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Outcomes of penicillin toxicities on DNA in rats

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Penicillin is a non-toxic class of antibiotics, which are used for most infections caused by Gram-negative cocci and majority Gram-positive bacteria. Sodium salt of penicillin G reaches a maximum of plasmatic concentration in 15 or 30 minutes. Although many indication of penicillin derivations are also clinically has toxic and adverse effects. Organ toxicity in urinary bladder,[1] DNA damage in children with rheumatic fever,[2] convulsions induced by penicillin and neurotoxic effects,[2,3] were studied in literature. However, no studies of any kind have investigated the effects of penicillin's toxicity on DNA quantity in muscles and organs. Thus, the purpose of this study was to show effect of penicillin derivates toxicity on DNA quantities in muscles and organs in rats with meningitis.

The study was conducted in the Faculty of Arts and Sciences, Dumlupinar University, Kutahya, Turkey between the year 2002-2004. This study was approved by the local Institutional Ethic Committee. Experiments were conducted in 160 adult female rats (Sprague Dawley). There were 10 rats in each group (12 experimental and 4 control groups). We tested a well-characterized rat's model of pneumococcal meningitis. Animals were weighed and meningitis was induced by transcutaneous intracisternal injection of 20 µl of 1×10^7 colony-forming units (cfu)/ml of *Streptococcus pneumoniae* type 3 after short-term anesthesia with halothane. All infected rats showed clinical signs of meningitis and the following day, penicillin derivatives have been injected as intraperitoneal 100 mg/kg/day to the experimental groups 3 times a week and during 60 day. After an overdose of thiopental, rats were perfused transcardially and the organs and muscles were extracted and rapidly frozen on dry ice. Certain grams (3 gm liver, 2.7 gm lung, 2.4 gm brain, 3.6 gm kidney, 1.02 gm tongue and 2.5 gm ovary, 5 gm from each muscle) of frozen organs or muscles were thawed, and washed in 5 times in 100 ml ice-cold portions of 0.1 M aqueous sodium citrate (pH 7.4) or 0.1 ethylenediaminetetraacetate (EDTA) (pH 7.35). The homogenate is centrifuged for 30 minutes at 1800 x g and the supernatant discarded. The final residue was extracted with 1000 ml of 2 M aqueous NaCl solution and was left at 4^0C for 1 day and then centrifuged for 1 hour at 1900 x g. To the opalescent supernatant 2 volume of absolute ethanol is added at a rate of 150 ml/ hr with constant slow swirling of the recipient flask; washed in 75% aqueous ethanol, drained, and redissolved, as described before, in a cooled tissue grinder in 1000 ml of 0.14 M aqueous NaCl solution made 0.015 M with respect to sodium citrate (pH 7.1), hereafter called "standard buffer". To this solution is added 1/9 volume of 5% Duponol in 45% aqueous ethanol and the mixture was stirred for 1 hour at room temperature. The mixture, which contains precipitated Duponol is then centrifuged for one hour at 31.000-x g to the clear supernatant 2 volume of ethanol redissolved in 1000 ml of standard buffer with 1/9 volume of 5% Duponol. Solid NaCl is then added to obtain 5% NaCl solution, and the mixture is stirred, stored, and centrifuged. To the final clear supernatant, 2 volume of absolute ethanol is slowly added, and the fibers are lifted, washed in 75% ethanol, and redissolved in standard buffer to yield a clear, very viscous solution, containing 1 - 2 microgram of DNA /per milliliter. The product is stored in the frozen state at

Table 1 - Antibiotics toxicities on DNA quantity of organ and muscles (intra-peritoneal injection of antibiotics per kg live weight and 3 times in a week and during 60 days).

