The effect of paclitaxel on rats following benzo(a)pyrene treatment

Didem Cosan, PhD, MSc, Ayse Basaran, PhD, Irfan Degirmenci, PhD, MSc, Hasan V. Gunes, PhD, MSc, Erinc Aral, PhD, MSc.

ABSTRACT

الأهداف: مراقبة آثار عقار باكليتاكسيل على الجرذان الذين تلقوا عقار بنزو– أي (benzo–a) بايرين.

الطريقة: في هذه الدراسة تم استعمال 45 ذكر جرذ تبلغ من العمر شهرين، من نوع سبراجو– داولي، بقسم الأحياء بكلية الطب بجامعة إسكيسهير عثمان غازي – تركيا، في عام 2006م. تم فحص البول والدم وعينات من أنسجة الكبد والكلى للجرذان الذين تلقوا عقار بنزو – أي (benzo–a) بايرين وعقار باكليتاكسيل في دراستنا هذه. قُيمت البيانات الإحيائية الكيميائية على عينات البول والدم، وعينات أنسجة الكبد والكلى على ضوء المجهر.

النتائج: كانت مستويات دسموتاس فوق الأكسدة، وأنشطة الكاتالاس وقيم مالونديالدهيد في المجوعة التي تلقت عقار بنزو– أي (benzo–a) بايرين مختلفة بشكل ملحوظ من مجموعة التحكم، و كانت معظم هذه القياسات قريبة من قيم التحكم في المجموعة التي تلقت عقار باكليتاكسيل عقب تلقي عقار بنزو– أي (benzo–a) بايرين. دعم الظهور النسيجي للعينات لجميع الجرذان أيضاً النتائج الحيوية الكيميائية.

خاتمة: أشارت الدراسة الحالية إلى أن تراكيب الكبد والكلى تتلف بواسطة عقار بنزو– أي (benzo–a) بايرين ويمكن استعادتها بواسطة عقار باكليتاكسيل.

Objective: To observe the effects of paclitaxel on rats that received benzo(a)pyrene.

Methods: In this study, 45 male Sprague-Dawley rats aged 2-month-old were used, which were housed at the Medical Biology Department of Eskisehir Osmangazi University, Eskisehir, Turkey in 2006. Urine, blood, liver, and kidney tissue samples of Sprague Dawley rats treated with benzo(a)pyrene and paclitaxel were examined in our study. Biochemical data were evaluated on urine and blood samples, and liver and kidney tissue samples were investigated by light microscopy.

Results: Superoxide dismutase, catalase activities, and malondialdehyde values in the group which received benzo(a)pyrene were significantly different than the control, and most of these parameters came close to control values in the group that received paclitaxel following benzo(a)pyrene application. Histological appearances of the samples of all rats also supported the biochemical results.

Conclusion: The present study indicated that liver and kidney structures damaged by benzo(a)pyrene may be restored by paclitaxel.

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From the Departments of Medical Biology (Cosan, Basaran, Degirmenci, Gunes), and Histology-Embryology (Aral), Medical Faculty, Eskisehir Osmangazi University, Eskisehir, Turkey.

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Address correspondence and reprint request to: Dr. Didem Cosan, Buyukdere Mh. Akdogan Sk. Birkonak Sitesi A Blok D 20, 26060 Eskisehir, Turkey. Tel. +90 (533) 3662680. Fax. +90 (222) 2392220. E-mail: dcosan@ogu.edu.tr

Benzo(a)pyrene [B(a)P] is a member of the polycyclic aromatic hydrocarbons (PAHs) group. Polycyclic aromatic hydrocarbons constitute the group that is researched as toxic and carcinogen substances, and many studies are also performed on this group as they are known to cause significant environmental pollution.[1,2] Polycyclic aromatic hydrocarbons are active carcinogen substances existing in coal tar. These chemical substances have been proven to cause cancer in test animals. Benzo(a)pyrene is the substance with the highest potential to cause cancer.[3] Furthermore, B(a)P has mutagenic and teratogenic effects.[1] Paclitaxel (Taxol), an anticancer drug that is highly efficacious in the treatment of several malignancies, is an antimicrotubule agent that stabilizes the microtubule network and inhibits the dynamics of microtubules.

