RESEARCH PAPERS

SOME PHARMACOLOGICAL PROPERTIES OF A NEW INTRAMUSCULAR IRON PREPARATION

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The pharmacological properties of a new intramuscular iron preparation containing 50 mg. of iron as an iron-sorbitol-citrate complex has been studied in animal experiments. The acute cardiovascular effects of intravenous injections, as well as toxicity in mice and rabbits, are reported. The preparation is stable *in vivo* and has no apparent antigenic properties.

PARENTERAL iron administration by the intramuscular route was made possible with the introduction of colloidal iron preparations which were well tolerated and produced no or minimal pain at the injection site. The intramuscular iron therapy has proved to be effective in the treatment of many iron-deficiency anemias. Disadvantages with this form of iron medication include pain and discolouration at the injection site (Karlefors and Nordén, 1958). The recent report of Haddow and Horning (1960) demonstrating sarcoma formation in animals after long-term administration of massive doses of iron-dextran has focused interest on the carcinogenic properties of intramuscular iron preparations. Though the exact cause of the sarcoma induction is not clear, inadequate absorption may be a factor of importance.

In the preparation of some new iron-sorbitol-citrate complexes in our laboratories (Lindvall and Anderson, 1961), special regard has been given to the absorption characteristics of these preparations. One such complex, Jectofer, is described by Lindvall and Andersson. In the present study some pharmacological properties of this complex will be presented and compared with those of an iron-dextran preparation.

METHODS

The iron-sorbitol-citrate complex contained 50 mg. of iron per ml. and was used as sterile solutions with a pH of 7·5 $\pm$ 0·2. For comparison, an iron-dextran preparation Imferon, pH 5·8 containing 50 mg. iron per ml. or a preparation of saccharated oxide of iron, Intrafer, pH 10·9 containing 20 mg. of iron per ml. were used. The iron preparations will be referred to as iron-sorbitol, iron-dextran and saccharated oxide of iron respectively.

Acute Effects in Anaesthetised Cats and Rabbits

Cats (2–3·5 kg.) were anaesthetised with sodium pentobarbitone (35 mg./kg.) and rabbits (2–2·75 kg.) with urethane (1·2 g./kg.). Blood pressure, respiration (pneumotachogram) and heart rate were measured with conventional transducers and recorded on a Grass Model 5 Polygraph. In some instances peripheral vascular effects were studied by the

insertion of a constant volume perfusion pump (Sigmamotor, Inc., Middleport, N.Y.) in one femoral artery. Injections were made intravenously in one external jugular vein and intra-arterially, proximally to the pump.

Acute Toxicity

Male albino mice (17–24 g.) of an inbred strain were divided in three series of 160, 140 and 120 animals and injected by the intraperitoneal, intravenous and subcutaneous routes respectively. In each series the animals were further divided into groups of 20, each group receiving the same dose per unit weight. Injections were made with a 0·5 per cent saline dilution of iron-sorbitol. The ratio between successive doses was held constant at 1:2. The observation period was 7 days. The LD50 values were calculated by the method specified in the Scandinavian pharmacopoeia. Student's "*t*" test was used for testing the significance of the differences between the LD50 values.

Male albino rabbits (2–2·5 kg.) were divided into two series of 45 animals each and injected intravenously and intramuscularly. In addition, 20 male albino rats received intramuscular injections.

Subchronic Toxicity

Forty male albino rabbits, initial weight 1·1–2·5 kg., were kept in separate cages and given a diet of hay, oats, rabbit pellets, green vegetables and water *ad libitum*. They were observed for 7 days before treatment. The iron-sorbitol complex corresponding to 5 mg. Fe/kg. was given, in series I, to 10 animals intramuscularly (deep intragluteally) and to 10 animals intravenously, while 5 rabbits were kept as controls. In the 9 weeks of treatment the total dose corresponded to 215 mg. Fe/kg. In series II, 4 animals were injected intramuscularly and 4 intravenously with the same dose 5 days a week as in series I. Seven animals were kept as controls. In the 5 weeks of treatment of this series a total of 110 mg. Fe/ kg. was given. Blood samples of approximately 0·3 ml. were taken weekly from all animals, 10 ml. (by heart puncture) twice from each animal in series I, and once weekly in series II. Urine was collected, measured, and analysed for protein qualitatively by Esbach's reagent and quantitatively by the biuret method (Kingsley, 1941) involving measurement of the extinction at 540 mμ with a Beckman DU spectrophotometer. The amount of protein was calculated from a standard curve obtained by Kjeldahl analysis.

