers, autoimmune diseases, and viral infections [29].

In this study; PAHs were found to induce activation of apoptosis where both wild type P53 (tumour suppressor gene) and Bax protein (pro-apoptotic protein) were over-expressed and Bcl-2 (the major anti-apoptotic protein) was downregulated which agree with [30-32]. The activated apoptosis act as a defense mechanism to prevent PAHs toxic effect as it helps exposed workers to get rid of harmed cells and to prevent progression to carcinoma. Increased P53 expression level suggests that PAHs induced apoptosis is triggered by the induced DNA damage in P53-dependent pathway. P53 in its natural form (wild-type) upregulates P21 causing cell cycle arrest to allow repair of damaged DNA [33]. Road paving asphalt fume was found by [34] to be mutagenic and induced

Immunostaining intensity / Marker		No. of +ve. cases	No. of cases with mild (weak) staining intensity	No. of cases with moderate staining intensity	No. of cases with strong staining intensity
PAHS exposed uninvolved skin (n=113)	P53	101	7	88	6
	Bcl-2	3	3	0	0
	Bax	107	6	38	63
SCC cases (n=9)	P53	9	0	7	2
	Bax	8	1	6	1
	Bcl-2	0	0	0	0

Table 2: Immunostaining intensity of P53, Bax and Bcl-2 in uninvolved skin and SCC cases of PAHs exposed group.

Score / Markers		No. Of + ve samples	< 1.0% (score 0)	1-10% (score1)	10 – 50% (score 2)	> 50% (score 3)
PAHS exposed uninvolved skin (n=113)	P53	101	12	15	80	6
	Bax	107	6	6	23	78
	Bcl-2	3	110	3	0	0
SCC cases (n=9)	P53	9	0	1	4	4
	Bax	8	0	0	2	6
	Bcl-2	0	9	0	0	0

Table 3: Apoptotic markers expression score in uninvolved skin and SCC cases of PAHs exposed group.

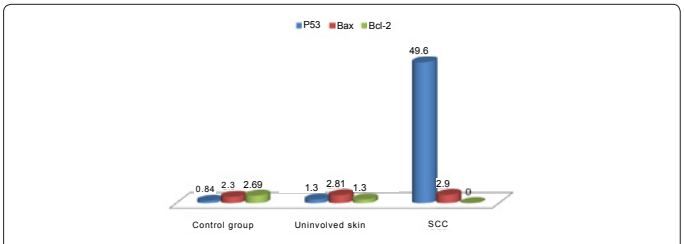

Figure 14: Immunostaining intensity of P53, Bcl-2 and Bax in uninvolved skin (A) and SCC (B) cases of PAHs exposed group.

DNA damage and adduct formation. DNA damage initiates a number of cell signaling pathways that may result in cell cycle delays, senescence or cell death. The key point of these changes is the tumour suppressor P53 [35]. Following DNA damage P53 is phosphorylated thereafter translocated to the nucleus where it may result in cell cycle arrest, allowing time for DNA repair or triggering apoptosis [36]. If damage is extensive, P53 induces apoptotic cell death by downregulating Bcl-2 and upregulating Bax expression [33]. Comparatively, we were able to further substantiate this hypothesis as we found an increased level of Bax which is involved in triggering the apoptotic process as Bax is known to be a downstream target of P53 and is often induced and/or translocated to mitochondria as a part of the apoptotic process [37]. The enhanced apoptosis was also confirmed from the observed lowered expression level of Bcl-2. Bcl-2 is a proto-oncogene that encodes the largest known anti- apoptotic protein. Bcl-2 regulates apoptosis through the formation of homodimer proteins with the other proteins belonging to the Bcl-2 group, especially the pro-apoptotic protein Bax [38].

