

PLASMA ATROPINE CONCENTRATIONS DETERMINED BY RADIOIMMUNOASSAY AFTER SINGLE-DOSE I.V. AND I.M. ADMINISTRATION

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SUMMARY

The plasma concentrations of atropine following i.v. or i.m. administration to surgical patients were determined by radioimmunoassay. When atropine sulphate 1 mg was given i.v. there was a rapid initial removal of the drug from the circulation in the first 10 min; thereafter the plasma concentration decreased more slowly. Atropine i.m. was rapidly absorbed with peak concentrations occurring at 30 min following injection. The plasma atropine concentration then decreased slowly, probably because of uptake of atropine by muscarinic cholinergic receptors. The chronotropic effect of atropine appeared to correspond to the concentration in plasma following i.m. administration. We conclude that i.m. atropine, as a premedication, should be given not later than 30 min before induction of anaesthesia.

Atropine is widely used as a premedicant for the reduction of secretions, protection against vagal overactivity and prevention of vomiting after operation (Kessel, 1974; Mirakhur et al., 1978). It is of interest to compare the therapeutic effect of the drug with its blood concentration. However, it has not been possible to determine the small concentrations of atropine found in plasma following typical doses, because of the lack of a sensitive method. Earlier investigations were performed with radio-labelled atropine. An obvious limitation of these studies was that the radioactivity rather than the parent compound was measured. Kalser and McLain (1970) administered ¹⁴C-labelled atropine i.m. to adult volunteers and found that the concentration of carbon-14 in the blood reached maximum concentration after 15–30 min. Beerman, Hellström and Rosén (1971) reported that, after i.v. administration of ³H-labelled atropine to humans, the plasma concentrations of radioactivity decreased rapidly. Oral administration on the other hand, resulted in maximum radioactivity in plasma after 1 h.

Recently, sensitive radioimmunoassay procedures for the determination of atropine have been reported

(Fath, Sollenberg and Sörbo, 1975; Wurzbürger et al., 1977). They have only been used for determinations of atropine in experimental animals.

In the present investigation we determined the plasma concentrations of atropine following a single-dose i.v. or i.m. administration of the drug as premedication to surgical patients. An improved version of the radioimmunoassay developed by Fath, Sollenberg and Sörbo (1975) was used.

METHODS

Patients, atropine administration and anaesthesia

The patients (ages 16–73 yr) underwent elective operations of comparable severity and duration (cholecystectomy, proximal vagotomy, mastectomy, internal fixation of tibial fracture, hemicolectomy). Single doses of atropine sulphate 1 mg were given as premedication to (a) six male patients i.v. immediately before induction of anaesthesia and (b) i.m. 30 min before the start of anaesthesia to two male and two female patients. The method of anaesthesia was the same for all patients: sodium thiopentone for induction followed by suxamethonium to permit intubation of the trachea. Fentanyl with 70% nitrous oxide in oxygen was given to maintain anaesthesia. When required, muscle relaxation was achieved with pancuronium.

Heparinized blood samples were taken immediately before atropine administration and then at intervals (figs 2, 3). Plasma was separated by centrifugation and stored frozen until analysed.

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Production of antiserum

The preparation of the immunogen (containing 21 residues of atropine per molecule of bovine serum albumin) has been described previously (Fasth, Sollenberg and Sörbo, 1975). Five male inbred chinchilla rabbits were immunized with immunogen 0.1 mg intradermally (Vaitukaitis et al., 1971). Blood samples were collected from the marginal ear vein 3 weeks after immunization and then at 1-week intervals. Serum was separated by centrifugation and assayed for anti-atropine antibodies (Fasth, Sollenberg and Sörbo, 1975). All animals responded with antibody production, which reached an optimum at 4–5 weeks after immunization. The concentration decreased slowly in four of the animals but in one it remained constant for 3 months. The antiserum used for the radioimmunoassay was harvested from the animals 9 weeks after the immunization.

Radioimmunoassay

A 2-ml aliquot of human plasma was mixed with 1 ml of sodium carbonate buffer 0.5 mol litre⁻¹ (pH 10.8) in an acid-washed test tube and extracted with 10 ml of benzene-1.5% isoamyl alcohol. The benzene phase was separated by centrifugation and atropine re-extracted into 0.5 ml of hydrochloric acid 1 mol litre⁻¹. The latter was evaporated to dryness under vacuum over-night. To the residue was added 0.2 ml of sodium phosphate buffer 0.12 mol litre⁻¹, pH 7.5, horse control serum 0.2 ml (Seronorm, Nyegaard & Co., Oslo, Norway) and ³H-atropine 10 µlitre containing atropine 20 pmol of specific activity 439 Ci mol⁻¹ (The Radiochemical Centre, Amersham). After thorough mixing, 0.1 ml of anti-atropine antiserum, diluted 1 : 20 with saline, was added. The sample was incubated at 25 °C for 60 min and saturated ammonium sulphate 0.5 ml was added. The precipitate was centrifuged 30 min later, washed twice on the centrifuge with 50% saturated ammonium sulphate 1 ml and dissolved in distilled water 1.5 ml. This solution was transferred to a counting vial containing 15 ml of a Triton X-100-toluene based scintillation liquid (Patterson and Greene, 1965) and the sample was counted in a liquid scintillation counter. The amount of atropine present in the serum samples was obtained from a standard curve, prepared by adding known amounts of atropine to human control serum (Hyland Division, Travenol Laboratories, Lessines, Belgium). Linearization of the standard curve was obtained by plotting the quotient of the radioactivity bound in the absence (B_0) and in the presence (B) of unlabelled