Haemoglobin concentration was determined according to King (1947), red cell counts by the method of Ellerman, hematocrit by conventional methods, iron in serum and iron binding capactity by procedures described by Lindvall and Andersson (1961) and serum proteins quantitatively by the biuret method as with urine. Paper electrophoresis was carried out on 10 μlitre aliquots of serum on 4 cm. paper strips (Whatman No. 1) in veronal buffer at pH 8·6 and a current of 0·5 mA/cm. for 16 hr. The strips were stained with bromphenol blue and scanned in a EEL densitometer.

All animals were killed 7 days after the last iron injection, the internal organs weighed and their iron content analysed. Histological specimens were fixed in ethanol, imbedded in paraffin and 10 μ sections were stained with hematoxylin-eosin, or for iron according to deVinal with the modification by Wöhler (1959) and counterstained with carmalum.

Antigenicity

Five guinea-pigs (209–243 g.) were injected intraperitoneally twice with 3 days interval with 0·35 ml. of a 1 : 50 saline dilution of iron-sorbitol (approx. 1·5 mg. Fe/kg.). Fourteen days later a challenging dose of about 2·5 mg Fe/kg. was given intracardially and observations made as recommended by the U.S. Pharmacopeia (1955). *In vitro* tests on isolated ilial segments from similarly treated guinea-pigs were also made. In

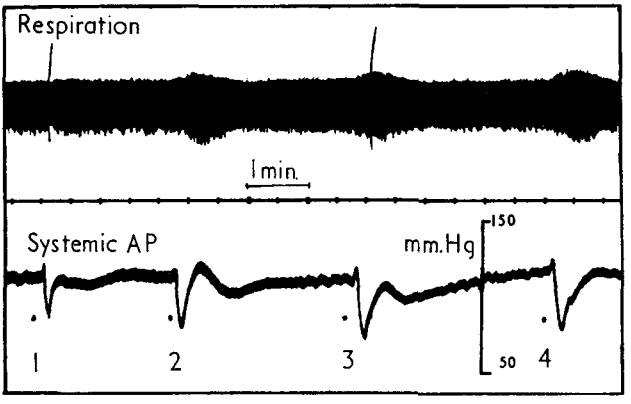

FIG. 1. Rabbit 2·65 kg. The hypotensive effect of increasing doses of intravenous iron-sorbitol. The dose at 1, 2, 3 and 4 corresponds to 2·8, 5·7, 11·3 and 22·6 mg. Fe/kg. respectively.

addition, antigenicity was studied by the agar gel-diffusion method (Ouchterlony, 1958) in 8 rabbits treated for 9 weeks with a total of 215 mg. Fe/kg. 1 ml. serum was tested against 1 ml. 0·5 or 0·1 per cent saline dilution of iron-sorbitol using the two-basin-plate technique.

RESULTS

Acute Effects in Anaesthetised Cats and Rabbits

Rapid intravenous injections in cats and rabbits of moderate doses (2–10 mg. Fe/kg. weight) of iron-sorbitol produced a fall in systemic blood pressure. The response was rapid, of short duration and, at the beginning of an experiment, often directly proportional to the dose injected (Fig. 1). The response of blood pressure and heart rate was qualitatively the same as that produced by acetylcholine (Fig. 2), and quantitatively, 3·5 mg. Fe/kg. as iron-sorbitol approximately corresponded to 0·1 mg. Fe/kg. as ferrous sulphate or 5·3 mg. Fe/kg. as iron-dextran. After repeated doses tachyphylaxis usually developed. The hypotensive effect was also greatly

influenced by the rate of injection. If the rate of injection was less than 1·5 mg. Fe/kg./min. the response was absent. The depressor effect of intravenous iron-sorbitol was not influenced by full doses of atropine or antihistamine drugs. Iron-sorbitol, like iron-dextran, antagonised the action of subsequently injected adrenaline. This antagonism lasted about

