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Variability of Mitomycin C Adsorption by Activated Charcoal

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Abstract

A saline suspension of mitomycin C adsorbed on activated charcoal and administered intraperitoneally has been reported to be safe and effective in the treatment of gastric carcinoma. Activated charcoal specifically targets tumour and lymph-node tissues and the sustained higher local drug concentration is thought to be beneficial. The charcoal particles used in these suspensions have varied in size from > 147 μm to < 20 nm in diameter, but no data have been published to show how this might affect drug adsorption and delivery. Any variability in drug adsorption could pose a serious clinical risk for drugs with a narrow therapeutic index. We have, therefore, investigated the adsorption of mitomycin C on activated charcoal in-vitro.

Activated charcoal was ground and sieved to yield four size-fractions between 180 and 53 μm. Adsorption isotherms ($n \geq 3$) were constructed and applied to the Freundlich model with 0–100 $\mu g\,mL^{-1}$ mitomycin C measured by HPLC with detection at 365 nm. Adsorption of mitomycin C by activated charcoal varied by a factor of three under identical conditions at room temperature (21°C) and at 37°C. The specific adsorption (μg mitomycin C (mg activated charcoal)$^{-1}$) was generally higher at 37°C than at room temperature. The variability of mitomycin C adsorption was greatly reduced by addition of the surface-active agent polyvinylpyrollidone, used to determine that adsorption of mitomycin C was independent of activated charcoal particle size.

The characteristics of adsorption of mitomycin C by activated charcoal are complex and should be thoroughly investigated to discover the critical controlling factors before submitting the suspensions for further clinical evaluation.

Mitomycin C adsorbed by a suspension of activated charcoal in saline has been used as a slow-release drug-delivery preparation to treat intraperitoneal tumours with the intention of giving a high local dose with low systemic toxicity (Markman 1991). Activated charcoal is said to target peritoneal-tumour and lymph-node tissues specifically, so that the mitomycin C dose is delivered at high concentrations to these sites and remains there for a prolonged period (Hagiwara et al 1992a; Takahashi et al 1994). Relatively little drug (10–100 times less than the intraperitoneal concentrations) has been detected systemically in animals or man (Hagiwara et al 1988a, 1992b; Cunliffe 1991). Other advantages claimed for this dosage form include simplicity, low cost, ease of preparation, dosage adjustment and administration as adjuvant treatment during surgery.

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The properties of activated charcoal make it an excellent candidate for a sustained-release drug-delivery system. Drug molecules are adsorbed by and desorbed from the surface of the activated charcoal in a state of dynamic equilibrium which is perturbed by changes in free (unadsorbed) drug concentration (C) because of drug disposition processes (Hagiwara & Takahashi 1987).

The combination of mitomycin C with activated charcoal of particle size from 147 μm downwards has been investigated in Japan (Hagiwara & Takahashi 1987), but the effect of activated charcoal particle size on mitomycin C adsorption has not been reported. Suspensions are often associated with difficulties related to the reproducibility of their properties (Storz & Kennon 1976), but published data adequate for assessment of the clinical variability of mitomycin C release from activated charcoal are also lacking. Variations in the adsorption–desorption properties of individual charcoal preparations could have important impli-

cations with regard to the clinical toxicity and efficacy of the activated charcoal-mitomycin C preparation, particularly because mitomycin C has a narrow therapeutic index. Wetting agents such as carmellose or polyvinylpyrollidone (PVP) have been used as suspension stabilizers. Polyvinylpyrollidone has been used with activated-charcoal preparations, but the effects on mitomycin C adsorption have not been published (Hagiwara & Takahashi 1987). We have therefore, studied the reproducibility of the adsorption process and the effect of polyvinylpyrollidone and charcoal particle size on mitomycin C adsorption.

Materials and Methods

Materials

Activated charcoal (CHR-30; Nakarai Chemical, Kyoto, Japan) was a kind gift from Professor T. Takahashi (Kyoto, Japan). Mitomycin C and polyvinylpyrollidone (40 000 MW, 28–32 cps viscosity) were purchased from Sigma (Poole, UK). HPLC-grade acetonitrile and methanol were purchased from Fisons (Loughborough, UK), and disodium hydrogen orthophosphate and potassium dihydrogen orthophosphate from BDH Chemicals (Poole, UK). Sodium chloride was from Philip Harris (Manchester, UK).