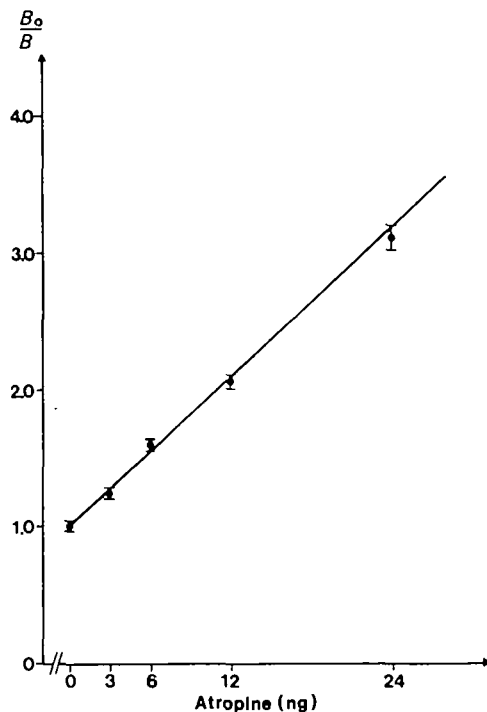


FIG. 1. Standard curves for atropine radioimmunoassay. The values presented are means \pm SEM of 19 separately determined standard curves. The quotient of the radioactivity bound in the absence (B_0) and in the presence (B) of unlabelled atropine *v.* the serum concentration of the latter is indicated. B_0 was $42.7 \pm 1.19\%$ (mean \pm SEM) of the total radioactivity present in the assay.

atropine *v.* the amount of the latter (fig. 1). The precision of the assay was evaluated by 19 repeated determinations of B_0/B for serum containing atropine 1.5 ng ml⁻¹. The result obtained was 1.24 ± 0.055 (mean \pm SD). The sensitivity of the method, defined as the amount of atropine giving a B_0/B quotient significantly greater than 1.00, was 0.9 ng in the assay, corresponding to an atropine concentration in plasma of 0.5 ng ml⁻¹.

RESULTS

Atropine in plasma

Following an injection of atropine i.v. there was a rapid reduction of plasma concentrations within the first 10 min (fig. 2). It was calculated that the amount of atropine remaining in the circulation at 10 min after injection corresponded to less than 5% of the dose administered. Plasma concentrations then decreased more slowly during the following 50 min.

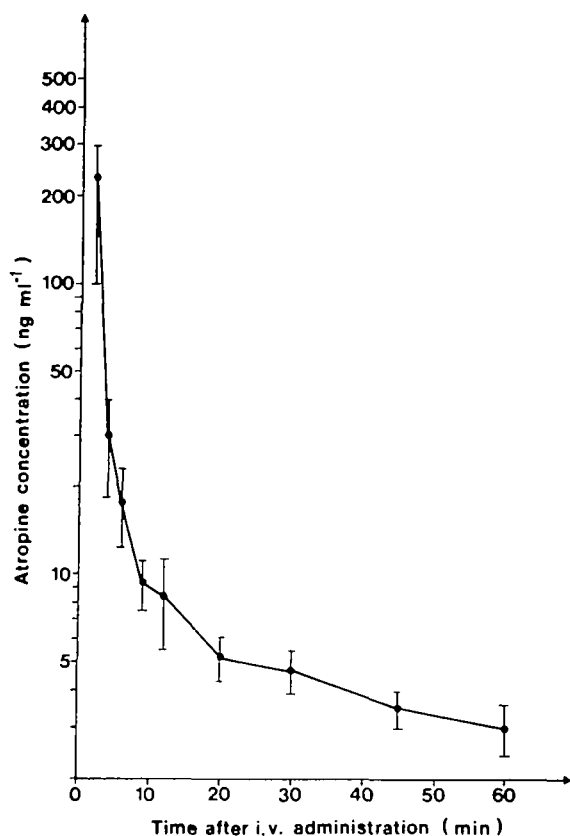


FIG. 2. Serum atropine concentrations after atropine sulphate 1 mg i.v. Each value represents the mean \pm SEM of the determinations in six male patients.