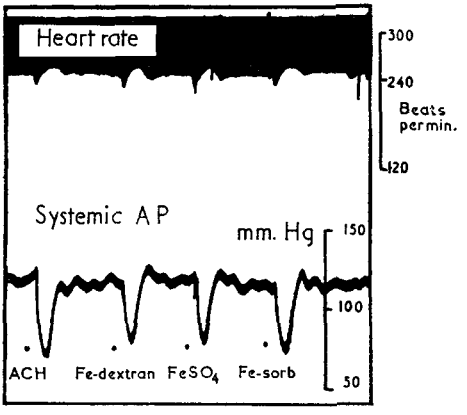

FIG. 2. Cat 2·85 kg. Comparison between the depressor effects of intravenous acetylcholine (ACH 1 μg.), iron(Fe)-dextran (5·3 mg. Fe/kg.), ferrous sulphate ($FeSO_4$, 0·1 mg. Fe/kg.) and iron(Fe)-sorbitol (5·3 mg. Fe/kg.).

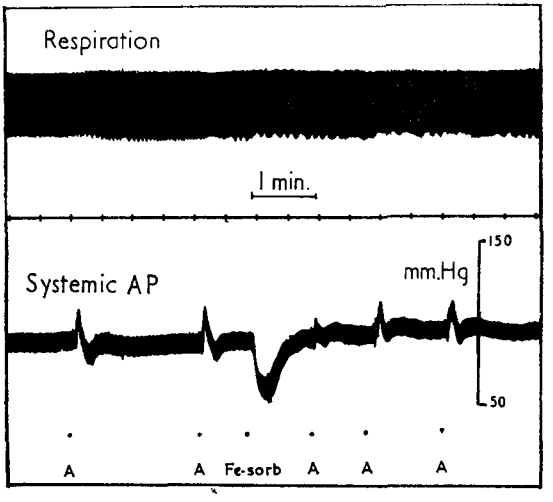

FIG. 3. Rabbit 2·75 kg. The adrenaline-antagonistic effect of i.v. iron-sorbitol (5·5 mg. Fe/kg.). At A, 4 μg. of adrenaline was administered intravenously.

two min. (Fig. 3). Recordings of the pressure in the femoral artery, perfused at constant rate, showed that local intra-arterial injection of iron-sorbitol (60 mg. Fe/kg.) was followed by only a slight fall in peripheral resistance. A similar result was obtained with iron-dextran. Fractionated doses of iron-sorbitol given by this route up to a total amount

of 100 mg. Fe/kg. did not elevate peripheral resistance as might have been expected if intravascular precipitation had taken place. On the other hand 1 mg. Fe/kg. given as ferric chloride, which precipitates when mixed with blood, and 30 mg. Fe/kg. as saccharated oxide of iron both caused an abrupt and long-lasting increase in peripheral resistance (Fig. 4).

With rapid intravenous injections of increasing doses of iron-sorbitol (3·5–350 mg. Fe/kg.) the magnitude of the fall in pressure was no longer related to the dose, but the duration of the pressure-drop progressively increased. When this occurred the duration of the acetylcholine response was also augmented. This would seem to indicate that large doses of the iron-sorbitol depressed the compensatory vasomotor mechanisms. However, considerable amounts of iron-sorbitol in fractionated doses,