PAHs-induced apoptosis may also have a role in cancer initiation and promotion. Results showed significantly increased expression levels of mutant P53 in SCC cases. The observed higher level of mutant P53 accompanied with high Bax expression may explain the aggressive behavior of SCC as the mutant type P53 protein was unable either to induce apoptosis or to suppress SCC development and progression [39]. Mutation in P53 gene leads to the loss of its repair function and thus apoptosis resistance of the DNA-damaged cell and hence cancer development [40]. Hussain and Harris [41] found that mutations in P53 gene are the most frequently observed genetic alteration in cancer. P53 mutation may occur due to chromosomal instability, reactive nitrogen and oxygen species and formation of reactive metabolites which induce G to T transversion mutations [42]. The lack of Bcl-2 expression in SCC confirms the hypothesis that most of tumor cells originate from the suprabasal keratinocytes which normally have negative Bcl-2 expression [43]. The negative Bcl-2 expression in SCC was unexpected as Bcl-2 is known to protect tumor cells from apoptosis [44]. This may be possibly compensated by over expression of other anti-apoptotic proteins (not included in the study). So, it is suggested that disturbed apoptosis and SCC formation in PAHs exposed workers is not Bcl-2 dependent. Although the relatively high expression level of Bax; it appears to be unable to suppress the tumor progression. This may be due to expression of functionally inactive Bax [45]. Another explanation to tumor formation was reported by Holme and Solhaug [46] where they stated that although apoptosis of cells exposed to PAHs is considered to function anti-carcinogenic since cells with extensive DNA damage will be removed, it is possible on other hand that removal of these cells may give survival and proliferating signals to surrounding cells with less DNA damage, increasing their probability of having mutations and give promotion signals to cell proliferation and hence cancer formation.

Conclusion and Recommendation

It could be concluded that chronic exposure to asphalt fumes may increase the risk for developing dermatotoxic and/or cancer which could be explained by PAHs exposure can disturb P53, Bax and Bcl-2 apoptotic protein controlling apoptosis. The asphalt fumes could be considered as a risk factor disturbs apoptosis; however this disturbance needs further thorough investigation on other apoptotic proteins as Bcl-xl, Caspases and Fas.

