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SUBCHRONIC EFFECTS OF INTRAPERITONEAL ADMINISTRATION OF MANGANUS CHLORIDE TO RABBITS

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ABSTRACT

Manganus chloride (2 mg/kg b.wt.) was given every other day intraperitoneally (i.p.) for a period of 5 weeks to mature male rabbits. Rabbits were challenged with Clostridia antisera as a stress factor. The results indicated significant elevation in the levels of ß and γ-globulins ($P < 0.001$), serum ALT and AST activities ($P < 0.01$) and significant decrease in the levels of α-globulins and urea (SUN) ($P < 0.001$). The blood picture was not significantly changed except for eosinophils and monocytes counts which showed a marked increase and decrease ($P < 0.001$) respectively. Whereas, a moderate increase ($P < 0.05$) was recorded in PO_2, O_2 SAT% and O_2-CT% of the blood. This study represents the hazardous effect of prolonged exposure to manganus chloide in both histotoxic anoxia and liver dysfunction.

INTRODUCTION

Manganese is an essential element in all living organisms. and is a co-factor for a number of enzymatic reactions particularly those involved in phosphorylation, cholesterol and fatty acid synthesis. While, it is present in urban air and in most water supplies, the principal portion of the intake is derived from food[1].

Industrial toxicity from exposure to inhalation, generally of manganese dioxide in mining or manufacturing, is of two types: The first, manganese pneumonitis, is the result of acute exposure. Men working in plants with high concentrations of manganese dust show an incidence of respiratory disease 30 times greater than the average rate. Pathologic changes include epithelial necrosis followed by mononuclear proliferation[2]. The second and more serious type of disease resulting from chronic inhalation of manganese dioxide, generally over a period of more than two years, involves the central nervous system[3].

Intrahepatic cholestasis can also be produced in rats by the administration of manganese sulfate intravenously[4]. Recently, manganese ingestion has been associated with hepatotoxicity in humans[5]. In rats, this response is associated with the development of necrotic lesions, which varies from focal necrosis to subtotal midzonal necrosis. Widespread dilatation of bile canaliculi with loss of microvilli is observed 20 hours after administration of manganese[2]. It was reported that the daily manganese intake ranges from 2-9 mg. Gastrointestinal absorption is less than 5%. It is transported in plasma bound to a ß-globulin, thought to be transferrin, and is widely distributed in the body.

Manganese accumulates in the mitochondria so that tissues rich in these organelles have the highest concentrations of manganese such as the pancreas, liver, kidney and intestine[2].

The present work was designed to further study the effect of manganese on the vital body functions. That could give better picture in both the biochemical and haematological changes caused manganese.

EXPERIMENTAL

Mature male rabbits weighing 1.5-2 kg were divided into 3 groups, each group contains six rabbits. They were acclimated for one week and allowed free access to standard pelleted food and drinking water. One group was given daily i.p. injections of distilled water and used as control. The other two groups were challenged with Clostridia antisera (0.2 ml/rabbit) as a stress factor given subcutaneously (s.c.) at once and left for one week. One of the latter groups was i.p. injected every other day with manganus chloride (BDH, England) in a dose of 2.0 mg/kg for 5 weeks. The other group was left as a control for the previous group receiving no treatment. The animals were then sacrificed by decapitation and blood samples were immediately collected. EDTA (disodium salt) was used as anticoagulant. Blood smears were freshly prepared, air dried, fixed in alcohol and stained with Leishman's stain.

Erythrocyte (RBC) and leucocyte (WBC) counts were determined in double improved Neubauer chamber[6]. Haemoglobin (Hb%) was determined by the acid haematin method[7]. Packed cell volume (PCV) was estimated in double capillary tube preparations using a microhaematocrit centrifuge.

Erythrocyte indicies were calculated[8] from the values of RBCs, PCV and Hb.

Serum biochemical variables were estimated using commercial kits supplied by BioMerieux (France). These included: alanine aminotransferase (ALT) and aspartate aminotransferase (AST)[9], serum total proteins[10], serum urea nitrogen (SUN)[11], cholesterol[12], triglycerides[13], bilirubin[14], uric acid[15], glucose[16], calcium^{2+}[17] and inorganic phosphorus [13].

Serum protein fractions were determined by electrophoresis, using 5 µl samples from the treated group and the control group. These were applied to agarose gel slabs (5401-001) hydragel protein (LKB-Sebia). Electrophoresis was run for 20 minutes using LKB equipment and methods (Sebia 91130 Issy Les Moulineaux, France).