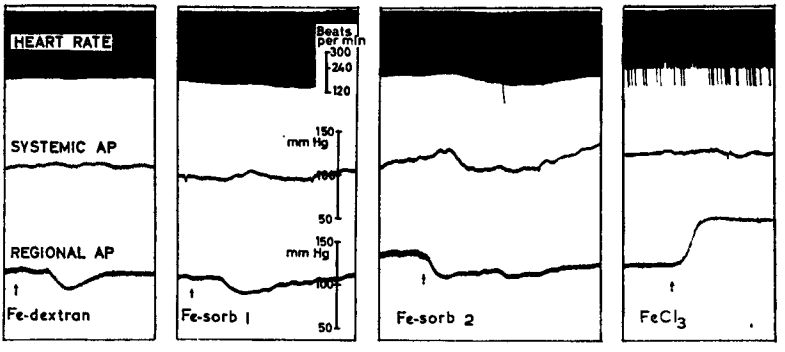

FIG. 4. Cat 2·4 kg. The effect on peripheral resistance of intra-arterial injections of iron(Fe)-dextran (4·5 mg. Fe/kg.), iron(Fe)-sorbitol (4·5 mg. Fe/kg. at 1 and 56·3 mg. Fe/kg. at 2) and ferric chloride ($FeCl_3$ 1 mg. Fe/kg.).

totalling up to 2 g. Fe/kg. in 3 hr., could be given in these acute experiments to the anaesthetised cats without significant changes in the resting blood-pressure level.

Respiration in rabbits was largely unaffected by intravenous iron-sorbitol in doses below 100 mg. Fe/kg., higher doses producing episodes of apnoea. Cats seemed less sensitive in this respect and doses of 170 mg. Fe/kg. given in rapid succession did not noticeably influence respiration. The normal E.E.G. pattern was not significantly influenced unless very high doses (1–2 g. Fe/kg.) were given, which produced a pronounced fall in blood pressure and imminent death.

Acute toxicity. In mice the intravenous LD50 was found to be 35·5 ± 1·33 (S.E.) mg. Fe/kg. weight. By the intraperitoneal and subcutaneous routes the average lethal doses were 50·25 ± 2·05 mg. Fe/kg. and 35·50 ± 1·08 mg. Fe/kg. respectively. In rabbits the intravenous and intramuscular LD50 values were 38·0 ± 5·3 and 38·0 ± 3·2 mg. Fe/kg. respectively. In rats the intramuscular LD50 was approximately 48 mg. Fe/kg.

Subchronic toxicity. The weight gain of the iron-sorbitol treated rabbits was similar to that of the control animals. The rabbits appeared healthy and had a normal appetite. The frequent injections seemed to

cause no discomfort. In series I (25 animals) given a total of 215 mg. Fe/kg., one rabbit in each of the intravenous, intramuscular and control groups died during the course of the experiment. The animal in the intravenous group died after 2 days, the other 2 animals died after 6 and 5 weeks respectively. All 3 animals had diarrhoea before death and except

TABLE I

THE EFFECT OF IRON-SORBITOL TREATMENT ON HAEMOGLOBIN CONCENTRATION (MG./100 ML.)

	Days of treatment	i.m. route	i.v. route	Control
Series I Total dose 215 mg. Fe/kg.	0	9·8 ± 0·1	9·6 ± 0·5	10·9 ± 0·5
	12	10·0 ± 0·2	10·2 ± 0·3	8·8 ± 0·5
	27	11·8 ± 0·3	10·9 ± 0·4	8·2 ± 1·1
	62	11·8 ± 0·3	11·4 ± 0·6	8·2 ± 0·3
Series II Total dose 110 mg. Fe/kg.	−2	9·9 ± 0·3	9·1 ± 0·2	10·4 ± 0·3
	8	10·3 ± 0·8	11·0 ± 0·4	10·4 ± 0·2
	14	8·6 ± 0·1	8·5 ± 0·2	9·4 ± 0·2
	22	9·3 ± 0·8	10·0 ± 0·7	10·1 ± 0·5
	28	11·7 ± 1·3	10·4 ± 0·4	10·0 ± 0·6
	36	10·7 ± 0·3	10·3 ± 0·3	11·0 ± 0·4

for signs of enteritis, postmortem examination was negative. In series II (15 rabbits) receiving a total of 110 mg. Fe/kg., 4 rabbits died as a result of the trauma of heart puncture.

