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The Effects of Benzo(A) Pyrene Doxorubicin and Paclitaxel on P170 Glycoprotein

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Abstract : B(a)P is a mutagenic, carcinogenic and teratogenic substance. Paclitaxel and doxorubicin are antineoplastic drugs widely used in cancer treatment. The purpose of this study is to observe the effects of doxorubicin and paclitaxel on p170 glycoprotein in rat liver and kidney tissue after administration of B(a)P. As is well known, p170 glycoprotein is an indicator of drug resistance.

We hypothesized that a combination of these antineoplastic drugs would cause lower p170 levels and thus would have a stronger effect than single drug administration. For confirmation, a combination of drugs was used to prevent the development of drug resistance.

In this study, Sprague-Dawley rats were selected from a group 3-4 months of age. After administration of BaP and subsequently single or combined antineoplastic drugs, rats were sacrificed and their liver and kidney tissues were removed. Immunohistochemical analyses for p170 glycoproteins were performed on tissue samples. Excessive staining (4+) was noted in groups which received single drug therapy; the lowest staining (1+) was noted in groups which received combined drug therapy.

Since the p170 level in the tissues increased when single antineoplastic drugs was administered, and it decreased when a combination of the two drugs (doxorubicin and paclitaxel) was given, it is our conclusion that a combined use of these drugs offers greater benefits in the treatment of carcinogenic diseases.

Key Words: benzo(a)pyrene, doxorubicin, paclitaxel, p170 glycoprotein, rat.

Introduction

Benzo(a)pyrene is a polycyclic aromatic hydrocarbon (PAH) which is an effective mutagen, together with +(a)-anti-BP-7,8-diol-9,10 epoxide, one of the metabolites formed as a product of metabolism in the liver. This epoxide forms a covalent bond with the guanine base of DNA, causing damage to the DNA structure (1-4).

The cancer-treatment drug, doxorubicin, is an anthracycline. Doxorubicin enters between base pairs within the double strands of DNA in rapidly proliferating cancer cells, and, forming a diagonal bond, binds to the sugar-phosphates of the DNA. In this way it causes breakages in the DNA strands, thus obstructing the synthesis of RNA (5-14).

Paclitaxel, which is used for cancer treatment, binds to microtubules and stimulates microtubule polymerization. This polymerization obstructs progress from metaphase into anaphase (15-17).

If the dose and duration of paclitaxel and doxorubicin are not sufficient, a resistance related to excessive synthesis of p170 glycoprotein will develop. p170 glycoprotein is a membrane bond protein which effects the rejection of foreign agents entering cells and is encoded by the mdr1 gene on chromosome 7q21-31 (18-27).

Immunohistochemical staining for p170 shows the expression of this glycoprotein in tissue samples, and if it rises there is drug resistance in cells.

To prevent the development of drug resistance, a combination of the drugs is used, each with its own effective mechanism. In this way the p170 level drops and the drugs have a stronger effect than when administered singly (28, 29).

In this study, toxicity was induced by benzo(a)pyrene, following which doxorubicin and paclitaxel were administered both singly and in combination. Their effectiveness on p170 in liver and renal tissues was then investigated.

Materials and Methods

All experimental procedures were performed in accordance with ethical rules. In this study, 250-350 g Sprague-Dawley male rats, 3-4 months of age, were used. For the duration of the experiments, all rats were kept in an air-conditioned and hygienic environment with sufficient food and water.

The 81 Sprague-Dawley rats were divided into 9 groups. Details of the applications for each group are shown in Table 1. Benzo(a)pyrene (Sigma) was dissolved in corn oil, and doxorubicin (Adriblastina-Deva) in distilled water. Paclitaxel (Taxol Bristol-Myers Squibb) was already available in solution. All materials were prepared freshly on a daily basis.

Rats were sacrificied 3 days after drug applications. For the purpose of immunohistochemical analysis, all liver and kidney samples were taken from the same anatomic region and placed in a neutral formaline. Then paraffin blocks were obtained and the sections prepared from these blocks were used for immunohistochemical analysis by the streptavidin-biotin-peroxidase staining method. HistoStain SP kit (Zymed) and its antibody (Mouse Anti-

Table 1. Doses of the drugs, with their duration and application types.