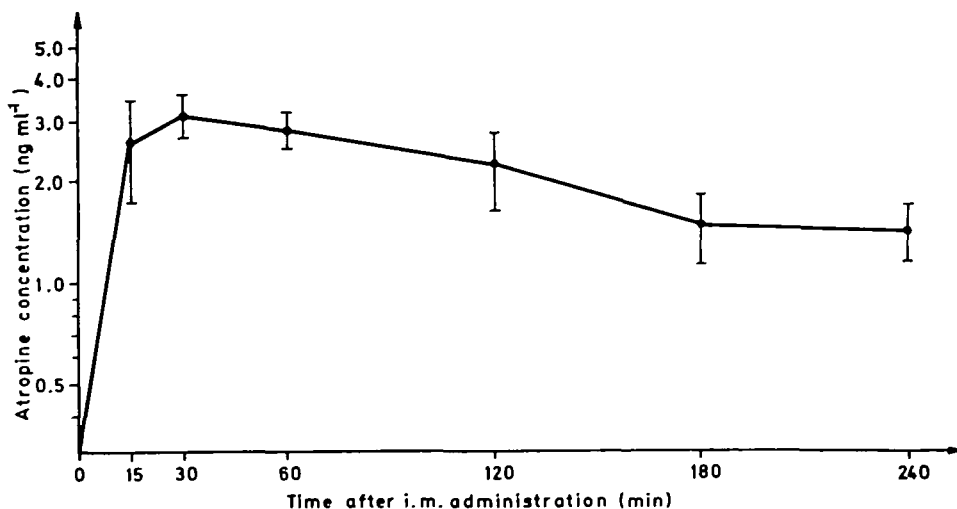


FIG. 3. Serum atropine concentration after atropine sulphate 1 mg i.m. Each value represents the mean \pm SEM of the determinations in four patients (two males and two females).

One hour after i.v. administration, the plasma concentration was approximately the same as after i.m.

I.m. atropine was rapidly absorbed, reaching peak concentrations in plasma at 30 min (fig. 3). Plasma concentrations then decreased slowly, but were still detectable at 240 min after injection. The concentration after 1 h corresponded to that after i.v. administration.

Radioimmunoassay

The antiserum used was raised by intradermal immunization and was of a slightly greater potency than that obtained earlier after subcutaneous administration of the antigen (Fasth, Sollenberg and Sörbo, 1975). It thus bound 40% of the labelled atropine at a final dilution of 1 : 100, whereas the same effect was obtained with the earlier antiserum at 1 : 40 final dilution under otherwise identical conditions. Furthermore, the present antiserum recognized (+)-hyoscyamine and (-)-hyoscyamine (the two isomers of atropine) with equal efficiency, whereas the antiserum studied previously was less effective with (-)-hyoscyamine. We also verified that the drugs used for anaesthesia (sodium thiopentone, suxamethonium, fentanyl and pancuronium) were not recognized by the antiserum. In order to increase the sensitivity of the assay a concentration step, based on solvent extraction, was included. Various solvents were explored for this purpose and benzene, containing a small amount of isoamyl alcohol, gave the best results. The addition of isoamyl alcohol to non-polar solvents for extraction of basic drugs was originally

suggested by Brodie, Udenfriend and Baer (1947) to prevent adsorption of the drug to glass surfaces. The yield of atropine in the concentration step was 84% but was corrected since both the standard and unknown plasma samples were taken through the same procedure.

DISCUSSION

A rapid initial removal of atropine from the circulation following injection i.v. was observed in the present investigation, confirming the findings of Beerman, Hellström and Rosén (1971) using ^3H -labelled atropine. However, these authors reported that the initial decrease was followed by a transient increase at 45 min, which was not observed by us. Wurzbürger and others (1977), also using radioimmunoassay, observed a rapid decrease of blood concentrations of atropine in the dog after i.v. injection. This is probably a result of tissue uptake by muscarinic cholinergic receptors, as demonstrated in animals using radio-labelled atropine (Albanus et al., 1968). Atropine is a mixture of two isomers, (+)- and (-)-hyoscyamine, and only the latter is pharmacologically active. We found that less than 5% of the administered dose of atropine was present in the circulation 10 min after i.v. administration. As our radioimmunoassay detects both (+)- and (-)-hyoscyamine with equal efficiency, this indicates that the pharmacologically inactive (+)-isomer also disappears from the circulation by tissue uptake. This interpretation is supported by a recent report (Aronstam, Abood and MacNeill, 1977) indicating that certain muscarinic cholinergic receptors bind anticholinergic drugs without stereo-selectivity.