Organ*	Penicillin V (Potassium salt)	Penicillin G (Sodium salt)	Cephapirin (Sodium salt)	Control (Healthy rats)	Cloxacillin (Sodium monohydrate salt)	Dicloxacillin (Sodium monohydrate salt)	Nafcillin (Sodium salt)	Control (Healthy rats)
Liver	764 ± 1.3^{a}	527 ± 2.9^{b}	408 ± 3^{c}	1314 ± 2^{d}	192 ± 1.2^{b}	112 ± 0.2^{a}	120 ± 2.2^{a}	829 ± 2.1^{c}
Lung	653 ± 2.8^{a}	532 ± 3.1^{b}	393 ± 4.7^{c}	1314 ± 1.7^{d}	189 ± 2.4^{b}	73 ± 0.7^{a}	82 ± 1^{a}	729 ± 2.1^{c}
Brain	853 ± 0.3^{a}	528 ± 6.2^{b}	402 ± 5^{c}	1321 ± 1^{d}	235 ± 2^{a}	231 ± 3.2^{a}	238 ± 3.2^{a}	772 ± 0.2^{b}
Kidney	749 ± 1.1^{a}	520 ± 4.4^{b}	401 ± 0.2^{c}	1299 ± 4.7^{d}	321 ± 3^{a}	324 ± 4^{a}	627 ± 4.9^{b}	928 ± 4.2^{c}
Tongue	675 ± 5.4^{a}	349 ± 4^{b}	273 ± 2.1^{c}	1161 ± 5.1^{d}	453 ± 0.9^{a}	678 ± 3.8^{b}	872 ± 4.9^{c}	1392 ± 4^{d}
Ovary	428 ± 2.4^{a}	198 ± 2.8^{b}	203 ± 5^{c}	1128 ± 5.8^{d}	738 ± 4.5^{a}	920 ± 4.2^{b}	927 ± 5.9^{b}	1820 ± 7.4^{c}
Heart	474 ± 3^{a}	273 ± 0.1^{a}	285 ± 2.8^{a}	1125 ± 6.1^{b}	773 ± 6.3^{a}	975 ± 4^{b}	963 ± 7.2^{b}	1798 ± 8.3^{c}
Muscles*								
Gluteus Maximus	666 ± 4.8^{a}	671 ± 2.2^{a}	673 ± 4.9^{a}	1295 ± 6.8^{b}	345 ± 4^{a}	653 ± 6^{b}	991 ± 3.8^{c}	1829 ± 7.8^{d}
Deltoid	882 ± 3.5^{a}	625 ± 3.5^{b}	478 ± 3^{c}	1328 ± 7^{d}	325 ± 4.2^{a}	638 ± 7.2^{b}	910 ± 5.1^{c}	1789 ± 4^{d}
Latissimus dorsi	914 ± 3.4^{a}	703 ± 4.9^{b}	598 ± 4.8^{c}	1419 ± 7^{d}	331 ± 1.1^{a}	630 ± 7.4^{b}	992 ± 5.3^{c}	1928 ± 3.9^{d}
Intercostals	988 ± 4.9^{a}	982 ± 5.1^{a}	709 ± 5.6^{b}	1347 ± 1^{c}	472 ± 1.2^{a}	720 ± 8.4^{b}	978 ± 7.8^{c}	1927 ± 7^{d}
Quadriceps femoris	991 ± 3.9^{a}	989 ± 4.1^{a}	738 ± 3.1^{b}	1728 ± 4.6^{d}	652 ± 3^{a}	863 ± 5.3^{b}	995 ± 5^{c}	1923 ± 4^{d}
Quadratus lumbarum	975 ± 6.3^{a}	977 ± 4.1^{a}	676 ± 4.2^{b}	1451 ± 6.2^{c}	527 ± 3.6^{a}	873 ± 8.9^{b}	994 ± 3.9^{c}	1978 ± 7.9^{d}
Pectoralis Muscles	598 ± 7.1^{a}	590 ± 4.3^{a}	473 ± 4.1^{a}	1300 ± 4.2^{b}	634 ± 4.2^{a}	829 ± 4.7^{b}	1302 ± 4.1^{c}	1900 ± 5^{d}
Teres major	539 ± 4.8^{a}	536 ± 1.1^{a}	329 ± 3.9^{b}	1281 ± 3.2^{c}	598 ± 3.1^{a}	892 ± 4^{b}	1293 ± 6.1^{c}	1913 ± 6.3^{d}
Tibialis anterior	779 ± 6.9^{a}	780 ± 3^{a}	962 ± 6.3^{b}	1432 ± 2.8^{c}	442 ± 0.1^{a}	872 ± 8.3^{b}	1116 ± 3.2^{c}	1962 ± 7.2^{d}
Gastro-Soleus	583 ± 3^{a}	590 ± 5^{a}	654 ± 3.2^{b}	1435 ± 2.1^{c}	465 ± 2.1^{a}	898 ± 2.4^{b}	993 ± 3.1^{c}	1973 ± 7.1^{d}
Hamistrings	882 ± 5^{a}	891 ± 6.1^{a}	375 ± 2^{a}	1189 ± 3^{b}	492 ± 3.9^{a}	920 ± 3.1^{b}	1298 ± 6^{c}	1989 ± 8.3^{d}
Abdominal	901 ± 6^{a}	894 ± 3.3^{a}	311 ± 2.2^{b}	1154 ± 2^{c}	476 ± 3.2^{a}	882 ± 3^{b}	1129 ± 7.9^{c}	1995 ± 7.9^{d}