GROUPS	DAY 1	DAY 11	DAY 21	DAY 31	DAY 41	DAY 42	DAY 43	DAY 44
I. CONTROL	0.3 ml water oral	0.3 ml water oral	0.3 ml water oral	0.3 ml water oral	0.3 ml distilled water i.p	0.3 ml distilled water i.p	0.3 ml distilled water i.p	0.3 ml physiologic saline i.v.
II. CORN OIL	0.3 ml corn oil oral	0.3 ml corn oil oral	0.3 ml corn oil oral	0.3 ml corn oil oral	0.3 ml distilled water i.p	0.3 ml distilled water i.p	0.3 ml distilled water i.p	0.3 ml physiologic saline i.v.
III. BENZO(A)PYRENE	10mg/kg benzo(a)-pyrene oral	10mg/kg benzo(a)-pyrene oral	10mg/kg benzo(a)-pyrene oral	10mg/kg benzo(a)-pyrene oral	0.3 ml distilled water i.p	0.3 ml distilled water i.p	0.3 ml distilled wateri.p	0.3 ml physiologic saline i.v.
IV. DOXORUBICIN	0.3 ml water oral	0.3 ml water oral	0.3 ml water oral	0.3 ml water oral	4mg/kg doxorubicin i.p.	4mg/kg doxorubicin i.p.	4mg/kg doxorubicin i.p.	0.3 ml physiologic saline i.v.
V. BENZO(A)PYRENE DOXORUBICIN	10mg/kg oral benzo(a)-pyrene oral	10mg/kg oral benzo(a)-pyrene oral	10mg/kg oral benzo(a)-pyrene oral	10mg/kg benzo(a)-pyrene oral	4mg/kg doxorubicin i.p.	4mg/kg doxorubicin i.p.	4mg/kg doxorubicin i.p.	0.3 ml physiologic saline i.v.
VI. PACLITAXEL	0.3 ml water oral	0.3 ml water oral	0.3 ml water oral	0.3 ml water oral	0.3 ml distilled water i.p	0.3 ml distilled water i.p	0.3 ml distilled water i.p	7.5 mg/kg paclitaxel i.v.
VII. BENZO(A)PYRENE PACLITAXEL	10mg/kg benzo(a)-pyrene oral	10mg/kg benzo(a)-pyrene oral	10mg/kg benzo(a)-pyrene oral	10mg/kg benzo(a)-pyrene oral	0.3 ml distilled water i.p	0.3 ml distilled water i.p	0.3 ml distilled wateri.p	7.5 mg/kg paclitaxel i.v.
VIII. DOXORUBICIN PACLITAXEL	0.3 ml water oral	0.3 ml water oral	0.3 ml water oral	0.3 ml water oral	4mg/kg doxorubicin i.p.	4mg/kg doxorubicin i.p.	4mg/kg doxorubicin i.p.	7.5 mg/kg paclitaxel i.v.
IX. BENZO(A)PYRENE DOXORUBICIN PACLITAXEL	10mg/kg benzo(a)-pyrene oral	10mg/kg benzo(a)-pyrene oral	10mg/kg benzo(a)-pyrene oral	10mg/kg benzo(a)-pyrene oral	4mg/kg doxorubicin i.p.	4mg/kg doxorubicin i.p.	4mg/kg doxorubicin i.p.	7.5 mg/kg paclitaxel i.v.

P-Glycoprotein, p170, Zymed) were used for this analysis. All procedures for staining were performed in accordance with the kit's instructions.

In all sections, enhancement of the staining revealed whether expression of p170 was excessive or lower. Assessment of the results was considered quantitatively and analyzed in a double-blind study. Evaluations of the stained preparations were made using light microscopy by three of the authors and scored independently. Levels of the staining were classified as minimal (1+), mild (2+), moderate (3+) or excessive (4+).

Results

Liver Results

In group I, the observed staining in hepatocytes was minimal (1+) (Figure 1). In group II, minimal staining (1+), similar to that of the control group, was observed in the hepatocytes. In group III, staining in the hepatocytes was observed to be greater (3+) than in the control group (Figure 2). In group IV, less staining (2+) was observed than for group III (Figure 3). The most staining was observed in group V (4+) (Figure 4). Staining in group VI was observed to be similar (2+) to that of IV, while staining in some hepatocytes in group VII was similar (4+) to that of group V. Groups VIII and IX had minimal staining (1+) similar to that of the control (Figure 5) (Table 1).