When atropine was administered by i.m. injection, the drug was rapidly absorbed with peak concentrations in plasma at 30 min. The plasma concentrations obtained at that time after i.m. injection were comparable to those found after i.v. administration. The clinical efficiency of the i.m. route for atropine is thus understandable. If the plasma concentration curve obtained after i.m. administration (fig. 3) is compared with the time course of the cardiac-accelerating effect of the drug (Sidell, Magness and Bollen, 1970), the heart rate closely follows the pattern set by the plasma concentrations. On the other hand, the depression of salivation is apparently a more delayed effect of atropine, reaching its peak effect about 100 min after i.m. administration (Murrin, 1973). This illustrates the well known fact that plasma concentrations of a drug may not always run parallel to its pharmacological effects.

A recent survey carried out in Great Britain and Ireland (Mirakhur et al., 1978) showed that atropine was the most popular anticholinergic premedicant. The principal mode of administration is i.m. and the i.v. route is second. The results of the present investigation indicate that, when atropine is given as premedication by i.m. injection, this should be performed at least 30 min before the start of anaesthesia.

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DETERMINATION DES CONCENTRATIONS D'ATROPINE DANS LE PLASMA PAR RADIOIMMUNOESSAI APRES ADMINISTRATION D'UNE SEULE DOSE D'ATROPINE PAR VOIE INTRAVEINEUSE ET PAR VOIE INTRAMUSCULAIRE

RESUME

Les concentrations d'atropine dans le plasma ont été déterminées par radioimmunoessai après l'administration par voie intraveineuse ou intramusculaire de ce médicament

à des opérés. Lorsqu'on a administré par voie intraveineuse du sulfate d'atropine à raison de 1 mg, on a constaté à l'origine un retrait rapide du médicament de la circulation au cours des 10 premières minutes; après quoi les concentrations dans le plasma ont baissé plus lentement. L'atropine administrée par voie intramusculaire a été rapidement absorbée, les concentrations de pointe se produisant 30 min après l'injection. Les concentrations d'atropine dans le plasma ont ensuite baissé lentement, probablement à cause de la fixation de l'atropine par les récepteurs cholinergiques muscariniques. L'effet chronotrope de l'atropine semble correspondre à la concentration dans le plasma après l'administration intramusculaire. Nous en concluons que l'atropine administrée par voie intraveineuse, en tant que prémédicament, doit être injectée au moins 30 min avant l'induction de l'anesthésie.

**ATROPIN-PLASMAKONZENTRATIONEN,
BESTIMMT DURCH RADIOIMMUNOTEST
NACH INTRAVENÖSER UND
INTRAMUSKULÄRER EINZELDOSIS**

ZUSAMMENFASSUNG

Die Atropin-Plasmakonzentrationen nach intravenöser oder intramuskulärer Verabreichung bei chirurgischen Patienten wurden mittels Radioimmunotest bestimmt. Wenn Atropinsulfat 1 mg intravenös gegeben wurde, kam es in den ersten 10 min zu einer rapiden Ausscheidung der Droge aus dem Kreislauf; danach sanken die Plasmakonzentrationen langsamer ab. Intramuskulär gegebenes Atropin wurde rapide absorbiert, mit Spitzenkonzentrationen 30 min nach der Injektion. Dann sanken die Plasma-

konzentrationen langsamer ab, wohl wegen der Aufnahme von Atropin durch die muscarinischen cholinergischen Rezeptoren. Der chronotrope Effekt von Atropin schien der Plasmakonzentration nach intramuskulärer Verabreichung zu entsprechen. Wir schliessen daraus, dass Atropin intramuskulär als Vorbehandlung nicht später als 30 min vor Anästhesieeinleitung verabreicht werden soll.

**CONCENTRACIONES DE ATROPINA EN EL
PLASMA DETERMINADAS POR
RADIOINMUNOPRUEBA DESPUES DE
ADMINISTRACION DE UNA DOSIS
UNICA I.V. E I.M.**

SUMARIO

Se determinó por radioinmunopruera las concentraciones de atropina en el plasma después de la administración i.v. o i.m. a pacientes sometidos a cirugía. Cuando se administró 1 mg de sulfato de atropina i.v., se produjo una remoción inicial rápida de la substancia en la circulación durante los primeros 10 min; después, la concentración en el plasma disminuyó más despacio. La atropina i.m. fue asimilada rápidamente con concentraciones máximas 30 min después de la inyección. Luego, la concentración de atropina en el plasma disminuyó despacio, probablemente por motivo de la captación de la atropina por los receptores colinérgicos muscarínicos. El efecto cronotrópico de la atropina pareció corresponder a la concentración en el plasma después de la administración i.m. Llegamos a la conclusión de que la atropina i.m., como premedicación, no debe administrarse sino a más tardar 30 min antes de la inducción de la anestesia.