Following staining with amidoblack and destaining in 5% acetic acid, the electrophoretograms were scanned for the percentages of albumin, α,-ß and γ-globulin fractions and the A/G ratio in LKB-5300 "Preference" densitometer programmed for protein analysis.

Blood gas analysis was done on freshly drawn venous blood collected in heparinized syringes promptly sealed hermetically and injected directly into the blood gas analysis system (2000 Eschweiler, West Germany).

Statistical analysis:

Data were statistically analysed using paired Student "t" test[18].

RESULTS

Tables (1 & 2) show the results of biochemical, haematological and blood gas analysis.

Regarding biochemical analysis, significantly increased levels were noted in ß and γ-globulins ($P < 0.001$), and serum ALT & AST activities ($P < 0.01$) whereas, marked decreases in serum α-globulins, urea (SUN) ($P < 0.001$) were recorded. Insignificant elevations in the levels of serum cholesterol, triglycerides, bilirubin, uric acid, inorganic phosphorus and Ca^{2+} were also noted. In addition, insignificant decreases were seen in the levels of serum total proteins, albumin, A/G ratio and glucose.

The blood picture showed a marked increase ($P < 0.001$) in eosinophils count and a marked decrease in monocytes counts. Insignificant decreases in RBCs, WBCs counts, Hb%, PCV%, neutrophils and lymphocytes counts were also recorded. A nonsignificant increase in the values of MCV, MCH and MCHC

Table (1): Serum chemical variables in rabbits treated with manganus chloride in a dose of 2 mg/kg b.wt. i.p. every other day for 5 weeks.

Variables	Control non vaccinated	Control vaccinated	Treated vaccinated	Variables	Control non vaccinated	Control vaccinated	Treated vaccinated
Total proteins g/L	64.96 ± 6.34	60.6 ±0.55	60.02 ±2.08	Bilirubin mg/L	37.9 ±2.96	37.3 ±1.17	39.1 ±0.78
Albumin g/L	39.36 ±1.24	39.05 ±2.83	37.62 ±1.65	Urea mg/L	619.7 ±39.14	697.0 ±10.55	564.85 ±14.4***b
α-globulins m/L	13.03 ±0.59	9.68 ±0.41**a	1.3 ±0.028***ab	Glucose mg/dl	86.07 ±6.98	82.9 ±13.3	66.72 ±8.58
ß-globulins g/L	6.38 ±0.19	3.06 ±0.06***a	4.88 ±0.23***ab	Uric acid mg/L	93.4 ±1.04	94.25 ±1.95	104.4 ±5.67
γ-globulins g/L	6.12 ±0.134	8.8 ±0.3***a	16.32 ±1.62***ab	Phosphorus mg/L	59.9 ±4.39	59.7 ±4.1	61.46 ±2.78
A/G ratio	1.57 ±0.2	2.165 ±0.59	1.78 ±0.32	ALT u/ml	14.5 ±3.25	19.62 ±2.37	29.62 ±2.68**ab
Cholesterol mg/dl.	105.2 ±7.85	103.16 ±6.87	110.75 ±3.37	AST u/ml	62.2 ±2.6	66.0 ±4.15	98.6 ±4.8***ab
Triglycerides g/L	1.9 ±0.26	2.12 ±0.185	2.22 ±0.15	Calcium mg/L	162.5 ±8.54	155.0 ±2.04	177.5 ±15.17

** P < 0.05
*** P < 0.01

a : significant from the control (nonvaccinated).
b : significant from the control (vaccinated).

Mean values ± S.E.
n = 6 Rabbits

was observed when compared with the control group.

The blood gas analysis revealed a significant increase ($P < 0.05$) in the values of PO_2, O_2-SAT% and O_2-CT%. An insignificant decrease in PCO_2, pH, HCO_3, TCO_2 and an increase in base excess (BE) was also reported.

DISCUSSION

Treatment with manganus chloride in a dose of 2.0 mg/kg. b.wt. of mature male rabbits every other day for 5 consecutive weeks resulted in some interesting findings.

The observed hyperglobulinaemia, as can be deduced from significantly decreased α-globulins and slight decrease in albumin and A/G ratio was observed associated with increased number of eosinophils. This combination of events appears to indicate enhanced immune response possibly as a reaction towards tissue damage seen in the liver[19].

Table (2): Blood picture & blood gas analysis of mature male rabbits given manganus chloride (2.0 mg/kg) every other day for 5 weeks.