Kidney Results

In group I, minimal staining (1+) was observed in the proximal tubule cells of the kidney (Figure 6). In group II,

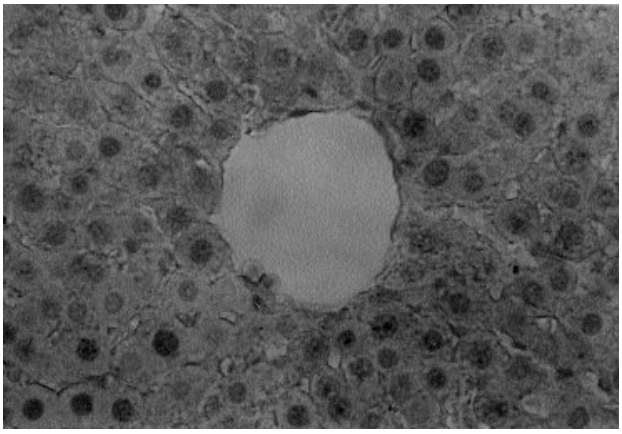

Figure 1. Minimal staining in hepatocytes of control group. Streptavidin-biotin-peroxidase staining method. x 132

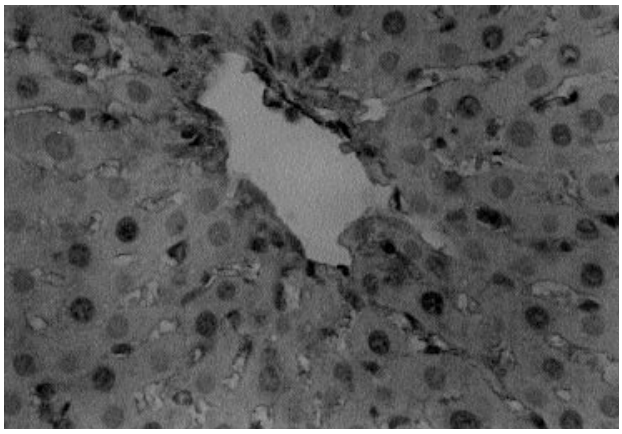

Figure 2. Moderate staining hepatocytes of benzo(a)pyrene group. Streptavidin-biotin-peroxidase staining method. x 132

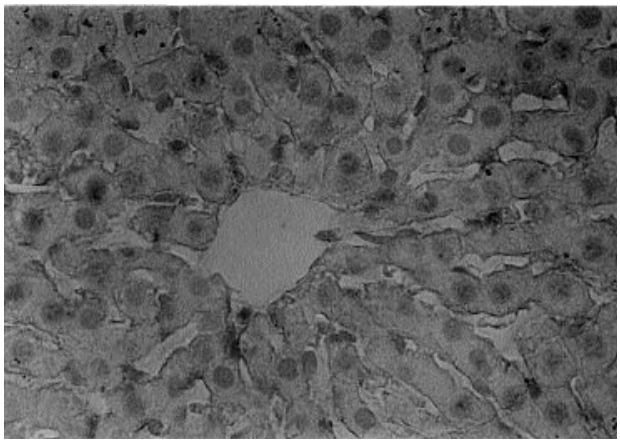

Figure 3. Mild staining in hepatocytes of doxorubicin group. Streptavidin-biotin-peroxidase staining method. x 132

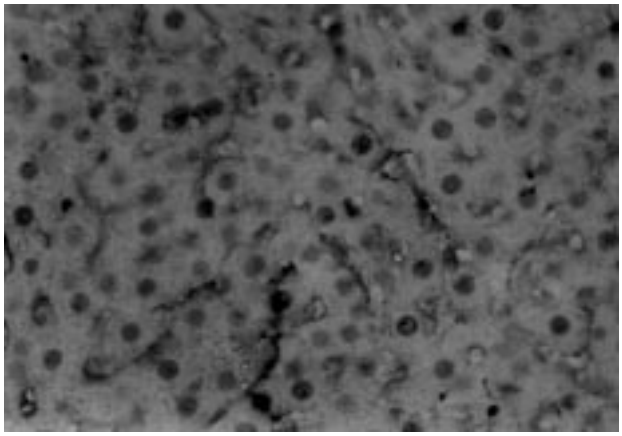

Figure 4. Excessive staining in hepatocytes of doxorubicin and benzo(a)pyrene group. Streptavidin-biotin-peroxidase staining method. x 132