Milling of activated charcoal

Activated charcoal was ground for 1 h in a 1-L porcelain pot with porcelain ball charges in a Pascal L9FS ball-mill (Pascal Engineering, Crawley, UK) as described by Parrott (1976); it was then collected for sieving.

Sieving

A series of 200-mm bronze-rimmed steel mesh sieves of 180, 150, 106, 75, 53 and 38-μm mesh sizes (Endecotts, London, UK) were assembled with lower sieves becoming progressively finer. Activated charcoal (15 g) was placed on the top sieve. The stack of sieves was then secured on to an Octagon 2000 variable-amplitude sieve-shaker (Endecotts, London, UK) and operated at half-maximum speed with an intermittent cycle of 6 s on, 2 s off for 3 h. The charcoal remaining on each of the sieves was then collected and weighed. The percentage of the total weight of activated charcoal on each sieve was calculated and the mean particle-size determined as described by Carstensen (1977).

BET gas adsorption analysis

The total surface area of each sample of activated charcoal was determined by the method of Brunauer et al (1938) with a Micromeritics ASAP 2000 analyser (Micromeritics, Norcros, USA) linked to a pentium 90 IBM-compatible PC (Escom, Liverpool, UK) which acquired and processed the data using DFT software (Micromeritics). The charcoal samples were freed from adsorbed gas by subjecting them to high-vacuum and then cooled to -196°C with liquid nitrogen. The amount of nitrogen adsorbed by activated charcoal samples was measured at a series of different partial pressures of nitrogen gas and the surface area g^{-1} was then calculated for each sample by use of the Brunauer-Emmett-Teller (BET) equation (Cooney 1995).

Determination of mitomycin C adsorption isotherms

Mixtures of activated charcoal with mitomycin C solutions of different concentration (0–100 μg mL^{-1}) were prepared by pipetting mitomycin C (200 $\mu g\,mL^{-1}$ in saline; 0–100 μL) into 1·5-mL polypropylene Eppendorf tubes, adding sieved activated charcoal (1 $mg\,mL^{-1}$ in saline; 50 μL), and then saline to a total volume of 200 μL. The tubes were vortex-mixed for 10 min with a SMI 81058 Multi-Tube Vortexer (SMI, Emeryville, USA) and centrifuged for 5 min in a MSE Microcentaur centrifuge at 12 000 g (Fisons, Crawley, UK). The free mitomycin C concentration in the clear supernatant was measured by HPLC. Standards of mitomycin C with no added activated charcoal were prepared similarly and a standard plot of mitomycin C peak area against mitomycin C concentration was used to determine the concentrations of mitomycin C detected in the supernatant. The bound concentration of mitomycin C was calculated as the difference between the starting concentration and the measured free mitomycin C concentration (Cooney 1995). The data were plotted as Freundlich isotherms by fitting the mathematical expression $Q = aC^n$ where Q is the specific adsorption (μg mitomycin C (mg activated charcoal)$^{-1}$), C is the free drug concentration ($\mu g\,mL^{-1}$), and a and n are constants.

The effect of polyvinylpyrollidone on the adsorption of mitomycin C by activated charcoal

The effect of polyvinylpyrollidone was investigated by adding the compound to activated charcoal in the concentration ratio 1:2·5 polyvinylpyrollidone/activated charcoal. Isotherms were constructed as described above but with polyvinylpyrollidone-treated activated charcoal which had been mixed for 1 h at 37°C.

Isotherm variability studies

The variability of the adsorption isotherms was tested both at 37°C and at room temperature (21 ± 2°C). Each isotherm was tested three or more

times on different days and each sampling point within an isotherm was tested in triplicate.

Measurement of mitomycin C

Mitomycin C was measured by HPLC (Kontron Instruments 325 System gradient solvent-delivery pump; 332 System UV-spectrophotometric detector set at 365 nm; 360 System autosampler with 20-μL sample loop and on-line CompuAdd 486DX33 computer for data acquisition and processing with Kontron PC integration pack chromatographic software; Kontron Instruments, Watford, UK). Chromatography was performed on a 150 × 3·2 mm i.d. Prodigy 5C8 mini-bore column (Phenomenex, Macclesfield, UK) protected by a 5C8 Hypersil guard column (HPLC Technologies, Macclesfield, UK); mitomycin C was eluted isocratically in 10 min at room temperature with 20% aqueous acetonitrile at a flow rate of 0·5 mL min^{-1}.

