Therapeutic Efficiency of Dimephosphone and Xydiphone in Experimental Pulse Therapy with Prednisolone

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Experiments on rats showed that pulse therapy with prednisolone (100 mg/kg intraperitoneally for 3 days) stimulated urinary excretion of hydroxyproline, increased the content of inorganic phosphorus, promoted the increase in the content of dienic conjugates and catalase activity, and decreased serum levels of MDA and ceruloplasmin. Ten-day treatment with dimephosphone (208 mg/kg) or xydiphone (45 mg/kg) after pulse therapy with prednisolone normalized urinary excretion of hydroxyproline and reduced the levels of dienic conjugates. Dimephosphone did not change, while xydiphone normalized the level of MDA decreased by prednisolone.

Key Words: *glucocorticosteroids; lipid peroxidation; hydroxyproline; dimephosphone; xydiphone (ethydronate)*

Pulse therapy (PT) with glucocorticosteroids (GCS), an intensive method for the treatment of systemic diseases of the connective tissue, is more effective that common methods of therapy. The use of PT is substantiated by inefficiency of nonsteroid and basic therapy. PT with prednisolone is a relatively safe method, but it can be associated with some side effects including impairment of phosphorus-calcium metabolism and LPO status [7,15]. We investigated changes in the mineral metabolism, LPO, and antioxidant system in rats receiving PT with prednisolone and evaluated the possibility of correcting these changes by dimephosphone and xydiphone, whose efficiency was previously demonstrated on the model of prednisolone-induced osteoporosis [4].

MATERIALS AND METHODS

Experiments were carried out on 32 random-bred albino rats of both sexes (4 groups, 4 males and 4 females per group) weighing 150-200 g. The animals were fed standard vivarium rations. Controls were daily (for 3 days) intraperitoneally injected with normal saline and then received the drug (1 ml/100 g) through a gastric tube for 10 days. PT was simulated in 3 experimental groups by daily (for 3 days) intraperitoneal injections of prednisolone in a dose of 100 mg/kg. After prednisolone PT group 1 animals received normal saline (1 ml/100 g) intragastrically (through a tube), while group 2 and 3 animals received dimephosphone (208 mg/kg: 2.08% solution, 1 ml/100 g) [4] and xydiphone (45 mg/kg (0.45% solution, 1 ml/100 g), respectively [1], for 10 days. On day 14 of the experiment the animals were decapitated under light ether narcosis. Total hydroxyproline in the urine was measured by spectrophotometry using the paradimethylaminobenzaldehyde reaction [9], serum alkaline phosphatase was measured using Bio-LACHEMA kit [6]. Inorganic phosphorus in the blood and urine was measured by colorimetry in the ammonium molybdate test [6], total calcium in the blood and urine was measured by colorimetry in the *o*-cresolphthaleine test [6], and total serum protein was measured by refractometry. Catalase activity in blood erythrocytes was measured using ammonium molybdate [5], peroxidase activity

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TABLE 1. Hydroxyproline, Total Calcium, and Inorganic Phosphate Levels in the Urine of Rats after a 3-Day Course of Prednisolone and Subsequent 10-Day Oral Treatment with Normal Saline, Dimephosphone, or Xydiphone (*M*±*m*, *n*=8)

Group		Hydroxyproline, μg/ml	Total calcium, mmol/liter	Inorganic phosphorus, mmol/liter
Control		4.16±0.50	0.17±0.02	10.3±1.0
1	prednisolone+normal saline	7.6±1.0* (183)	0.18±0.02 (106)	5.4±1.6 (52)
2	prednisolone+dimephosphone	4.9±0.3 (118)	0.10±0.01* (59)	10.7±0.8 (104)
3	prednisolone+xydiphone	3.9±0.6 (94)	0.12±0.01* (71)	6.5±3.3 (63)

Note. Here and in Tables 2, 3: *$p<0.05$ *vs.* control. % of control is shown in parentheses.