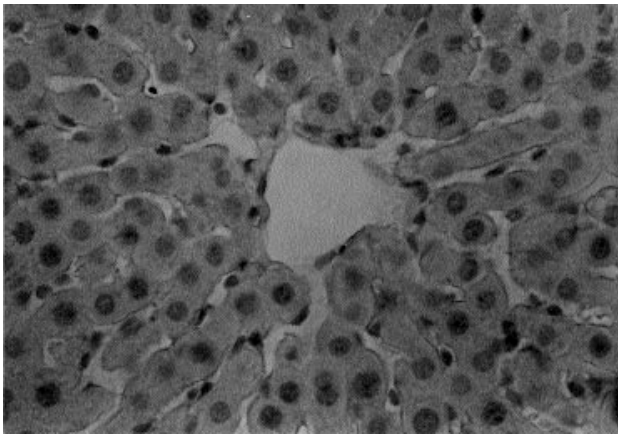

Figure 5. Minimal staining in benzo(a)pyrene, doxorubicin and paclitaxel groups' hepatocytes was similar to that of the control group. Streptavidin-biotin-peroxidase staining method. x 132

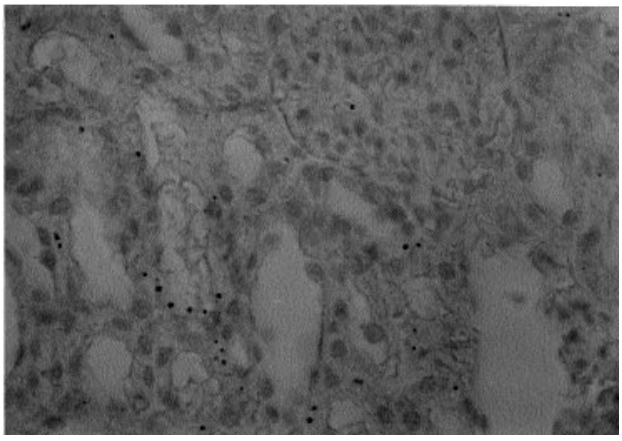

Figure 6. Minimal staining in proximal tubule cells of control group. Streptavidin-biotin-peroxidase staining method. x 132

staining was minimal and similar (1+) to that of the control group. In group III, staining was greater (3+) than in the control group (Figure 7) and in group IV, staining was less (2+) than in group III (Figure 8). The most staining (4+) was observed in group V (Figure 9). Staining in the tubule cells of groups IV and VI were observed to be similar (2+). In the lumen-facing surfaces of the proximal tubule cells in group VII excessive staining (4+) was observed, similar to that of group V. In group IX and the tubule cells of group VIII, minimal staining (1+), similar to that of the control, was observed (Figure 10) (Table 2).

Discussion

When the drugs are individually administered, the immunohistochemical examination showed p170 glycoprotein, which functions in the pumping out of foreign material entering the cell, to be highest in groups V and VII, and to be slightly higher than the control in groups III, IV and VI. When the drugs are given in combination, the results in group IX are observed to be similar to those of the control. Antineoplastic drugs (doxorubicin or paclitaxel) were administered alone, and p170 glycoprotein production greatly increased. This was a result of p170 gene amplification (30,31). The

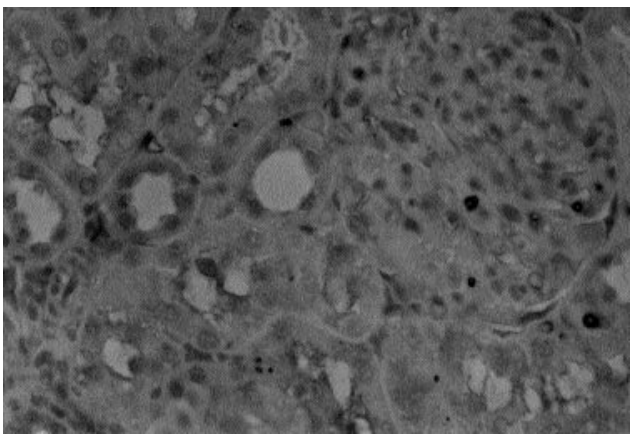

Figure 7. Moderate staining in proximal tubule cells of benzo(a)pyrene group. Streptavidin-biotin-peroxidase staining method. x 132