Statistics

Results are presented as the means ±95% confidence limits, standard deviation (s.d.) or standard error of the means (s.e.m.) ($n \geq 3$). Calculations and data analysis were performed with Microsoft Excel 5·0 (Microsoft Corporation, USA).

Results

Sieve analysis

Four activated charcoal fractions (180–150, 150–106, 106–75 and 75–53 μm) were isolated and used in our experiments. The 180-μm sieve retained activated charcoal without defining an upper size limit and none remained on the finest sieves. The mean diameter, defined as the sieve aperture size that retained 50% cumulative weight of activated charcoal, was 142 μm and is in good agreement with the previously published value of < 147 μm using the same charcoal preparation (Hagiwara et al 1987a,b).

Gas adsorption

The total activated charcoal adsorption area including that of pores showed the activated charcoal fractions to have similar values (Table 1). The surface areas found (481–497 m^2 g^{-1}) were consistent with an activation time during manufacture of approximately 10 to 15 min (Cooney 1995).

Mitomycin C adsorption isotherms

The adsorption isotherms of the different-sized activated-charcoal fractions (Figure 1) showed no correlation with particle size. The amount of mitomycin C adsorbed was greatest for 180–150 μm particles, was significantly less for 150–106 μm and 106–75 μm particles, but increased again for 75–53 μm particles. Threefold variation was found in mitomycin C adsorption isotherms determined on four different occasions with 75–53 μm activated charcoal particles at 37°C (Figure 2).

Adsorption of mitomycin C by 75–53 μm particles was significantly higher at 37°C than at room temperature but elevating the temperature to 50°C did not enhance mitomycin C adsorption (Figure 3).

Table 1. Analysis of the surface area of the activated charcoal fractions by the BET gas-adsorption method (Brunauer et al 1938).

Charcoal fraction (μm)	BET surface area (m^2 g^{-1})
180–150	487
150–106	497
106–75	490
75–53	481

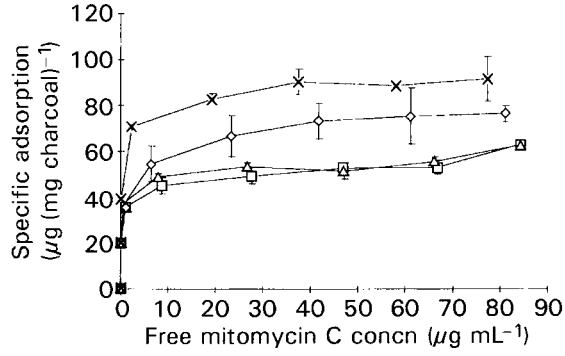

Figure 1. Mitomycin C adsorption isotherms for charcoal of different sizes: ◇, 75–53 μm; □, 106–75 μm; △, 150–106 μm; ×, 180–150 μm. Results are means ±95% confidence limits of triplicate determinations at 21°C.

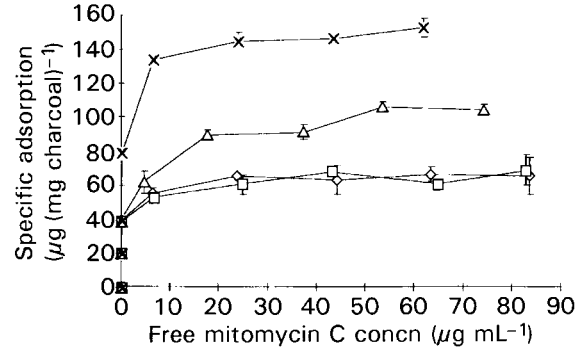

Figure 2. Inter-day variability of adsorption of mitomycin C by 75–53 μm activated charcoal at 37°C. ×, Day 1; △, day 2; ◇, day 3; □, day 4.

