WHOLE BLOOD METAHAEMOGLOBIN CONCENTRATION IN RABBIT DURING A LONG TERM TREATMENT WITH $MnSO_4$

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INTRODUCTION: Manganese takes part in several enzymatic systems whether an activator (or inhibitor) or an essential constituent of the enzymatic system itself. Enzymes belonging to the first class present specific manganese linkage places rapidly reversible with catalityc and regulating functions. Enzymes containing manganese as essential constituent are represented by arginase, Mn-dipendent superoxide dismutase that functions as acceptor of electrons. Therefore, manganese ions take part in radicalic redox reactions. Among these, Mn(II) defences against cellular damages, while the couple Mn(II)-Mn(III) promotes radical (oxidative) damage against cellular components (Wedler, 1993). Mithocondrial Mn-dipendent superoxide dismutase is the best explored containing manganese enzymatic system and it protects against the damage provoked by the superoxide radical. In bibliography, it is reported in which manner free Mn(II) in solution scavanges radicals product either during metabolic processes (expecially those involving NADPH) or by chemioterapic agents or ionizing radiations (Wedler, 1993). Mn(II) bound and transported to the tissues by metallothioneins can offer protection against free radicals, as demonstred in vitro by Wedler (1993). To verify the *in vivo* antioxidant capability of Mn(II) the Authors dosed the level of metahemoglobin in rabbits during a long time treatment with $MnSO_4$ administred per os by feed. The ration was not addictioned with oxidative substances and was formulated on the basis of the requirement of the considered species and ages.

MATERIALS AND METHODS: The experimental design was carried out during 101 days and 12 Grimaud females rabbit 50 days old were employed. Subject were divided randomly in two groups (experimental and control) constituted by 6 animals and each animal was housed alone. Supplementation by Mn(II) was realised through drinking water, previously tested for the presence of Mn(II) and determined in 64 µg/l. 1 ml/l of a solution of monohydrate manganese sulphate at the concentration of 15000 mg/l in distillated water was added to the drinking water. To each group, water was administred ad libitum. The average quantity of water drunk by the rabbit belonging to the experimental group was 0.73 l/sub/day corresponding to a total assumption of manganese of 4.47 mg/subj/day. The subjects of the control group assumed 0.65 l/sub/day of water corresponding to 0.42 mg/subj/day of manganese(II). In order to assess the total content of Mn(II) assumed by rabbits, the commercial integrated feed administred ad libitum to rabbit was analysed, revealing a content of Mn(II) of 40 ppm. The average consumption of feed per experimental subject every day was 0.205 Kg (total consumption of Mn(II) = 8.2 mg/subj/day), while for the control subject every day was 0.178 Kg/sub/day (total consumption of Mn(II) = 7.1 mg/subj/day).

Totally, the experimental group assumed about 12.67 mg/subj/day of Mn(II), while the control group assumed 7.57 mg/subj/day.

To verify the metabolic effect of Mn(II) the subjects were sampled through the cardiac puncture adding blood with anticoagulant (Li-heparin). The samples were processed within one hour. Samplings were realised after 1 month from the beginning of the experimental work and at the end of the period of treatment. This paper concerns the haematological results and particularly RBC, WBC, differential blood cells count, Hct, Hb. To verify the antioxidative capability of the manganese we perform the determination of metahaemoglobin based on the method by Tietz (1974). Method is based on the principle that metahemoglobin spectrum absorbs at a characteristic between 620 and 640 nm. The high peak decreases when the metahemoglobin is converted in cianmetahemoglobin (cyanMetahb) by adding CN and the decrease of absorbance is proportional

to Metahb concentration. Determinations of the concentration of Mn in plasma and whole blood were performed by atomic absorption spectrometry

RESULTS AND DISCUSSION: Comparison between haematological results obtained by subjects treated with $MnSO_4$ and by control subjects did not revealed statistical significant differences, while Metahb concentrations showed a statistical significant differences between the two groups.

rabbit	HCT %	HB g/dl	RBC 10^6cell/mm^3	MCV flt	MCH pg	MCHC %	WCB 10^3cell/mm^3	Metahb %
AVE. TREATED	43,50	15,13	7,66	56,90	19,80	34,81	7,30	0,406
ST.DEV.	1,64	0,52	0,44	2,35	0,82	1,11	1,75	0,134
AVE. CONTROL	43,60	14,68	7,54	57,86	19,48	33,69	7,74	1,745
ST.DEV.	1,52	0,43	0,32	1,86	0,62	1,14	1,97	0,787
P	***0,919***	***0,154***	***0,631***	***0,477***	***0,500***	***0,135***	***0,704***	***0,003***

The average quantity of Mn(II) assumed by treated subjects differs from that assumed by control subjects in reason of 5.1 mg/subj/day. This quantity, even if apparently little, when administred chronically to rabbits is able to influence in a significant manner on the concentration of Metahb. Decrease in Metahb concentration in treated animals respect to controls is expressed as 76.7%, that represents an interesting metabolic influence. The capability of Mn(II) to act as an antioxidant drug was studied in the horse. In this species, the administration of a bivalen salt of Mn showed a antioxidant action to be compared to that expressed by superoxidedesmutase enzyme. The experiment executed in horse utilised liver cells, while in our work the antioxidative action of Mn was evaluated in vivo in red blood cells. It is known that the level of Metahb in erythrocytes increases following assumption of substances with oxidative action. In our work, no oxidative material was voluntarily added to feed administred to rabbits, just for this reason the Mn expresses a severe antioxidant action. Indeed, Mn shows its antioxidant capability even in presence of poor quantities of oxidative substances, reducing the Metahb concentration at physiological values in treated subjects. Following to the results we obtained during the experimental design, it is possible to stress that a correct food integration with this ion permits a defence against oxidant agents. The results we reported show the possibility to verify the effective antioxidant capability of the Mn(II) in animals assuming feed naturally rich or artificially added of oxidant agents.