Paclitaxel induces mitotic block and apoptosis.[4,5] In our study, we aimed to induce toxicity in rats by using B(a)P, then to examine the therapeutic effects of paclitaxel both biochemical and histological.

Methods. All experimental procedures were performed in accordance with the National Institute of Health's Principles of Laboratory Animal Care. In the present study, 45 male Sprague-Dawley rats aged 2-3-months old housed at the Medical Biology Department of Eskisehir Osmangazi University, Eskisehir, Turkey in 2006, were used. Sprague-Dawley rats of 250-350g weights were divided into 5 groups containing 9 rats each. The first group received saline [intravenously (IV), 0.4ml/kg], the second group received corn oil [intragastrically (IG), 1.2 ml/kg] and the third group received 10 mg/kg B(a)P (Sigma-Aldrich Inc., St. Louis, USA) IG once in 10 days for 40 days (total 40 mg/kg). In the fourth group, a single dose of 7.5 mg/kg paclitaxel (Bristol-Myers Squibb) IV was injected into the tail vein under the ether anesthesia. The fifth group received a single dose of 7.5 mg/kg paclitaxel (antineoplastic) IV followed by a total of 40 mg/kg B(a)P (toxic and carcinogen) IG application. While B(a)P dissolved in the corn oil, paclitaxel was available in dissolved form. Substances were prepared freshly on the day they were applied to the rats. Throughout the period of study, the rats were kept in an air-contained room and fed with a commercial standard diet and water ad libitum. In the conventional room, the light cycle was 12 hours light/12 hours dark, the temperature range was 22-24°C, and the range of air humidity was 40-60%. After the 24 days from paclitaxel treatment, all rats were taken into urine collection cages and 24 hours urine samples were collected in a bottle placed on ice. Blood samples were collected from the heart under ether anesthesia. Then liver and kidney samples of all rats were quickly fixed in neutral formalin. Levels of superoxide dismutase (SOD),[6] catalase activity (CAT),[7] and malondialdehyde (MDA),[8] which are products of lipid peroxidation were determined by preparing hemolysates of blood samples taken into ethylene diamine triacetic acid (EDTA) tubes. Hemoglobin (oxihemoglobin method) and hematocrit values were also determined. Serum creatinine, serum glutamic oxaloacetic transaminase (SGOT, Chroma test kit), serum glutamic pyruvic transaminase (SGPT, Chroma test kit) and alkaline phosphatase (ALP, Chroma test kit) activities were measured spectrophotometrically in other blood samples. After determining volume and pH values of collected urine samples, uric acid (Caraway's modified method), urea nitrogen (Urease-nesslerization method), and creatinine (Jaffe alkaline picrate assay) values were also measured spectrophotometrically. All spectrophotometric measurements were performed by means of Shimadzu UV-1601 digital spectrophotometer (Schimadzu Corp., Kyoto, Japan). Five micrometers thick tissue cross sections were selected and stained by hematoxylin-eosin after routine follow-up procedures on tissues. Randomly selected cross sections were examined by light microscope. This study was approved by the local ethics committee of Eskisehir Osmangazi University of Animal Experiments (no. 43/2007).

Statistical analysis. All data were expressed as means ± standard deviation. Differences between the means of various results were assessed for statistical significance by analysis of variance (ANOVA), followed by Tukey's multiple comparison tests. Statistical analyses were performed using the Statistical Package for Social Sciences version 10.0 for windows (SPSS Inc., Chicago, IL, USA). A p value <0.05 was considered to indicate statistical significance.

Results. All parameters were found similar between the control group (first group) and the corn oil group (second group). Superoxide dismutase increased in the third (p<0.001) and fifth (p<0.05) groups, CAT decreased in the third (p<0.001), fourth (p<0.05), and fifth (p<0.01) groups and MDA increased in the third (p<0.001), fourth (p<0.05), and fifth (p<0.01) groups compared to the control groups (**Table 1**). There was no difference between the control and other groups in terms of hemoglobin and hematocrit values. While SGOT enzyme activity increased only in the third group (p<0.001), SGPT enzyme activity increased in the third (p<0.001) and fifth (p<0.05) groups and ALP decreased in the third (p<0.001), fourth (p<0.05), and fifth (p<0.01) groups compared to the control groups (**Table 2**). While urine volume increased in the fourth (p<0.001) and fifth (p<0.01) groups, pH level decreased only in the third group (p<0.001) compared to the control groups. Urine uric acid, urine urea nitrogen,

Table 1 - Superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) levels of all groups (N=9 in each group).