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References

1. Schreiner CA (2011) Review of mechanistic studies relevant to the potential carcinogenicity of asphalts. Regul Toxicol Pharmacol 59: 270-284.
2. Michael F (1999) Asphalt. Chemical Engineering 77: 81.
3. King RW, Puzinauskas VP, Holdsworth CE (1984) Asphalt composition and health effects: A critical review. American Petroleum Institute Technical Publication, Washington, DC: 1-11.
4. Harrison RJ (2004) Polycyclic aromatic hydrocarbons. In: Ladou J (2006) (4th Edn) Current Occupational and Environmental Medicine. McGraw Hill, New York.
5. Yvonne CMS (2007) Polycyclic aromatic hydrocarbon induced effects on gene expression in relation to carcinogenic potency. Universitaire Pers Maastricht.
6. Bull SCC, HPA (2008) Polycyclic aromatic hydrocarbons (Benzo[a]pyrene): Toxicological overview 2008 Version 1.
7. Wang S, Sheng Y, Feng M, Leszczynski J, Wang L, et al. (2007) Light-induced cytotoxicity of 16 polycyclic aromatic hydrocarbons on the US EPA priority pollutant list in human skin HaCaT keratinocytes: relationship between phototoxicity and excited state properties. Environ Toxicol 22: 318-327.
8. (1995) Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological Profile for Polycyclic Aromatic Hydrocarbons (PAH). US Department of Health and Human Services, Atlanta, US.
9. Boffetta P, Jourenkova N, Gustavsson P (1997) Cancer risk from occupational and environmental exposure to polycyclic aromatic hydrocarbons. Cancer Causes Control 8: 444-472.
10. Sivak A, Niemeier R, Lynch D, Beltis K, Simon S, et al. (1997) Skin carcinogenicity of condensed asphalt roofing fumes and their fractions following dermal application to mice. Cancer Lett 117: 113-123.
11. Boffetta P, Burstyn I, Partanen T, Kromhout H, Svane O, et al. (2003) Cancer mortality Among European asphalt workers: an international epidemiological study. II. Exposure to bitumen fume and other agents. Am J Ind Med 43: 28-39.
12. Okona-Mensah KB, Battershill J, Boobis A, Fielder R (2005) An approach to investigating the importance of high potency polycyclic aromatic hydrocarbons (PAHs) in the induction of lung cancer by air pollution. Food Chem Toxicol 43: 1103-1116.
13. Zhao HW, Yin XJ, Frazer D, Barger MW, Siegel PD, et al. (2004) Effects of paving asphalt fume exposure on genotoxic and mutagenic activities in the rat lung. Mutat Res 557: 137-149.
14. Parker CM, Schreiner CA, Hallmark N, Kriech AJ, Osborn LV, et al. (2011) Evaluation of reproductive/developmental and repeated dose (subchronic) toxicity and cytogenetic effects in rats of a roofing asphalt fume condensate by nose-only inhalation. Regul Toxicol Pharmacol 59: 445-453.
15. Raulf-Heimsoth M, Marczynski B, Spickenheuer A, Pesch B, Welge P, et al. (2011) Bitumen workers handling mastic versus rolled asphalt in a tunnel: assessment of exposure and biomarkers of irritation and genotoxicity. Arch Toxicol 85: S81-S87.
16. Timothy D (2009) Apoptosis. Skin cancers. Medical Review Board.
17. Erb P, Ji J, Wernil M, Kump E, Glaser A, et al. (2005) Role of apoptosis in basal cell and squamous cell carcinoma formation. Immunol Lett 100: 68-72.
18. Lei W, Yu R, Mandlekar S, Kong AN (1998) Induction of apoptosis and activation of interleukin 1 beta-converting enzyme/Ced-3 protease (caspase-3) and c-Jun NH2-terminal kinase 1 by benzo (a) pyrene. Cancer Res 58: 2102-2106.
19. Salas VM, Burchiel SW (1998) Apoptosis in Daudi human B cells in response to benzo[a]pyrene and benzo[a]pyrene-7, 8-dihydrodiol. Toxicol Appl Pharmacol 151: 367-376.
20. Chin BY, Choi ME, Burdick MD, Strieter RM, Risby TH, et al. (1998) Induction of apoptosis by particulate matter: role of TNF-alpha and MAPK. Am J Physiol 275: L942-L949.
21. Gosset P, Garçon G, Casset A, Fleurisse L, Hannothiaux MH, et al. (2003) Benzo(a)pyrene-coated onto Fe2O3 particles-induced apoptotic events in the lungs of Sprague-Dawley rats. Toxicol Lett 143: 223-232.
22. Petrusz A, Ordronneau C (1983) Immunocytochemistry Practical applications in pathology and biology. Polak &Van noordeen, Bristol wright, PSG: 212.

23. Liang SB, Ohtsuki Y, Furihata M, Takeuchi T, Iwata J, et al. (1999) Sun-exposure- and aging-dependent P53 protein accumulation results in growth advantage for tumor cells in carcinogenesis of nonmelanocytic skin cancer. Virchows Arch 434: 193-199.

24. McClean MD, Wiencke JK, Kelsey KT, Varkonyi A, Ngo L, et al. (2007) DNA Adducts among Asphalt Paving Workers. Ann Occup Hyg 51: 27-34.

25. Manestar-Blazić T, Batinac T, Hadzisejdić I, Brajac I (2007) Apoptosis and immune response are responsible for the site-specific incidence of non-melanoma skin cancer. Med Hypotheses 68: 853-855.

26. Partanen T, Boffetta P (1994) Cancer risk in asphalt workers and roofers: review and meta-analysis of epidemiologic studies. Am J Ind Med 26: 721-740.

27. Voelter MS, Scheriau R, Zwahr G, Koch B, Escobar PLC, et al. (2007) Skin tumors among employees of a tar refinery: the current data and their implications. Int Arch Occup Environ Health 80: 485-495.

