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EFFECTS OF THERAPEUTIC ULTRASOUND ON CLEARANCE RATE OF BLOOD BORNE COLLOIDAL PARTICLES *IN VIVO*

A. H. SAAD AND A. R. WILLIAMS

From the Department of Medical Biophysics, University of Manchester Medical School, Oxford Road, Manchester M13 9PL

SMALL PARTICLES injected into the blood stream are rapidly removed by the reticulo-endothelial system (RES), now generally referred to as the mononuclear phagocyte system, which is distributed mainly within the liver, spleen, bone marrow, alimentary canal and lymph nodes. The rate of removal of the particulate material is a measure of the phagocytic activity of the RES.

An ideal particulate material which is to be used to obtain an estimate of the efficiency of the RES should have the following characteristics:

(1) It should be readily available as a sterile and stable colloidal suspension.
(2) It should not interact with tissue components and be unable to cross the normal capillary endothelium.
(3) There should be a narrow range of particle sizes.
(4) Uptake should be selective by the RE cells alone.
(5) It should be non-toxic.
(6) It should be easily measured in blood and tissue samples.

Sulphur colloid labelled with ^{99m}Technetium meets these criteria (Larson & Nelp, 1966) and, being radioactive, its rate of removal from the blood may be followed by placing a sodium Iodide (NaI) detector over a muscular part of the body which does not contain significant amounts of RE tissue and does not take up detectable amounts of the colloid from the blood. Thus the level of colloid in the blood can be monitored continuously and accurately by placing a NaI detector over the upper thigh thus avoiding the shaft of the femur, the marrow of which contains RE cells, by shielding it and other parts of the body with lead. The study undertaken here is to test the effect of therapeutic intensities of ultrasound upon the functioning of the RES as measured by the rate of removal of labelled sulphur colloid from the blood stream of rats.

METHOD

(a) *Preparation of ^{99m}Technetium labelled sulphur colloid.*—Sodium thiosulphate (0·4 ml of 10 mg/ml), potassium perrhenate (0·4 m of 2·5 mg/ml) and hydrochloric acid (0·4 ml of 1M solution) were mixed as described by Larson & Nelp (1966) and sodium pertechnetate (3mCi) in isotonic saline added to a final volume of 3·2 ml. The mixture was heated for 3 min in a water bath at 100°C and 0·8 ml of phosphate buffered saline (pH 7·4) added to neutralize the mixture and stop the reaction.

(b) *Measurement of the half life of removal of colloid from blood.*—Female rats weighting between 200–250 g were anaesthetized with ether and injected *via* a tail vein with 0·3 ml of sulphur colloid. The amount of colloid in the blood was monitored using a sodium iodide detector placed directly over the thigh of the animal, other parts of the body being shielded by lead. Ten second counts were obtained every 30 sec until there was no detectable change. The half life of clearance of colloid was obtained from the plot of log counts against the time after injection.

(c) *Application of ultrasound.*—The upper abdominal area of the rat was shaved, liberally coated with ultrasound couplant cream (Halas Laboratories Ltd. Wetherby, Yorks) and the "umbilical" region of the abdomen irradiated from the ventral aspect with 1·65 MHz ultrasound at a variety of space-averaged intensities. The labelled colloid (0·3 ml) was administered 5 min after the ultrasonic exposure. Control experiments were also carried out in which sulphur colloid (0·3 ml) was injected intravenously 5 min after mock-irradiation of the umbilical region of the abdomen of female rats was performed.

(d) *Heparin administration.*—Heparinized saline was injected intravenously; after 3 min labelled colloid (0·3 ml) was administered.

RESULTS

(a) *Calculation of half-life of removal of colloid*

A plot of log counts against time shows that the removal of sulphur colloid from the blood is biexponential. Extrapolation of the slower phase to zero time would enable the fast phase of removal of colloid to be stripped off from the biexponential plot. From a semilogarithmic plot of this fast phase, the $T_{\frac{1}{2}}$ of removal of colloid could be calculated.

(b) Four serial administrations of 0·3 ml colloid into one animal results in half-lives of removal of colloid of 0·8, 0·935, 1·23 and 1·13 min respectively.

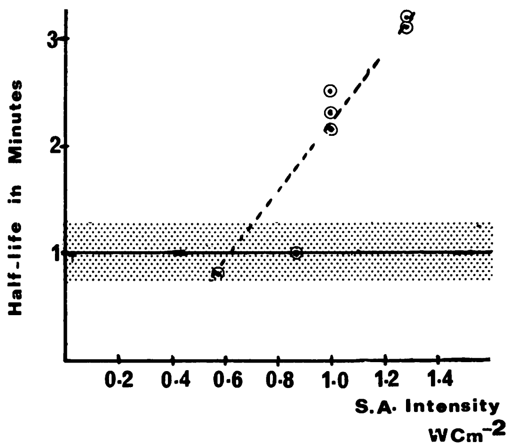

FIG. 1.—A plot of the intensity of ultrasound applied over the upper abdomen of rats (for 5 min) against the resulting $T_{\frac{1}{2}}$ of removal of blood-borne colloid. The line and the dotted zone indicate the mean $T_{\frac{1}{2}} \pm$ s.d. of removal of blood-borne sulphur colloid in unsonicated rats (n = 15).