Iron-sorbitol did not cause any obvious change in urine production nor did it produce proteinuria. During the 7 days' observation period before treatment small amounts (<50 mg./24 hr.) of protein were found in the urine of 4 rabbits on five occasions. During iron-sorbitol treatment the

TABLE II

THE EFFECT OF IRON-SORBITOL TREATMENT ON HEMATOCRIT (H) AND RED CELL COUNT (RBC) IN SERIES II, RECEIVING A TOTAL OF 110 MG. FE/KG.

Days of treatment	Administration				Control	
	i.m.		i.v.			
	H	RBC × 10^6	H	RBC × 10^6	H	RBC × 10^6
−2	29·0 ± 14·8	5·4 ± 0·1	30·0 ± 2·0	5·3 ± 0·1	34·0 ± 3·5	5·3 ± 0·1
8	30·3 ± 3·0	5·2 ± 0·2	28·3 ± 2·8	5·1 ± 0·1	32·0 ± 1·6	5·6 ± 0·2
14	31·2 ± 0·8	5·0 ± 0·1	31·3 ± 0·9	4·7 ± 0·1	31·0 ± 1·3	5·3 ± 0·3
22	35·5 ± 2·5	4·8 ± 0·5	37·3 ± 2·7	4·7 ± 0·1	36·2 ± 1·7	4·8 ± 0·2
28	42·3 ± 3·6	5·1 ± 0·6	40·0 ± 0·6	5·3 ± 0·1	36·2 ± 1·7	4·8 ± 0·1
36	40·5 ± 1·4	5·2 ± 0·3	38·7 ± 0·7	5·1 ± 0·1	41·2 ± 1·6	5·0 ± 0·3

urine from 3 different rabbits showed a positive protein test on five different occasions. The amount of protein excreted in these few instances was less than 80 mg. per 24 hr. By paper electrophoresis and chemical estimation no qualitative or quantitative changes in serum proteins were found in the treated animals.

The haematological effects of the iron-sorbitol treatment are summarised in Tables I and II. No consistent changes were found in either haemoglobin concentration, hematocrit value or red cell count. The intramuscular and intravenous groups did not differ significantly from each other or from the control group (Student's "*t*"-test).

In the rabbits of series I, the transferrin, when determined after a total of 215 mg. Fe/kg., was found to be saturated in 5 out of 6 animals. The mean value for iron in serum in these 5 animals was 290 ± 20 μg. per cent. The values for iron in serum and the unsaturated iron-binding capacity in series II showed much individual variation both before and during treatment and no consistent changes could be attributed to the iron-sorbitol administration.

At post-mortem examination no excess pleural or peritoneal fluid was observed. There was no discolouration of the internal organs except for the livers, which in some animals showed a deeper reddish-brown colour than those of the controls. In the intramuscularly treated rabbits the lymph nodes in the groin displayed a light-brown colour. At the sites of injection the muscles were markedly brown coloured and in 3 animals necrotic areas were observed. The organ: body weight ratios for liver, spleen and kidney of the treated rabbits did not differ significantly from

TABLE III

THE EFFECT OF IRON-SORBITOL TREATMENT ON IRON CONTENT (MG./G. WET WEIGHT) AND TISSUE/WEIGHT RATIO OF INTERNAL ORGANS. SERIES I.

Tissue	Administration				Control	
	i.m.		i.v.			
	Fe	weight ratio	Fe	weight ratio	Fe	weight ratio
Liver	0·59 ± 0·03	3·53 ± 0·16	0·54 ± 0·06	3·86 ± 0·21	0·06 ± 0·01	3·30 ± 0·27
Kidney ..	0·28 ± 0·03	0·58 ± 0·03	0·25 ± 0·03	0·61 ± 0·01	0·05 ± 0·01	0·67 ± 0·04
Spleen ..	0·29 ± 0·02	0·07 ± 0	0·32 ± 0·14	0·07 ± 0	0·05 ± 0·01	0·08 ± 0·01
Lungs ..	0·086 ± 0·014	0·47 ± 0·02	0·103 ± 0·011	0·51 ± 0·02	0·03	0·61 ± 0·06
Heart.. ..	0·03	0·35 ± 0·05	0·03	0·37 ± 0·04	0·04	0·30 ± 0·02
Inj. site ..	0·54 ± 0·05	—	—	—	0·02	—

those of the control animals (Table III). The greatest concentration of iron was found at the injection site in the muscle and in the liver (Table III). Iron values well above the control were also seen in spleen, kidney and lungs, while the iron level in the heart was not significantly changed.