Table 1 - *(Continuation)*

Organ*	Cefazolin (Sodium salt)	Cefoperazone (Sodium salt)	Oxacillin (Sodium monohydrate salt)	Control (Healthy rats)	Cephalonium	Benzylpenicillin	Phenoxyme-thylpenicillin	Control
Liver	885 ± 3.2^{a}	510 ± 2.9^{b}	408 ± 4^{c}	1325 ± 2.2^{d}	125 ± 1.2^{a}	103 ± 0.1^{a}	128 ± 1.3^{a}	1203 ± 4.1^{b}
Lung	771 ± 2.1^{a}	533 ± 3.1^{b}	404 ± 3.2^{c}	1317 ± 4.3^{d}	143 ± 2^{b}	98 ± 1.2^{a}	125 ± 2.5^{a}	1192 ± 4.9^{c}
Brain	765 ± 2.4^{a}	525 ± 5^{b}	409 ± 5.9^{c}	1327 ± 4.9^{d}	82 ± 0.1^{a}	81 ± 1.1^{a}	85 ± 1^{a}	552 ± 2.1^{b}
Kidney	753 ± 2.1^{a}	507 ± 6.2^{b}	398 ± 6^{c}	1335 ± 6.2^{d}	73 ± 2.3^{a}	65 ± 1^{a}	197 ± 2.1^{b}	991 ± 2.1^{c}
Tongue	682 ± 5.3^{a}	365 ± 2.6^{b}	267 ± 6.2^{c}	1165 ± 4.4^{d}	167 ± 1.9^{a}	173 ± 1.9^{a}	185 ± 3.7^{a}	1226 ± 5.4^{b}
Ovary	298 ± 3.7^{a}	217 ± 3^{b}	201 ± 5.3^{c}	1124 ± 6.1^{d}	389 ± 2.9^{c}	112 ± 1.3^{a}	221 ± 6.2^{b}	1720 ± 7.2^{d}
Heart	287 ± 2.1^{a}	271 ± 4.4^{a}	262 ± 1.3^{a}	1132 ± 4^{b}	421 ± 3.3^{c}	109 ± 1^{a}	234 ± 2.9^{b}	1892 ± 3.9^{d}
Muscles*								
Gluteus Maximus	662 ± 3.9^{a}	675 ± 4.7^{a}	672 ± 6.3^{a}	1290 ± 4.2^{b}	533 ± 4^{b}	227 ± 3^{a}	752 ± 0.2^{c}	1902 ± 4.6^{d}
Deltoid	888 ± 1.1^{a}	618 ± 3.3^{b}	492 ± 2.5^{c}	1318 ± 1.1^{d}	528 ± 2^{b}	298 ± 2.1^{a}	878 ± 3.2^{c}	1899 ± 7^{d}
Latissimus dorsi	906 ± 2^{a}	721 ± 4.7^{b}	611 ± 2^{c}	1426 ± 3.9^{d}	782 ± 3.3^{b}	226 ± 1.3^{a}	983 ± 4.3^{c}	1872 ± 7.5^{d}
Intercostals	989 ± 3.8^{a}	982 ± 5.2^{a}	721 ± 5.3^{b}	1355 ± 6^{c}	653 ± 4.5^{b}	302 ± 3.6^{a}	830 ± 3.3^{c}	1779 ± 6.9^{d}
Quadriceps femoris	910 ± 1^{a}	903 ± 5^{a}	736 ± 3.4^{b}	1733 ± 3.1^{d}	542 ± 4.2^{b}	287 ± 2.5^{a}	775 ± 1.4^{c}	1100 ± 2.9^{d}
Quadratus lumbarum	981 ± 5^{a}	985 ± 2.1^{a}	682 ± 2^{b}	1453 ± 6^{c}	440 ± 2.1^{b}	265 ± 1^{a}	862 ± 5.4^{c}	1992 ± 2^{d}
Pectoralis Muscles	460 ± 2^{a}	466 ± 0.3^{a}	483 ± 3.9^{a}	1309 ± 5.3^{b}	469 ± 4.9^{b}	129 ± 1.1^{a}	926 ± 5.4^{c}	1525 ± 5.3^{d}
Teres major	527 ± 6^{a}	534 ± 1^{a}	315 ± 1.1^{b}	1288 ± 5^{c}	490 ± 3.2^{b}	217 ± 1.3^{a}	885 ± 2^{c}	1302 ± 3.9^{d}
Tibialis anterior	784 ± 1^{a}	777 ± 6^{a}	966 ± 3.6^{b}	1438 ± 2^{c}	312 ± 2.2^{b}	105 ± 1.2^{a}	765 ± 4.6^{c}	1420 ± 4^{d}
Gastro-Soleus	428 ± 3.8^{a}	425 ± 3.2^{a}	641 ± 4.3^{b}	1965 ± 3.3^{c}	319 ± 1.1^{b}	98 ± 0.2^{a}	698 ± 4.1^{c}	1398 ± 2.9^{d}
Hamistrings	345 ± 3.8^{a}	355 ± 1.1^{a}	388 ± 4.4^{a}	1973 ± 2.9^{b}	329 ± 2.1^{b}	126 ± 2.1^{a}	752 ± 6.2^{c}	1572 ± 3.8^{d}
Abdominal	425 ± 3.5^{a}	433 ± 5.2^{a}	312 ± 3.9^{b}	1964 ± 4.4^{c}	120 ± 2.4^{a}	489 ± 3.4^{b}	777 ± 2^{c}	1673 ± 5.8^{d}