Groups	SOD (U/gHb)	Erythrocyte CAT (U/l) Mean ± SD	MDA (U/gHb)
1	3408 ± 350	210.99 ± 9.11	97.80 ± 5.33
2	3581 ± 360	210.70 ± 4.42	95.15 ± 5.11
3	5742 ± 569‡	138.13 ± 6.68‡	132.28 ± 17.89‡
4	3580 ± 105	202.13 ± 4.51*	103.28 ± 2.45*
5	3781 ± 355*	198.53 ± 6.44†	105.03 ± 3.94†

*p<0.05, †p<0.01, ‡p<0.001

Table 2 - Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) . and alkaline phosphatase (ALP) levels of all groups (N=9 in each group).

Groups	Hemoglobin (g/dl)	Hematocrit (%)	SGOT (U/l)	SGPT (U/l)	ALP (U/l)
			Mean ± SD		
1	24.33 ± 3.85	47.70 ± 2.26	50.88 ± 7.16	14.84 ± 3.72	63.43 ± 5.85
2	26.60 ± 6.93	48.40 ± 6.39	50.49 ± 7.85	14.05 ± 1.83	59.72 ± 6.41
3	27.96 ± 5.36	48.73 ± 2.65	68.21 ± 4.02‡	28.64 ± 7.47‡	36.92 ± 2.69‡
4	25.00 ± 3.25	48.75 ± 2.83	52.68 ± 3.52	16.98 ± 2.07	58.04 ± 3.12*
5	26.89 ± 1.78	49.00 ± 2.51	51.71 ± 5.39	20.56 ± 7.13*	55.44 ± 3.55†

*p<0.05, †p<0.01, ‡p<0.001

Table 3 - Urine and creatinine values of all groups (N=9 in each group).

Groups	Urine volume (ml)	pH	Uric acid (mg/dl)	Urea nitrogen (mg/dl)	Urine creatinine (mg/dl)	Creatinine clearance (ml/min)	Serum creatinine (mg/dl)
				Mean ± SD			
1	09.75 ± 0.14	07.55 ± 0.99	10.32 ± 0.79	1690 ± 69	75.98 ± 3.19	0.38 ± 0.09	1.60 ± 0.32
2	09.69 ± 0.24	07.55 ± 0.50	10.36 ± 0.97	1702 ± 78	75.39 ± 2.42	0.33 ± 0.09	1.65 ± 0.24
3	09.83 ± 0.26	06.06 ± 0.49‡	08.92 ± 0.54‡	1548 ± 62‡	65.14 ± 2.65‡	0.19 ± 0.05‡	2.71 ± 0.30‡
4	09.11 ± 0.35‡	07.42 ± 0.95	09.50 ± 0.73*	1606 ± 87*	71.63 ± 4.26*	0.28 ± 0,06*	2.03 ± 0.43*
5	09.40 ± 0.25†	08.11 ± 0.95	09.25 ± 0.50†	1596 ± 63†	70.51 ± 2.72†	0.25 ± 0.09†	2.13 ± 0.29†

*p<0.05, †p<0.01, ‡p<0.001

urine creatinine, and creatinine clearance of the third (p<0.001), fourth (p<0.05), and fifth (p<0.01) groups decreased compared to the control groups, however, serum creatinine value increased in the third (p<0.001), fourth (p<0.05), and fifth (p<0.01) groups (**Table 3**).