Variables	Control non vaccinated	Control vaccinated	Treated vaccinated	Variables	Control non vaccinated	Control vaccinated	Treated vaccinated
RBCs 10^{-6}	6.65 ±0.28	7.6 ±0.81	6.6 ±0.34	Monocytes count	88.0 ±0.0	85.0 ±0.35***a	39.0 ±0.22***ab
WBCs No/µl	8830 ±430	8500 ±650	7750 ±50	PO_2 mmHg	50.15 ±3.18	45.12 ±0.9	78.2 ±10.2**b
HB %	10.2 ±0.16	9.63 ±0.37	9.42 ±0.28	PCO_2 mmHg	98.4 ±25.44	186.3 ±26.22	147.7 ±23.21
PCV %	35.25 ±2.62	35.5 ±5.55	22.33 ±6.78	pH	6.17 ±0.16	6.35 ±0.1	6.31 ±0.14
MCV Cu µ	52.95 ±3.05	48.35 ±9.89	53.78 ±9.07	BE mmol/L	43.85 ±4.7	33.72 ±4.87	37.8 ±4.86
MCH u µg	15.48 ±0.46	13.13 ±1.6	4.45 ±1.11	HCO_3 A mmol/L	4.15 ±1.77	11.3 ±2.83	7.85 ±2.0
MCHC %	29.58 ±2.23	29.45 ±5.21	29.8 ±6.8	HCO_3 S mmol/L	4.93 ±2.76	21.1 ±5.82	9.07 ±2.41
Neutrophils count	4481 ±64.08	4866 ±262.8	4572 ±239.1	TCO_2 mmol/L	6.62 ±2.3	15.95 ±3.32	11.55 ±1.7
Eosinophils count	88.0 ±0.51	21.0 ±0.05***a	58.1 ±0.29***ab	O_2-SAT %	22.9 ±4.54	24.27 ±7.33	48.12 ±2.53*ab
Basophils count	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	O_2-CT %	3.05 ±0.62	3.27 ±0.44	6.67 ±0.37*ab
Lymphocytes count	41.72 ±0.75	3527 ±178.5	3080.6 ±176.8				

* P < 0.05
** P < 0.01
*** P < 0.001

a : significant from the control nonvaccinated.
b : significant from the control nonvaccinated.
HCO_3 A = Actual. HCO_3 S = saturation. O_2 - SAT = saturation.
BE = Base excess. O_2-CT = content. TCO_2 = (Total CO_2).
Mean values ± S.E. n = 6 in each group.

This result was confirmed by the significant increase in serum ALT and AST activities. It was reported that since the liver of mature horses, cattle and sheep do not contain significant levels of GPT (now known as ALT), only very small elevations in serum GPT occur from hepatic necrosis in these species. Significant elevations in GOT (known now as AST) are liver specific only in small animals and primates. It was also indicated that elvations in SGOT activity can be associated with alterations in cell necrosis of many tissues[19].

The same author reported that severe liver insufficiency causes decreased serum urea (SUN), apparently because of impaired urea synthesis, since the liver is the main site of urea synthesis[19].

The increased γ-globulins could partly be accounted for by an increase in IgE suggested by the increased number of eosinophils indicating an allergic mainfestation. The increased γ-globulins are most likely attributed to chronic hepatitis, chronic inflammatory disease and glomerulonephritis[19]. Chronic hepatitis also exhibited changes characteristic of chronic disease where the polyclonal increase of immunoglobulins is more marked and the hypoalbuminaemia may merely be a reflection of the severity of the disease process and the more intense antigenic response generated[20].

The present results are in full agreement with the data previously reported[2]. It was reported that ingestion of manganese has been associated with hepatotoxicity in humans[2]. In rats this response is associated with the development of necrotic lesions, which varies from focal necrosis to subtotal midzonal necrosis. Manganese accumulates in mitochondria, so that tissues rich in these organelles have the highest concentrations of manganese including pancreas, liver, kidney and intestine[2].

The increase in globulin fractions especially the ß-and γ-globulins indicate that a state of immunopotentiation has possibly occurred as a result of treatment with manganese chloride.

The decreased monocytes count might be attributed to depression of the bone marrow as evidenced in our study by the slight decrease in RBCs and WBCs counts. It is also interesting to note that increased PO_2, O_2-content and O_2-saturation occurred concurrently with slight hypochromic anaemia in this experiment. This indicates appropriate pulmonary ventillation, and diminished oxygen uptake by tissues. In a state similar to mild histotoxic anoxia, occurring due to intake of principals partially impairing the function of cytochrome oxidase.

REFERENCES

(1) **Underwood, E.J.;** "Trace elements in Human and Animal Nutrition", 4th Ed., Academic Press, Inc. New York (1977).

(2) **Klaassen, C.D.; Amdur, M.D. and Doull, J.;** "Casarett and Doull's Toxicology". The basic Science of Poisons. 3rd., ed., MaCmillan Publishing Company: New York, Toronto, London, pp. 298, 614 (1986).