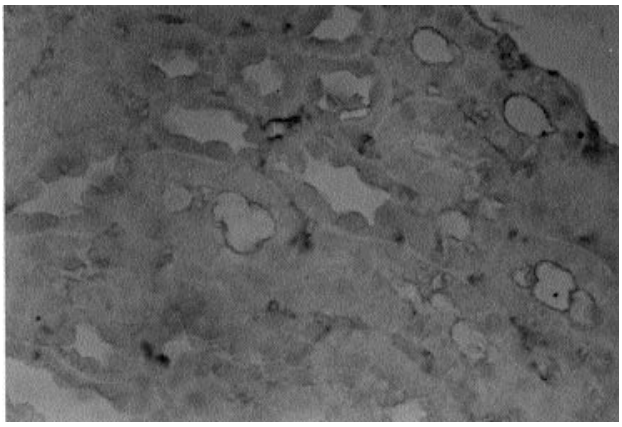

Figure 8. Mild staining in tubule cells of doxorubicin group. Streptavidin-biotin-peroxidase staining method. x 132

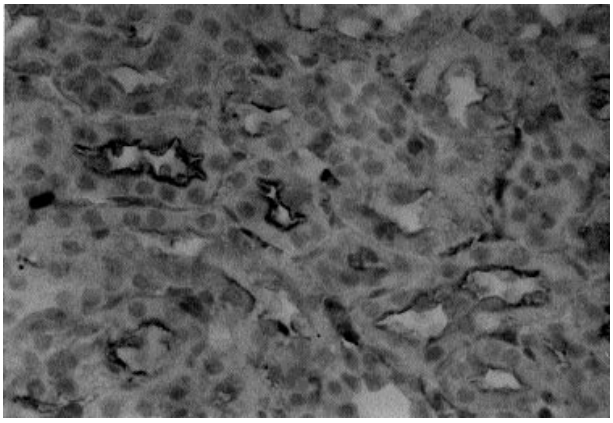

Figure 9. Excessive staining in tubule cells of doxorubicin and benzo(a)pyrene group. Streptavidin-biotin-peroxidase staining method. x 132

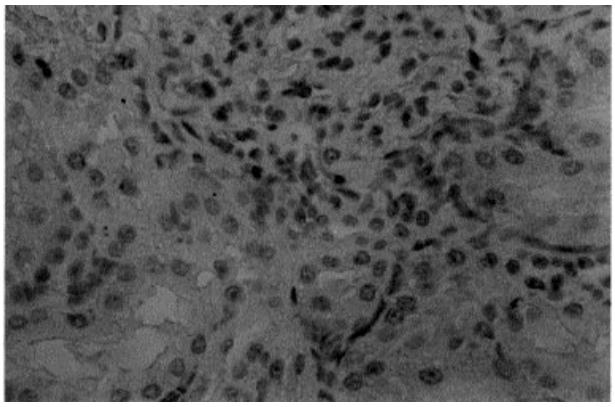

Figure 10. Minimal staining in benzo(a)pyrene, doxorubicin and paclitaxel groups' tubule cells was similar to that of the control group. Streptavidin-biotin-peroxidase staining method. x 132

Table 2. Classifed results of the levels of immunohistochemical staining for p170 glycoprotein renal and liver tissue samples.

CONTROL (n=9)	CORN OIL (n=9)	B(A)P (n=9)	DXR (n=9)	B(A)P+ DXR (n=9)	PLX (n=9)	B(A)P+ PLX (n=9)	DXL+ PLX (n=9)	B(A)P+ DXL + PLX (n=9)
+	+	+++	++	++++	++	++++	+	+

B(A)P: Benzo(a)pyrene, DXR: Doxorubicin, PLX: Paclitaxel

increased quantities of p170 glycoprotein cause the drugs to be pumped out of the cells before they can take effect sufficiently (31,32). It has been established that some antineoplastic drugs other than doxorubicin and paclitaxel have different effects on the development of multidrug resistance, depending on whether they are administered alone or in combinations (25, 30, 33). For this reason, in order to prevent the development of resistance to treatment, a combination of drugs with different mechanisms, each known to be effective singly against its particular tumor type, is recommended (28). In our study, combined administration of paclitaxel and doxorubicin was shown to be more effective than individual administration of either.

In our study, objective evaluation of the staining intensity was difficult, but we believe that our classification in the staining levels gave an indication of p170 glycoprotein expression (34).

Conclusion

Although further studies on the resistance of these drugs in human beings are needed, our study indicates that the doxorubicin-paclitaxel combination prevents the excessive expression of p170 glycoprotein.

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