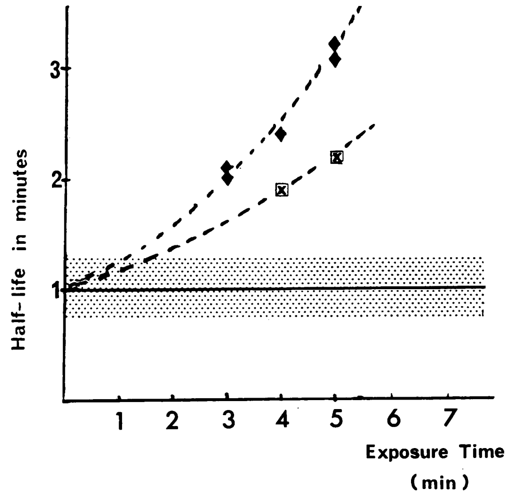

FIG. 2.—A plot of the length of the period of application of ultrasound (intensity being constant) against the resulting $T_{\frac{1}{2}}$ of removal of blood-borne colloid. The line and the dotted zone indicate the mean $T_{\frac{1}{2}} \pm$ s.d. of removal of blood-borne sulphur colloid in unsonicated rats. (◆ = 1·28 W/cm^2, ⊠ = 1·04 W/cm^2).

(c) *Effects of ultrasound on the RES*

Fig. 1 shows the effect of application of various intensities of continuous wave ultrasound for 5 min on the rate of removal of sulphur colloid. Fig. 2 shows the effect of application of 1·28 W/cm^2 and 1·04 W/cm^2 continuous wave ultrasound for various times on the rate of removal of sulphur colloid.

No significant increase in the $T_{\frac{1}{2}}$ of removal of blood-borne sulphur colloid was obtained following mock-irradiation.

The above results demonstrate that ultrasound (c.w., f = 1·65 MHz) reduced the rate of removal of colloid, (*i.e.* increased the $T_{\frac{1}{2}}$ of removal) at therapeutic intensities. The time of exposure also contributed to this effect.

(d) Sonication for 5 min at 1·28 W/cm^2 caused the $T_{\frac{1}{2}}$ of removal of colloid to increase to 3·1 min from 1·2 min. After 3 h, the $T_{\frac{1}{2}}$ of removal fell to 2·1 min.

(e) *Effects of heparin*

Heparin (PULARIN®) in saline injected intravenously (i.v.) was found to

TABLE.—*The effects of i.v. injections of heparin upon the $T_{\frac{1}{2}}$ of removal of* ^{99m}*Technetium-labelled sulphur colloid. The magnitude of the increase in $T_{\frac{1}{2}}$ of removal of sulphur colloid following administration of heparin is more than the magnitude of the increase in $T_{\frac{1}{2}}$ of removal following serial administration of sulphur colloid*

$T_{\frac{1}{2}}$ of control (min) Injection No. one	two	Procedure	$T_{\frac{1}{2}}$ of test Injection No. three
1·24	1·50	150 units of heparin injected	2·92
—	—	100 units of heparin injected	2·07
0·81	1·10	100 units of heparin injected	1·90
1·03	1·20	80 units of heparin injected	1·75

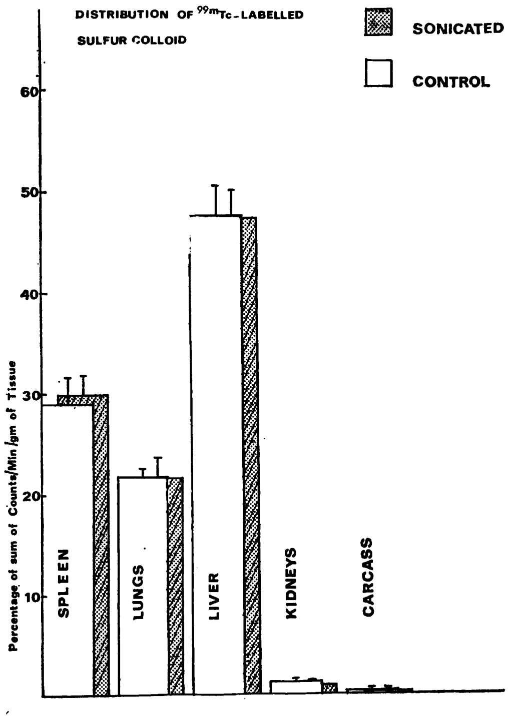

FIG. 3.—Histogram showing the distribution of ^{99m}Technetium-labelled sulphur colloid following removal from the blood of normal (*i.e.* mock-sonicated) and sonicated rats (n = 6).

increase the $T_{\frac{1}{2}}$ of removal of colloid. The Table shows these effects.