(3) **Mena, I.; Kazuko, H.; Burke, K. and Cotzias, G.C.;** Neurology, 19: 1000-1006 (1969).

(4) **Witzleben, C.L.;** Am. J. Pathol., 66: 577-582 (1972).

(5) **Lustig, S.; Pittik, S.D. and Rosenfeld, J.B.;** Arch. Intern. Med., 142: 405-406 (1982).

(6) **Wintrobe, M.M.;** Clinical Haematology. 5th ed., London, Henry Kimpton (1961).

(7) **Lynch, M.J.; Raphael, S.S.; Meller, MD.; Spare, P.D. and Inwood, M.J.H.;** Medical Laboratory Technology and Clinical Pathology. 2nd Ed., Philadelphia, London, Toronto: W.B. Saunders Company (1969).

(8) **Baker, F.J.; Silverton R.E. and Luckcock, E.D.;** An introduction to Medical Laboratory Technology. 4th ed., London: Butterworth (1969).

(9) **Reitman, S. and Frankel, S.;** Am. J. Clin. Pathol., 28, 56-63 (1957).

(10) **Reinhold, J.;** Standard Methods of Clinical Chemistry. Reiner, N. (Ed.). New York and London Academic Press (1953).

(11) **Chaney, A.L. and Marbach, E.P.;** Clin. Chem., 130 (1962).

(12) **Steinberg, D.;** "Athero-sclerosis and Coronary Heart Diseases", 1 (2) Elsevier North Holland, pp. 31-48 (1981).

(13) **Varley, H.; Gowenlock, A.H. and Bell, M.;** Practical Clinical Biochemistry. Vol. 1. General Topics and Commoner tests. 5th ed., London, William Heinmann Medical Books Ltd., pp. 675, 887 (1980).

(14) **Colombo, J.;** Clin. Chem. Acta. 15, 217. Cited from: Practical Clinical Biochemistry Varley, H., Gowenlock, A.H. and Bell., M.C. (eds) (1980), 5th ed., London William Heinmann Medical Books. LTd., pp. 1018-1020 (1974).

(15) **Caraway, W.T.;** Standard Methods of Clinical Chemistry, edited by Seligson, D., Academic Press, New York and London, 4, pp. 239 (1963).

(16) **Trinder, A.;** Ann. Clin. Biochem., 6, 24. Cited from: Practical Clinical Biochemistry Varley, H., Gowenlock, A.H. and Bell., M.C. (eds) (1980), 5th ed., London William Heinmann Medical Books. LTd., pp. 389-391 (1969).

(17) **Connerty, H. and Briggs, A.;** Am. J. Clin. Pathol., 45, 290 (1966).

(18) **Snedecor, G.W.;** Statistical Methods, Ames, Iowa, USA: The Iowa State University Press (1969).

(19) **Kaneko, J.J. (1980):** "Clinical Biochemistry of Domestic Anaimals". 3rd Ed., Academic Press Inc.

(20) **Rumbaugh, G.E.; Smith, B.P. and Carlson, G.P.;** Am. Vet. Med. Assoc., 172, 304. Cited in Kaneko, J.J. (1980). "Clinical Biochemistry of Domestic Animals". 3rd. ed., Academic Press Inc (1978 b).

التأثيرات تحت المزمنة الناشئة عن حقن كلوريد المنجنيز في الغشاء البريتونى للأرانب

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قسم علم الحيوان - كلية العلوم - جامعة الزقازيق - مصر

فى هذا البحث تم اعطاء كلوريد المنجنيز لذكور الأرانب البالغة عن طريق الحقن داخل البريتونى يوم بعد يوم لمدة خمسة أسابيع فى جرعة مقدارها ٢مجم/كجم من وزن الجسم . تم تحصين الأرانب بمصل الكلوستريديا وذلك لتنشيط انتاج الجلوبيولينات المناعية . وقد أظهرت النتائج وجود زيادة معنوية فى مستوى البيتا والجاما جلوبيولينات وفى نشاط إنزيمى الكبد ALT & AST كما كان هناك نقصا معنويا فى الألفا جلوبيولينات والبولينا فى المصل. وأظهرت صورة الدم وجود تغيرات غير معنوية فيما عدا عدد كرات الدم البيضاء الحامضية التى أظهرت زيادة معنوية وكذا الكرات المللتهمة الكبيرة والتى أظهرت نقصاً واضحاً بينما كان هناك زيادة ملحوظة فى ضغط ونسبة التشبع وكذا محتوى الدم من الأكسجين.