Appearance and histological structures of liver and kidney cross-sections of animals in the control and corn oil groups were normal. Deformations occurred in the cord such as arrangements of hepatocytes of liver cross sections in the B(a)P group, and sinusoid congestion and Kupffer cell hyperplasia was observed in some areas. Furthermore, vacuolar degenerations were observed in some hepatocytes (**Figure 1**). Polymorph nuclear cell (PMNL) and mononuclear (MNL) cell increases also occurred in some areas. There was hydropic degeneration, and sinusoidal congestion in some hepatocytes of liver cross sections of the group treated with paclitaxel. Dilation of some distal tubules and hydropic degeneration of tubule cells was also observed in kidney cross-sections. Sinusoidal congestion was seen in paclitaxel-B(a)P liver cross sections and in a few liver lobules. There was no prevalent parenchymal degeneration in hepatocytes despite existence of hydropic degeneration (**Figure 2**). Mild hyperemia of kidney cross-sections and hydropic degeneration of some tubule cells were observed. All degenerative changes were significantly reduced compared to the third group (**Figure 3**).

Discussion. In the present study, urine, blood, liver, and kidney tissue samples treated with benzo(a)pyrene and paclitaxel were evaluated. Superoxide dismutase, CAT, and MDA in the hemolyzed blood were examined to investigate systemic results. Superoxide dismutase is found in all normal cells and mostly in erythrocytes. Superoxide dismutase protects cells against superoxide and hydrogen peroxide-mediated lipid peroxidation. Studies have reported decreasing SOD activity in some malignancies.[9] Superoxide dismutase activity of Sprague-Dawley rats were increased at the sixth and twenty-fourth hours, and reached the control levels at the ninety-sixth hours after they have received 20 mg/kg B(a)P.[10] The results of our study indicated a similar increase in SOD activity in case of toxicity formed by B(a)P in line with the above mentioned study. The enzyme antioxidant CAT is extensively found in

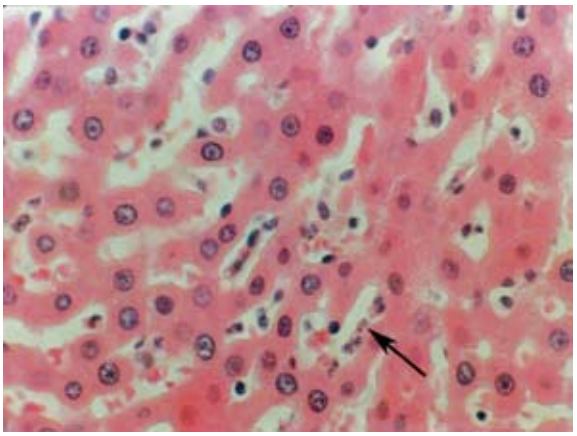

Figure 1 - Liver section in group 3. Note the deformation in the cord, such as arrangements of hepatocytes and polymorph nuclear cell infiltrations (arrow) (hematoxylin-eosin x132).

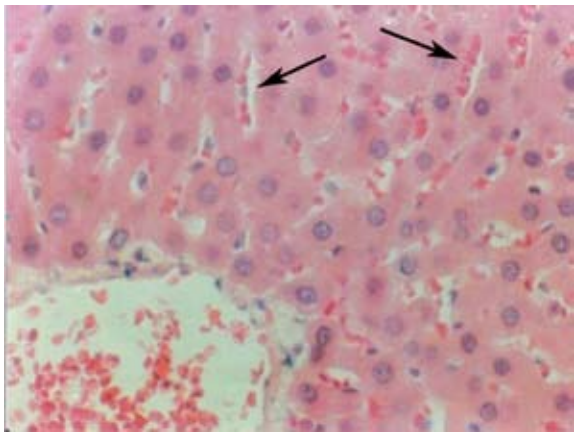

Figure 2 - Liver section in group 5. Note the sinusoidal dilatation and congestion (arrows) (hematoxylin-eosin x132).

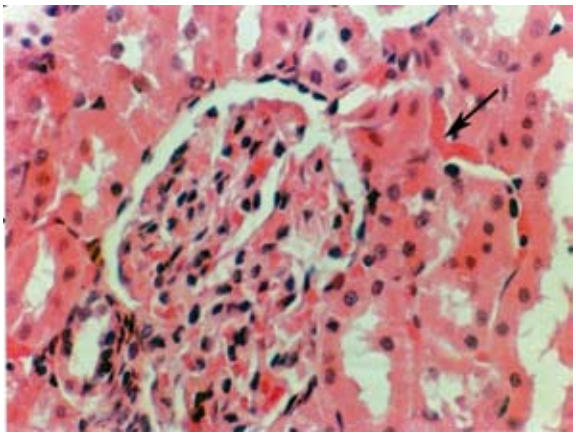

Figure 3 - Section of renal cortex in group 5. Note the mild hyperemia and degenerations of tubule cells (arrow) (hematoxylin-eosin x132).