28. Sinha Hikim AP, Swerdloff RS (1999) Hormonal and genetic control of germ cell apoptosis in the testis. Rev Reprod 4: 38-47.

29. Bivik C, Rosdahl I, Ollinger K (2007) Hsp70 protects against UVB induced apoptosis by preventing release of cathepsins and cytochrome c in human melanocytes. Carcinogenesis 28: 537-544.

30. Ponten F, Lundeberg J (2003) Principles of tumor biology and pathogenesis of basal cell carcinoma and squamous cell carcinoma. (1st edn), JL Bolognia, JL Jorizzo, RP Rapini Dermatology. Mosby, Spain 2: 1663.

31. Solhaug A, Refsnes M, Låg M, Schwarze PE, Husoy T, et al. (2004) Polycyclic aromatic hydrocarbons induce both apoptotic and anti-apoptotic signals in Hepa1c1c7 cells. Carcinogenesis 25: 809-819.

32. Moll UM, Wolff S, Speidel D, Deppert W (2005) Transcription-independent pro-apoptotic functions of p53. Curr Opin Cell Biol 17: 631-636.

33. Barakat M, Abd-Elghany M (2007) Expression of Apoptosis and cell cycle regulatory proteins in cutaneous Squamous Cell Carcinoma. PALD 18: 31-41.

34. De Meo M, Genevois C, Brandt H, Laget M, Bartsch H, et al. (1996) In vitro studies of the genotoxic effects of bitumen and coal-tar fume condensates: comparison of data obtained by mutagenicity testing and DNA adduct analysis by 32P-postlabelling. Chem Biol Interact 101: 73-88.

35. Niida H, Nakanishi M (2006) DNA damage checkpoints in mammals. Mutagenesis 21: 3-9.

36. Efeyan AM, Serrano M (2007) p53: guardian of the genome and policeman of the oncogenes. Cell Cycle 6: 1006-1010.

37. Landvik NE, Arlt VM, Nagy E, Solhaug A, Tekpli X, et al. (2010) 3-Nitrobenzanthrone and 3-aminobenzanthrone induce DNA damage and cell signalling in Hepa1c1c7 cells. Mutat Res 684: 11-23.

38. Verhaegh ME, Sanders CJ, Arends JW, Neumann HA (1995) Expression of the apoptosis-suppressing protein Bcl-2 in non-melanoma skin cancer. Br J Dermatol 132: 740-744.

39. Chan S, Jong O, Kim I (1995) Detection of Bcl-2 family and Bax in head and neck cancers. J Korean Cancer Association 27: 570-577.

40. Erb P, Ji J, Wernli M, Kump E, Glaser A, et al. (2005) Role of apoptosis in basal cell and squamous cell carcinoma formation. Immunol Lett 100: 68-72.

41. Hussain SP, Harris CC (2007) Inflammation and cancer: an ancient link with novel potentials. Int J Cancer 121: 2373–2380.

42. vom Brocke J, Krais A, Whibley C, Hollstein MC, Schmeiser HH (2009) The carcinogenic air pollutant 3-nitrobenzanthrone induces GC to TA transversion mutations in human p53 sequences. Mutagenesis 24: 17-23.

43. Lu QL, Poulsom R, Wong L, Hanby AM (1993) Bcl-2 expression in adult and embryonic non-haematopoietic tissues. J Pathol 169: 431-437.

44. Xie X, Clausen OP, De Angelis P, Boysen M (1999) The prognostic value of spontaneous apoptosis, Bax, Bcl-2 and p53 in oral squamous cell carcinoma of the tongue. Cancer 86: 913-920.

45. Kurabayashi A, Furihata M, Matsumoto M, Ohtsuki Y, Sasaguri S, et al. (2001) Expression of Bax and apoptosis-related proteins in human esophageal squamous cell carcinoma including dysplasia. Mod Pathol 14: 741-747.

46. Holme JA, Solhaug A (2005) PAH-induced apoptosis. Abstracts/Toxicology Letters 158: S21-S258.