Data is shown with mean ± SEM, *Values shown with different letters in the same horizontal column are significant (p<0.01); values shown with same letter are not significant (p>0.05).

-15°C. Storage in nonbuffered of low ionic strength as well as drying was avoided, as they invariably lead to a degraded product. All the steps are performed at 0-4 °C, with glass, rubber, or plastic tools and vessels. According to calf's thymus standard DNA (D-1501), was purchased from Sigma, our DNA samples obtained from organ and muscles was 87% purified.[4,5] Statistical analysis was performed with SPSS software (version 10, Chicago, USA). The mean ± SEM results of groups were calculated by one-way analysis of variance. A probability value of $p<0.05$ was considered significant.

Penicillin derivates toxicity were statistically decreased in DNA quantity than healthy control group rats (**Table 1**) ($p<0.01$). According to healthy control group's DNA quantity, penicillin's were decreased minimum 32.4% and maximum 94.2% in DNA quantity ($p<0.01$). In all penicillin's toxicity, the highest DNA damage were observed in Benzyl penicillin toxicity in organ (85.3 - 94.2%) and the muscle tissue (83.3-93%) (**Table 1**). The neurotoxic potential of intravenous benzylpenicillin, nonetheless, is not simply related to the administered dose or serum levels. The weight of current evidence, largely from animal studies, suggests that brain tissue antibiotic concentrations rather than the cerebrospinal fluid concentrations are predictive of neurotoxicity.[2,3] The parent compound of imipenem itself was subsequently found to be neurotoxin and most probably shares the Gamma amino butyric acid (GABA)-mediated mechanism with penicillins. As a matter of fact, the higher incidence of neurotoxicity with imipenem-cilastatin could have been related to the side chain on the second carbon atom; this side chain is more basic (compared with that of meropenem) and hence, has higher binding avidity to GABA receptors.[6] In our study, brain DNA damages 35.4 - 85.3% were observed with penicillin derivates toxicity. Results of this study are important and in concordance with Schliamser et al[2,3] studies. In patients with chronic diseases and those who routinely received penicillin are under DNA damages in multiple organs and muscles. Some investigators studied 13-week toxicity in F344/N rats and B6C3F1 mice to determine the general toxicity and target organ toxicity with amphetamine sulfate, ampicillin trihydrate, codeine, 8-methoxyp-soralen, alpha-methyldopa sesquihydrate, penicillin VK, and propantheline bromide.[1] The dose levels administered to rodents with doses used in the treatment of human disease. At higher doses, target organ toxicity are seen which includes the urinary bladder in male rats after propantheline bromide; the glandular stomach in rats and mice after penicillin VK; the liver and adrenals in rats after 8-methoxypsoralen; and the kidney in mice and rats after alpha-methyldopa. In this present study, the muscle DNA damage results are reflected to muscle atrophy and decreased in strength. Thus, further studies are needed to investigate penicillin toxicity in human DNA. On the otherhand, patients treated with penicillin should have special exercises to prevent muscle atrophy. Consequently it should be noted that penicillin derivates toxicity can cause muscles and organs DNA damages in rats with meningitis.

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