DISCUSSION

Ultrasound applied over the umbilical region of the female rat causes an increase in the $T_{\frac{1}{2}}$ of removal of ^{99m}Tc-labelled sulphur colloid from the blood. This increase is dependent on the intensity as well as the period of sonication.

The possible mechanisms by which ultrasound causes such effects are:

(1) *Biophysical mechanisms*

The blood pressure was monitored while ultrasound was applied, it was found that sonication at 1·28 W/cm^2 for 4 min caused a drop in the blood pressure of the anaesthetized rat (anaesthetized with INACTIN®) from 110/90 mmHg to 75/65 mmHg. Assuming that the blood vessel resistance remained constant, then such a drop in blood pressure could cause a drop in blood flow which would lead to a decrease in the rate of removal of colloid. To test this hypothesis, the rate of removal of colloid was measured in a rat anaesthetized with INACTIN® (INACTIN® was used in order to maintain a steady blood pressure) and the blood pressure was then reduced from 120/110 mmHg to 90/80 mmHg by increasing the dose of anaesthetic. This resulted in an increase in the $T_{\frac{1}{2}}$ of removal of colloid from 0·97 min to 1·31 min. This increase in the $T_{\frac{1}{2}}$ of removal of blood-borne sulphur colloid is considered insignificant. It is reasonable to predict that further reduction in blood pressure using the anaesthetic INACTIN®, such that the blood pressure reduction obtained with anaesthetic alone is equal to that obtained when ultrasound was applied, is unlikely to lead to a significant increase in the $T_{\frac{1}{2}}$ of removal of colloid as was obtained following sonication at 1·28 W/cm^2 for 4 min. Therefore, ultrasound must exert another effect in addition to this decrease in blood pressure, which could account for the effect upon the RES.

The ultrasound could have caused the vessels supplying the liver, for example the hepatic portal vein (PV) to constrict, hence less blood would be carried to the liver (the PV is the vessel which supplies 80% of the liver blood flow). Such a constriction would cause a decrease in the rate of removal of colloid. However, this effect could be discounted on the basis of the observation that ^{99m}Tc-labelled sulphur colloid prepared with 0·8M HCl (instead of the 1M HCl) was removed from the blood at an unchanged rate following sonication. The two types of colloid have different physical properties (Saad & Williams, unpublished observation). Heparin injected i.v. similarly does not alter the rate of removal of the sulphur colloid prepared with 0·8M HCl.

The ultrasound could have damaged or inactivated the macrophages. However, distribution studies showed that sonication did not cause a significant change in the amount of colloid removed by either the spleen, lungs or liver (Fig. 3).

(2) *Biochemical mechanisms*

Opsonins are globulins which somehow cause an increase in the phagocytic activity of macrophages for foreign substances (Saba, 1970). Ultrasound could be changing the rate of removal of colloid by changing either the concentration or the activity of these opsonins. The two types of sulphur colloid could be opsonized by different globulins. Sonication may affect the activity or plasma concentration of one of these opsonins. Also evidence exists which shows that an opsonin binds to heparin (Mossesson, 1978; Saba *et al.*, 1978) This could explain the effect of ultrasound and heparin on the rate of removal of the sulphur colloid prepared with 1M HCl and with 0·8M HCl. However, the time of onset of the effect of ultrasound (after 5 min following the end of time of sonication) would tend to discredit the above theory. An alternative theory is that the two types of colloid are phagocytozed by different populations of macrophages (Vernon-Roberts, 1972) and that ultrasound and heparin affect only one of these populations of macrophages by inhibiting uptake. Ultrasound could be inhibiting the uptake of colloid by the macrophages if it is causing tissue damage which is resulting in the release of significant quantities of tissue debris. This debris (*e.g.* free haemoglobin from disrupted erythrocytes) would be competing with the colloid for removal by the R.E.S. Consequently, the rate at which the colloid is removed would be changed but its final distribution would be unaltered.

In summary, continuous wave ultrasound applied at therapeutic intensities causes a decrease in the rate of removal of certain colloids from the blood of rats. This decrease has been shown to be at least partially reversible. Further work needs to be done to determine the mechanism by which ultrasound causes its effect upon the RES.

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