was evaluated by color reaction with indigo carmine [8]. Serum ceruloplasmin was measured using paraphenylenediamine hydrochloride [13]. Total antioxidant activity (AOA) was measured in the yolk lipoproteins prepared by mixing chicken yolk with phosphate buffer (1:50). This model contained lipid protein complexes corresponding by composition to LDL and VLDL. Addition of iron sulfate accelerated oxidation of unsaturated fatty acids in the lipids and the formation of LPO products [2]. Plasma content of unsaturated higher fatty acid dienic conjugates was measured by spectrophotometry [3]. Serum content of TBA-reactive products was measured as described previously [12]. The results were processed using Student's *t* test and nonparametrical Fisher's test.

RESULTS

In experimental group 1 ten days after PT with prednisolone urinary concentration of hydroxyproline increased by 83% in comparison with the control, while the total calcium content virtually did not change (Table 1). Therapy with both dimephosphone and xydiphone normalized hydroxyproline excretion increased by prednisolone, while urinary calcium level dropped below the control values in groups 2 and 3. In group 1 the level of inorganic phosphorus decreased in comparison with the control, while therapy with phosphonates normalized this parameter.

No significant changes in the serum levels of total calcium and alkaline phosphatase activity were detected in three experimental groups in comparison with the control (Table 2). Reduced level of total protein and increased level of dienic conjugates in the serum, resultant from prednisolone PT, normalized in both groups 2 and 3. In contrast to dimephosphone, xydiphone led to normalization of prednisolone-elevated content of inorganic phosphorus and decreased serum MDA level (in the group treated with dimephosphone these parameters remained at the same level as in untreated animals (Tables 2, 3).

Catalase activity in erythrocytes increased by 20-30% and peroxidase activity did not change in all experimental groups in comparison with the control. Phosphonate therapy normalized prednisolone-decreased level of ceruloplasmin and led to a reduction of the serum AOA (Table 3).

Biochemical markers (hydroxyproline, total calcium, inorganic phosphorus, alkaline phosphatase) reflect changes in bone tissue metabolism, namely, resorption and bone formation. They are essential for the detection of remodeling disorders in osteoporosis and for monitoring the treatment efficiency [11]. Hydroxyproline is an integral indicator of collagen metabolism, informing about connective tissue disorganization and synthesis [11].

We previously showed that intragastric administration of prednisolone in a dose of 50 mg/kg increa-

TABLE 2. Serum Phosphorus-Calcium Metabolism and Total Protein after 3-Day Prednisolone Course and Subsequent 10-Day Oral Treatment with Normal Saline, Dimephosphone, or Xydiphone (*M*±*m*, *n*=8)

Group		Total calcium, mmol/liter	Inorganic phosphorus, mmol/liter	Alkaline phosphatase, mcat/liter	Total protein, g (%)
Control		1.14±0.10	2.2±0.2	2.8±0.3	6.2±0.3
1	prednisolone+normal saline	1.0±0.1 (88)	2.7±0.1* (122)	2.5±0.4 (89)	5.3±0.2* (85)
2	prednisolone+dimephosphone	1.1±0.1 (97)	2.7±0.1* (122)	3.4±0.9 (121)	6.5±0.4 (105)
3	prednisolone+xydiphone	1.30±0.03 (114)	2.30±0.03 (105)	2.4±0.2 (86)	5.7±0.3 (92)

TABLE 3. Serum Content of LPO Products and Antioxidant Status of Rats after a 3-Day Course of Prednisolone and Subsequent 10-Day Oral Treatment with Normal Saline, Dimephosphone, or Xydiphone (*M*±*m*, *n*=8)