The liver contained large quantities of stainable iron mainly localised to the portal areas. Conglomerates or iron granules were contained within the Kupffer cells and also in the sinusoidal lining cells. In the peripheri of the lobules, the parenchymal cells were filled with smaller iron granules. There were no signs of tissue damage. In the spleen, large iron deposits were observed in the capsule and in the fibrous trabeculae, sometimes to such an extent that the cell limits were difficult to distinguish. Aggregates of iron granules were observed in macrophages and apparently also extracellularly in the sinusoids of the red pulp. The sinusoidal lining substance displayed a diffuse blue colour. None or only small quantities had entered the lymphoid tissue which seemed to be normal in total amount. The general pattern for the distribution of iron in these organs did not differ essentially from that previously described for the iron-dextran and saccharated oxide of iron complexes (Nissim 1953, Pinninger 1956).

In the kidney the iron content was confined to the cortical substance. The epithelial cells of the proximal tubules contained fine iron granules

in the cytoplasm. Some of these cells showed disintegration of the cell membrane. This lesion is probably of the same nature as that observed by Golberg (1958) since the amount of damage seemed to be related to the time elapsed between autopsy and fixation of the tissue sample. In the heart and brain no significant amounts of iron and no pathological changes were observed.

The injection site showed large deposits of iron mainly in the connective tissue surrounding the muscle bundles. A large number of iron-filled macrophages were observed in the fatty tissue and occasionally interstitially in the muscle tissue.

Antigenicity. In none of the three different tests could any antigenic properties of iron-sorbitol be observed. There were no signs of anaphylaxis in the sensitised guinea-pigs when they were injected with the challenging dose intracardially. The isolated ilial strips from sensitised guinea-pigs did not respond to iron-sorbitol added to the bath. In the gel-diffusion experiments no reaction was observed when iron-sorbitol was tested against sera from the rabbits treated for 9 weeks with a total of 215 mg. Fe/kg.

Discussion

The acute toxicity of the iron-sorbitol is high when compared with the iron-dextran or the best preparations of saccharated oxide of iron (Martin and others 1955). This is presumably the consequence of the high diffusibility of the complex (Lindvall and Andersson 1961). Intravenous or intra-arterial injections to anaesthetised cats and rabbits are well tolerated and the effects are comparable to those of iron-dextran. After rapid i.v. injections to cats equi-effective depressor doses of iron-dextran, iron-sorbitol and ferrous sulphate showed a 60:40:1 relationship. The hypotensive effect of iron-sorbitol and iron-dextran seems to be due to the presence in small amounts of ferrous iron, which has been shown by Rajapurkar (1960) to exert an adrenergic blocking action. Chemical analysis by Dr. Lindvall revealed that both iron-dextran and iron-sorbitol contain ferrous iron and although the depressor effect is not directly proportional to the amount it shows the expected relationship.

The acute experiments on anaesthetised animals further demonstrated a good *in vivo* stability of the iron-sorbitol complex. When injected intra-arterially into the hind leg of the cat perfused with blood at a constant rate the peripheral resistance did not increase. Saccharated oxide of iron, and ferric chloride, in similar experiments augmented the peripheral resistance presumably as a result of intravascular precipitation. The absence of proteinurea in the subchronic experiments in rabbits also indicates that the iron-sorbitol complex is stable *in vivo* in the sense that it does not precipitate or cause precipitation of blood constituents. For the ultimate usefulness of iron-sorbitol the apparent lack of antigenic properties will also be of importance.

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