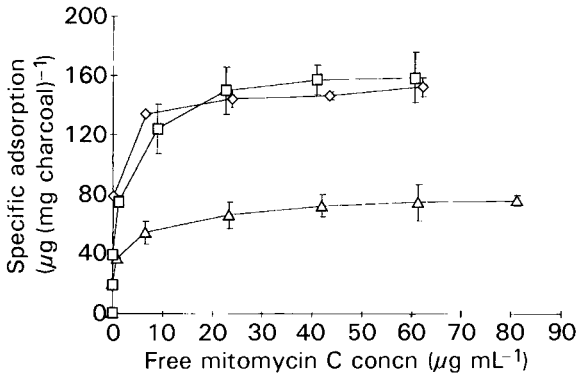

Figure 3. The effect of temperature (◇, 37°C; □, 50°C; △, 21°C) on the adsorption of mitomycin C by 75–53 μm activated charcoal particles. Results are means ± 95% confidence limits (n = 3).

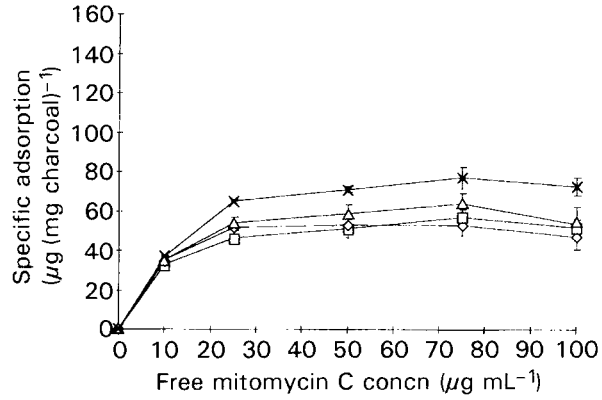

Figure 4. Reduction in the inter-day variability of adsorption of mitomycin C by 75–53 μm activated charcoal by incorporation of polyvinylpyrollidone: ◇, day 1; □, day 2; △, day 3; ×, day 4.

The amount adsorbed by each activated charcoal size-fraction at both temperatures varied by a factor of three (Table 2). The ranges of the maximum specific adsorption (Q; μg (mg activated charcoal)$^{-1}$) of the isotherms determined under identical conditions (at 21°C and 37°C) are also shown in Table 2.

Effect of polyvinylpyrollidone on mitomycin C adsorption

Polyvinylpyrollidone added to activated charcoal at a concentration ratio of 1:2·5 polyvinylpyrollidone/activated charcoal reduced the variability and capacity of adsorption of mitomycin C by 75–53 μm activated charcoal at 37°C (Figures 2 and 4). When polyvinylpyrollidone was added to the charcoal the adsorptive capacities for mitomycin C were similar for the four different size fractions (Figure 5).

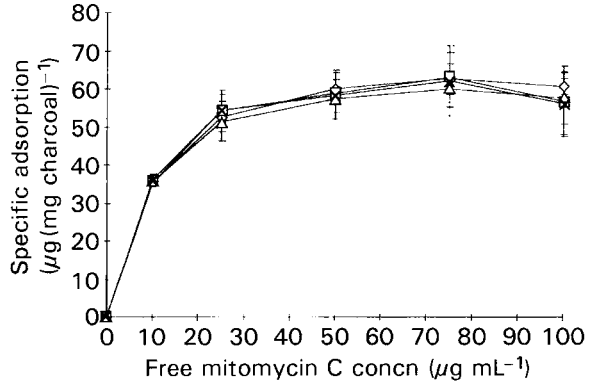

Figure 5. The effect of polyvinylpyrollidone on the inter-day variability of adsorption of mitomycin C by different-sized activated charcoal: ◇, 180–150 μm; □, 150–106 μm; △, 106–75 μm; ×, 75–53 μm. Results are the means ± s.e. of n = 4 isotherms.

Discussion

An important property of charcoals is their extended branching network of internal pores; compared with this the external surface area contributes almost negligible total adsorption (Cooney 1995). These pores can vary in size from millimetre dimensions (macro-pores) to nanometres (micro-pores) and their contribution to the total adsorption area can vary with comminution. The results of gas adsorption analysis, which measures the total adsorption area of the activated charcoal, including that of the pores, showed that the various activated charcoal fractions had similar values (Table 1).

Table 2. Variability of the specific adsorption of mitomycin C by activated charcoal.