Group		Dienic conjugates, μmol/liter	MDA, μmol/liter	AOA, %	Catalase, mcat/liter×100	Peroxidase, μmol/min/liter	Ceruloplasmin, mg (%)
Control		4.6±0.3	5.4±0.2	17.4±1.5	3.5±0.2	105.2±3.5	32.9±2.5
1	prednisolone+normal saline	6.1±0.4*	4.30±0.14*	21.5±1.0	4.40±0.13*	101.9±1.1	24.3±0.9*
		(132)	(76)	(124)	(126)	(97)	(74)
2	prednisolone+dimephosphone	4.3±0.2	4.4±0.1*	14.3±2.2	4.20±0.16*	98.9±1.0	26.6±2.7
		(93)	(82)	(82)	(120)	(94)	(81)
3	prednisolone+xydiphone	5.8±0.7	5.6±0.3	14.3±2.2	4.60±0.19*	99.4±3.5	39.8±4.0
		(126)	(105)	(82)	(131)	(95)	(121)

sed urinary excretion of hydroxyproline by 60% in comparison with the control [4]. The results of experiments described in this paper showed that urinary excretion of hydroxyproline (a marker of bone resorption) [11] also increased after prednisolone PT (Table 1). Interestingly, activity of alkaline phosphatase (bone formation marker) was virtually normal in all the groups.

Relative safety of prednisolone PT for mineral metabolism parameters is confirmed by the absence of changes in total calcium levels in the serum and urine. This is in line with previous reports [7] indicating that blood calcium level remains normal in primary osteoporosis. We observed an increase of serum calcium concentration and its urinary excretion during long-term intragastric treatment with prednisolone [4]. The increase of serum inorganic phosphate level 10 days after prednisolone PT is one more evidence of greater safety of PT, because 14-day intragastric treatment with prednisolone in a dose of 50 mg/kg significantly reducted its level [4]. The differences in prednisolone effects in different modes of its administration manifested in urinary excretion of inorganic phosphate. Long-term (14-day) intragastric treatment with prednisolone (50 mg/kg) induced a 113% increase in urinary excretion of inorganic phosphate [4], while in the present study we observed a decrease in inorganic phosphate concentration in the urine. Prednisolone PT caused a transitory increase in total protein concentration in the blood. Ten days after prednisolone treatment serum concentration of total protein decreased, which together with the increase in urinary excretion of hydroxyproline was a biochemical proof of a stable catabolic effect of GCS.

Prednisolone induced an increase in the levels of intermediate LPO products (dienic conjugates). It obviously led to an increase in activity of antioxidant enzyme (blood catalase). Presumably, decreased level of MDA (one of the end products of LPO) was due to activation of the antioxidant system.

Prednisolone PT was associated with a decrease of ceruloplasmin level, while long-term intragastric treatment with prednisolone led to its increase [4]. The increase in the blood erythrocyte catalase activity 10 days after prednisolone PT could be due to the involvement of compensatory mechanisms aimed at deceleration of dienic conjugate formation in the serum. Serum MDA content decreased, which seemed to be a positive effect of pulse therapy with GCS, promoting a higher therapeutic effect [15].

Phosphonates used in our study had similar effects on the studied parameters of mineral metabolism and LPO. Differences in the effects were observed for inorganic phosphate and MDA: xydiphone normalized these parameters after prednisolone PT, while a similar course of dimephosphone caused no significant changes in comparison with the levels in untreated rats. This is in line with published data on xydiphone capacity to stimulate LPO [10].

Antiresorptive effect of xydiphone is widely used in the treatment of osteoporosis [10,11,14]. We showed previously that dimephosphone monophosphonate and xydiphone biphosphonate are equally effective in the correction of disorders in hydroxyproline excretion caused by long-tern intragastric treatment with GCS [4]. These effects of dimephosphone and xydiphone were confirmed after PT with prednisolone: both phosphonates normalized urinary excretion of hydroxyproline increased after prednisolone therapy.

Hence, the proposed mode of prednisolone PT induced changes in mineral metabolism and LPO parameters, the majority of which could be normalized by dimephosphone monophosphonate or xydiphone biphosphonate.

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