all cells, and it catalyzes disintegration of hydrogen peroxide that is generated by tumor cells.[9] Low levels of CAT are expressed on tumor cells.[11] Studies have indicated that decreasing CAT activity in cancer may be due to the consumption of this enzyme, which catalyzes excessive production of hydrogen peroxide by cancer cells.[9] Another study has indicated that CAT activity in erythrocytes of Sprague-Dawley rats that received 20 mg/kg B(a)P significantly increased at the end of the twelfth and twenty-fourth hours, and reached control levels at the ninety-sixth hour.[10] Our study examined the effects of long term B(a)P treatment on CAT, and concluded that CAT activity might have decreased. As reported in a published study,[9] reason for the decrease in CAT activity may be the increase in lipid peroxides within the cell cycle.The number of MDA and various aldehydes increases as a result of peroxidation of membrane lipids.[12] The increase in product levels of lipid peroxidation was reported to play a role in the early phases of the tumor.[9] Another study has indicated that 20 mg/kg B(a)P led to increases in erythrocyte MDA levels at the twelfth hour and kept increased MDA levels constant for more than 96 hours.[10] Results of our study indicated that MDA levels of rats, which received long-term B(a)P increased compared to the control group. Although there is no study examining the effects of paclitaxel treatment following B(a)P on SOD and CAT activities and MDA level, there was a study in which paclitaxel was administered following 7,12 dimethyl benz(a)anthracene (DMBA). In this study, Sprague-Dawley rats received DMBA for inducing experimental breast cancer and they received 33 mg/kg paclitaxel once a week for 4 weeks. Superoxide dismutase and CAT values in breast and liver tissues of the group, which were treated with DMBA following paclitaxel had increased, and MDA levels had decreased.[9] Examination of SOD, CAT, and MDA values of the groups, which were administered paclitaxel following B(a)P in our study indicated a decrease in SOD activity and MDA values and an increase in CAT activity compared to the B(a)P treated group. The difference between our study and the research with DMBA is the decrease in erythrocyte SOD activity caused by paclitaxel treatment.

A study performed on Wistar rats has reported increases in SGOT, SGPT, and ALP enzyme activities in the groups that were treated with 10 mg/rat B(a)P.[13] As our study indicated a decrease in ALP, this research and our study do not resemble in terms of ALP. Although there is no study evaluating paclitaxel, SGOT, and ALP values together, ALP and SGOT values that are indicative of the liver function have been reported to decrease with treatment of docetaxel, a similar drug to paclitaxel.[14] Similarly, ALP values also decreased in our study. Although there is no study examining uric acid

and urea nitrogen in urine following B(a)P treatment, 6-nitrochrysene (6-NC) that is an environmental contaminant and carcinogen such as B(a)P was used in a research study. In this study, following the Syrian golden hamsters receiving 5 mg/kg 6-NC IP daily for 3 days, uric acid and blood urea nitrogen values in serums decreased.[15] Our study indicated that urine uric acid and urea nitrogen values decreased by benzo(a)pyrene treatment. In another study, there was no difference between blood urea nitrogen, and serum creatinine values of the control group, and Sprague-Dawley rats, which were administered 10 mg/kg B(a)P IP once a week for 5 weeks.[16] In our study, serum creatinine value in B(a)P treated rats increased. There is no research indicating that urine creatinine and creatinine clearance decrease with B(a)P treatment. Histological, degenerative changes in liver, and kidney cross section of the group, in which paclitaxel was administered followed by B(a)P were significantly restored.

In conclusion, the B(a)P treatment in rats damaged liver and kidney tissue structures and functions considering urine parameters, liver function tests, and histological appearances. The results indicated that paclitaxel treatment following B(a)P improves liver and kidney tissue degeneration considering biochemical parameters and histological appearances of tissues. Comparative data obtained from further investigations would be advanced to support our results.

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