Temperature (°C)	Range and maximum specific adsorption (Q) for fractions of activated charcoal of size range (μm):			
	180–150	150–106	106–75	75–53
21°C Equation:	$Q = 41C^{0.19} - 65C^{0.21}$	$Q = 35C^{0.13} - 72C^{0.22}$	$Q = 33C^{0.13-37}C^{0.15}$	$Q = 32C^{0.08} - 33C^{0.19}$
Q (μg mitomycin C (mg activated charcoal)$^{-1}$):	87–145	48–162	63–78	57–68
37°C Equation:	$Q = 36C^{0.12} - 69C^{0.20}$	$Q = 41C^{0.13} - 87C^{0.22}$	$Q = 40C^{0.14} - 50C^{0.17}$	$Q = 40C^{0.13} - 75C^{0.2}$
Q (μg mitomycin C (mg activated charcoal)$^{-1}$):	58–154	72–181	89–107	66–106

Mitomycin C adsorption isotherms for 75–53 μm activated charcoal particles under identical conditions (37°C, n = 4) varied by a factor of three (Figure 2). Such variation is disconcerting clinically because delivery of the dose in-vivo would presumably be subject to the same variation. Patients not being monitored would be at risk of either toxicity or therapeutic failure, especially for cytotoxic drugs with a narrow therapeutic index.

Adsorption of mitomycin C was greater at 37°C than at 21°C (Figure 3) which was unexpected because at higher temperatures molecules generally have more energy and would be expected to be desorbed (Cooney 1995). It is possible that the drug molecules with their higher energies penetrate the activated charcoal pore structure more easily and then become trapped. The temperature-dependence was noted again with activated charcoal particles of other sizes, but the threefold variation remained, implying that some other factor is responsible for this. Freundlich isotherms do not yield a true maximum specific adsorption value because of their exponential nature and the values shown are those obtained under the experimental conditions employed, which were identical for the respective isotherms (Gessner & Hasan 1987). Because the mitomycin C concentrations were always referred to standard curves obtained in the absence of activated charcoal, the variability was caused by the charcoal particles. We postulated that treating the charcoal with a wetting agent, surfactant or suspension stabilizer might alter the charcoal surface characteristics and reduce the variability of adsorption.

Polyvinylpyrollidone is a synthetic polymer of the monomer *N*-vinylpyrollidone. It is a flexible molecule similar to dextran and has been used as a plasma expander, dispersant, solubilizer and emulsion-stabilizer (Hewan-Lowe et al 1994). It is excreted by the kidneys if the molecular weight is smaller than 70 kDa. Larger molecules are phagocytosed by the reticuloendothelial system. Although polyvinylpyrollidone has low toxicity it can accumulate in tissues if injected systemically and can also interfere with certain diagnostic tests (Hewan-Lowe et al 1994).

When polyvinylpyrollidone was added to activated charcoal samples in a polyvinylpyrollidone/charcoal concentration ratio of 1 : 2·5, as used by Hagiwara et al (1988b) and Ito et al (1990), the variability of the mitomycin C adsorption isotherms was greatly reduced (Figure 4) compared with charcoal samples containing no polyvinylpyrollidone (Figure 2). However, overall the specific adsorption of mitomycin C by the activated charcoal was also reduced. Therefore, a higher quantity of activated charcoal would be needed to adsorb a given dose of drug. This inhibitory effect of polyvinylpyrollidone has been noted previously for other systems (Larionova et al 1985). By reducing the variability of the isotherm, the different activated charcoal size fractions were found to have similar mitomycin C adsorption capacities, consistent with the gas adsorption data shown in Table 1.

Although polyvinylpyrollidone clearly improved the reproducibility of the adsorption properties of the suspensions, its mode of action is uncertain. The suspension was visibly more stable and the charcoal particles remained suspended for much longer than without polyvinylpyrollidone. This supports the belief that thorough mixing of the suspension in the absence of polyvinylpyrollidone is critical for accurate dosing (Hagiwara & Takahashi 1987). A simple buoying (retarding) action, however, does not explain the reduced mitomycin C adsorption by activated charcoal and a surface interaction is implicated.

Activated charcoal adsorbs mitomycin C in a temperature-dependent manner. The variation in adsorption can be reduced with a suitable wetting agent such as polyvinylpyrollidone and the size of activated charcoal particles does not effect the amount of drug adsorbed. Activated charcoal is a promising drug-delivery system with many advantages over presently available alternatives, but its variable adsorption properties are cause for concern over its efficacy and safety in clinical use when it is simply suspended in saline with neither a wetting agent nor a buffer to control pH.

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